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Poster

433. Cortical Neurogenesis and Development

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 433.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: “Research Cooperability” Program of the Croatian Science Foundation funded by the European Union from the European Social Fund under the Operational Programme Efficient Human Resources 2014-2020 PSZ-2019-02-4710 (ZK) Croatian Science Foundation project DOK-2020-01-5029 (AJ)

Title: First condensation of cortical plate is characterized by the expression of projection neuron markers of the deep layers during early human brain development

Authors: ***J. KOPIC**¹, **A. JUNAKOVIC**¹, **M.-R. RASIN**², **I. KOSTOVIC**¹, **Z. KRŠNIK**¹;
¹Med. Sch. Zagreb, Univ. of Zagreb, Med. Sch. Zagreb, Univ. of Zagreb, Zagreb, Croatia;
²Robert Wood Johnson Med. School, Rutgers Univ., Rutgers, NJ

Abstract: Transition from embryonal to fetal human cortical development at 8 postconceptional weeks (8 PCW) is an event marked by the initial formation of the cortical plate (CP). At that point, cortical neurons align in a radial fashion in order to form first columns. Eventually, CP becomes more compact and increases in size that leading to primary condensation of the CP. As a precursor of the future six-layered cerebral neocortex, early CP contains projection neurons gathered in this transient fetal compartment. Importantly, future projection neurons need to reach their cortical destination, i.e. the exact laminar position to mature and obtain their molecular profile. Here we aimed to follow the expression pattern of molecular markers of the future projection neurons (CELFI, TBR1, CTIP2, TLE4) during an initial cortical plate formation at the early fetal developmental stages of the human telencephalic wall. We performed immunofluorescence (IF) stainings to reveal projection neuron markers pattern on prenatal postmortem human brain tissue. Our results showed that at the early period of primary CP condensation, projection neuron markers are already expressed in the CP, suggesting their potential role in neurogenetic processes responsible for correct layering of the future cerebral neocortex.

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Poster

433. Cortical Neurogenesis and Development

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Program #/Poster #: 433.02

Topic: A.01. Neurogenesis and Gliogenesis

Title: Polymicrogyria: an intersection of pathology and tensegrity

Authors: *R. HAMMOND¹, C. DUNHAM²;

¹Pathology and Lab. Med. and Clin. Neurolog. Sci., Univ. of Western Ontario, London, ON, Canada; ²Pathology and Lab. Med., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Polymicrogyria is an anomaly of cortical development. It may be pure but it is usually associated with other brain malformations. Polymicrogyria is etiologically diverse and associated with a spectrum of neurological consequences ranging from mild to severe in proportion to its extent and associated abnormalities. While there is no debate that the responsible insult acts on the developing brain, there has been a long and healthy one on timing and the significance of particular etiologies, inviting a variety of mechanisms to account for its peculiar architecture. One of the more reliable causes of polymicrogyria in an otherwise healthy fetus is congenital cytomegalovirus (CMV) infection. We studied nine cases of polymicrogyria in the setting of congenital CMV encephalitis in fetuses ranging from 16 to 39 weeks gestational age. Several pathological findings were almost invariable: necrosis of the subventricular zone, infection of subventricular germinal cells, radial glial disruption and injuries to the pial-glial border. Subcortical neuronal heterotopias and leptomeningeal heterotopias were also common. Clinical details and pathological features of these cases indicate that the responsible insults occurred before neuronal migration was complete, lasted several weeks and resulted in a variety of structural perturbations to the developing brain. These cases add to the evidence that polymicrogyria is not limited to late gestation and that a variety of injuries (and timings) can replicate its relatively stereotyped morphology. A categorical and reductionist focus on timing, etiology, specific polymorphisms and syndromes has distracted from a more fundamental and versatile construct. Into this void steps an attractive concept, tensegrity, whereby the brain's unique morphology (and disruptions of the same) are explained on the basis of forces at play at the cellular and macromolecular levels. Tensegrity is proposed as a unifying basis for polymicrogyria, embracing variables of timing and etiology.

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Title: WITHDRAWN

Poster

433. Cortical Neurogenesis and Development

Location: SDCC Halls B-H

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Program #/Poster #: 433.04

Topic: A.01. Neurogenesis and Gliogenesis

Title: Genetic fate mapping techniques based on Tbr2 expression identify neuronal variety in the deep projection layers of the cortex

Authors: *Z. ATAK, T. F. HAYDAR;

Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC

Abstract: Neuronal progenitors within the neocortex are highly varied in their characteristics. While the brain in general, and the cerebral cortex in particular, have a great diversity of neurons, the mechanism by which this diversity originates remains an open and fundamental question. Excitatory neurons, which constitute almost 80% of the entire neocortical neuron population, are either generated directly or indirectly. This origin discrepancy plays a crucial role in shaping the morphometric and physiological neuronal diversity observed across cerebro-cortical layers. Prior work from our group has demonstrated that Tbr2-expressing neural precursors generate neurons with different morphology and function compared to their Tbr2-negative counterparts. To determine whether Tbr2 and non-Tbr2 lineages also contribute to the neuronal diversity within the deep projection layers of the cortex (layer V and VI), we used in utero electroporation (IUE) to introduce Tbr2-Cre driver plasmids with dual fluorescent reporters to fate-map neural precursors and their progeny. This strategy enables fate mapping of Tbr2 lineage neurons from birth to maturity. **RESULTS:** Developing mouse embryos were electroporated at E11.5 and E12.5 and neural precursors as well as mature neurons were assessed at 24- and 48-hours post-electroporation and at the third postnatal week (P21), respectively. The electroporated Tbr2 lineage precursors showed a high degree of co-localization with TBR2 in contrast to non-Tbr2 lineage cells. At 24 hours, both Tbr2-expressing and non-Tbr2 precursors contributed to doublecortin-immunoreactive (Dcx+) immature neurons. The majority of electroporated cells at this stage displayed no Sox2 immunoreactivity, suggestive of successful differentiation into immature neurons. Approximately 40% of the IUE-derived neurons localized to layer VI while the remainder was located in layer V at P21. Interestingly, within layer VI, Tbr2 lineage cells generated neurons with distinct soma size and arborization compared to the non-Tbr2 expressing lineage. Future work will assess the projection patterns and functional differences between Tbr2 and non-Tbr2 lineages in the deep cortical layers (V and VI).

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH NINDS 4R00NS111731
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T32GM008243

Title: Examining the molecular determinants of cortical arealization with single-cell organoid perturbation

Authors: *P. R. NANO, C. V. NGUYEN, J. MIL, S. WANG, W. GE, E. FAZZARI, A. BHADURI;
Biol. Chem., David Geffen Sch. of Medicine, Univ. of California Los Angeles, Los Angeles, CA

Abstract: The cerebral cortex derives its power from areas specializing in judgment, movement, and other functions, and area-specific phenotypes characterize virtually all neurodevelopmental and neuropsychiatric disorders. These typically emerge during development, suggesting a link with the patterning of cortical areas. Despite a century of work characterizing cortical areas, therapeutic insights into neurological disorders remain limited by gaps in the molecular understanding of how these areas form. We have addressed this with single-cell transcriptomic profiling, revealing the genes that distinguish areas with high cell type resolution. These data provide testable hypotheses regarding cortical arealization, including *which* area-specific transcription factors drive arealization, *when* they commit cells to areal fates, and *if* they interact with extrinsic cues. We have addressed these hypotheses by integrating organoid and CRISPR technologies, focusing on the pre-frontal cortical area (PFC), which has the most distinct molecular signature and is specifically targeted in autism and schizophrenia. We have established a single-cell CRISPR activation platform in cortical organoids that will (1) screen for determinants of PFC-specific gene expression patterns, (2) measure the developmental time window of the activity of these determinants, and (3) evaluate their sensitivity to determinants of other cortical areas. These studies seek to reveal which PFC markers prime cells towards PFC identity in early development and determine whether these factors can act in later developmental stages to commit cells to PFC fate. Broadly, our findings will identify networks that might be implicated in neurological disease and establish a platform for the molecular analysis of other cortical regions.

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Korea Institute of Toxicology KK-2205

Title: A single-cell Transcriptomic analysis of gyrencephalic cortex development in the miniature pig

Authors: *K.-T. KIM¹, H.-W. JEONG², J. SONG¹, Y. KIM¹, J. HWANG¹, S.-C. HAN¹;
¹Korea institute of toxicology, Korea institute of toxicology, Jeongeup, Korea, Republic of;
²Max-Planck Inst. for Mol. Biomedicine, Muenster, Germany

Abstract: The swine's central nervous system (CNS) has anatomical features similar to the human brain. In particular, swine have a gyrencephalic brain, mainly composed of gyrus and sulcus with a developmental stage similar to humans. In particular, swine has a gyrencephalic brain, mainly composed of white and gray matter with developmental stages similar to humans. To understand gyrencephalic cortex development, we analyzed the gene expression by scRNA sequencing using 12 Yucatan miniature pig embryos between 4 weeks of fertilization and postnatal day 0. We identified significant subtypes of 10 progenitors and 7 neurons in the PFC region and revealed the specific gene expression similar to human development at the single-cell resolution. And it was confirmed that the gyrencephalic brain structure of the cerebral cortex is very similar to the developing human brain. Especially, it was confirmed that the inner subventricular zone (iSVZ) and the external subventricular zone (oSVZ) are similar to developing human brains, and the neuronal differentiation is very active in the early stage of cortex with the gyrus and sulcus formation. Thus, the cortical structure, gene expression, and cell types and functions were similar to the human brain, thereby verifying the possibility of a neurodevelopmental research model.

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01NS123263
NSF GRFP fellowship

Title: Mapping differentiation patterns of human radial glia using single cell lineage tracing

Authors: *M. R. STEYERT¹, M. G. KEEFE¹, R. N. DELGADO², D. E. ALLEN¹, T. J. NOWAKOWSKI¹;

¹Univ. of California San Francisco, San Francisco, CA; ²Harvard Med. Sch., Boston, MA

Abstract: The human neocortex is composed of a variety of cell types organized in a discrete cytoarchitecture which is set up during prenatal development. Both neurons and most glia are generated by progenitors known as radial glia that span the cortical wall and serve as the stem

cells of the cortex. At around mid-neurogenesis in human cortical development, radial glia undergo a dramatic separation into two distinct subtypes that occupy different niches: truncated radial glia (tRG) that maintain contact with the ventricle and outer radial glia (oRG) that maintain contact with the pial surface. On the population level, radial glia give rise to cell types in a defined order starting with deep layer neurons followed by upper layer neurons and eventually astrocytes and oligodendrocytes. However, the lineage relationships between these cell types and the lineage potential of individual radial glia has remained unknown. Recently, we developed a novel lineage tracing tool called STICR that marks individual cells with a highly diverse, heritable DNA barcode that is expressed by the infected cell and its progeny. We identified that individual radial glia are multipotent: many produced both neurons and glia. Surprisingly, we found that cortical radial glia that produced excitatory neurons frequently also gave rise to inhibitory neurons, identifying a novel mechanism for generating neuronal diversity in the cortex and expanding the known potency of human radial glia. We further assayed the lineage potential of radial glia occupying distinct niches in the germinal zone and found that they gave rise to previously unappreciated subtypes of astrocytes that are molecularly, morphologically, and positionally unique. Finally, we found that radial glia output is determined by subtype and is influenced by extracellular signaling factors, providing a potential explanation for how the generation of cortical cells is regulated *in vivo*. Together, these studies illuminate the heterogeneous nature of human cortical radial glia and the means by which they contribute to cellular diversity in the neocortex.

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Topic: A.01. Neurogenesis and Gliogenesis

Support: R01MH124808
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Title: Myt1l suppresses early neuronal differentiation programs to determine neuronal fate

Authors: *J. CHEN¹, N. FUHLER², K. K. NOGUCHI², J. D. DOUGHERTY¹;
¹Genet. and Psychiatry, ²Psychiatry, Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Myelin Transcriptional Factor 1 Like (MYT1L) is a pro-neuronal transcriptional factor, which has been implicated in human neurodevelopmental disorders (NDDs), like ASD, ID, and ADHD. Although both *in vitro* overexpression model and *in vivo* mouse model have confirmed its potent roles in neuronal differentiation, MYT1L's genomic targets, molecular and cellular functions across normal brain development remain poorly understood. Here, we adopted

newly developed Cut and Run technology to profile MYT1L targets at different developmental time points in the mouse brain. Leveraging the MYT1L germline knockout mouse, we identified highly confident and specific MYT1L binding targets both in developing cortex and adult prefrontal cortex (PFC), without noticing obvious binding changes between the two. Motif analysis revealed different TF co-occupancies at MYT1L bound promoter and enhancer regions, where motifs of transcriptional activators (e.g., SP1 and ELK1) and neurogenic factors (e.g., NEUROD1 and NEUROD2) were significantly enriched respectively. We next integrated Cut & Run data with ATAC-seq datasets and found MYT1L binding has subtle effects on chromatin accessibility, while MYT1L loss dramatically affects histone modification landscapes at its binding sites. Combining with RNA-seq datasets, we found such alternation results in the consistent activation of early neuronal differentiation programs across mouse brain development and the subsequent pre-mature neuronal transcriptomic profiles in the adult PFC. Notably, MYT1L directly regulates the expression of genes associated with neuronal identity in different cortical layers (e.g., CTIP2). Further immunohistochemistry experiments revealed an up-regulated ratio of CTIP2+/BRN2+ neurons in MYT1L heterozygous mice cortex, echoing MYT1L's role in determining neuronal identity between deep and up cortical layers. Together, this study identified MYT1L targets across developmental stages and defines its molecular and cellular functions *in vivo*, providing valuable insights to how its loss of functions lead to human disease pathogenesis.

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Topic: A.01. Neurogenesis and Gliogenesis

Title: Characterizing Extrinsic Thalamic Signals that Influence Cortical Fate Specification in Fused Organoids

Authors: *C. NGUYEN¹, P. NANO², J. MIL², A. BHADURI²;
¹Neurosci., ²Biol. Chem., UCLA, Los Angeles, CA

Abstract: The thalamus has largely been thought of as a relay center for sensory information destined for the cortex. However, the thalamus also plays a vital developmental role. It grows in parallel with the cortex and together these areas form early reciprocal thalamocortical afferents (TCAs). According to the protocortex hypothesis, extrinsic thalamic signaling is necessary for refining cortical areas and cell types. However, the specific contribution of thalamic input and TCA-derived cues remain unknown. Organoids, 3D structures generated from stem cells, provide a tractable system to answer these types of questions as they recapitulate aspects of early human development. Thus, they can be used to assess the factors required to transition pluripotent cells into differentiated cell types. Furthermore, organoids allow us to visualize cortical development

in isolation or with extrinsic inputs by fusing cortical and thalamic organoids. In this study, we generate cortical and thalamic organoids to investigate thalamic signaling and TCA-derived molecular cues involved in cortical patterning. In our thalamic organoids, we induce discrete morphogenetic gradients to enrich for neuronal populations of interest. Furthermore, we validate a protocol to fuse organoids resembling early developing human cortex and thalamus into thalamocortical assembloids. Using single-cell RNA sequencing and immunohistochemistry techniques, we reveal the ability of reciprocal TCAs to induce arealization of human cortex and thalamus. Revealing the contributions of early thalamic signaling can enhance our knowledge of neurodevelopmental disorders characterized by thalamocortical dysregulation, including schizophrenia and autism. Elucidating the mechanisms underlying this developmental divergence may help inform future therapeutics.

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Topic: A.01. Neurogenesis and Gliogenesis

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Title: Crosstalk between Protocadherin 8 and transcription factor Dbx1 regulate cell fate in the developing cerebral cortex

Authors: *A. W. CWETSCH^{1,2,3}, J. GILABERT JUAN⁴, S. FERREIRA^{3,2}, M. X. MOREAU^{2,3}, Y. SAILLOUR^{2,3}, E. DELBERGHE², J. GONZÁLEZ MARTÍNEZ⁵, S. NÉDÉLEC⁶, U. BORELLO⁷, S. THOMAS², F. CAUSERET^{2,3}, A. PIERANI^{2,3};

¹Univ. of Valencia, Univ. of Valencia, Valencia, Burjassot, Spain; ²Inst. Imagine, Paris, France; ³Inst. Psychiatry And Neurosci. De Paris - Inserm Univ. Paris City, Paris, France; ⁴Univ. Autónoma de Madrid, Madrid, Spain; ⁵Natl. Cancer Res. Ctr. (Centro Nacional de Investigaciones Oncológicas), Madrid, Spain; ⁶Inst. du Fer à moulin, Paris, France; ⁷Univ. of Pisa, Pisa, Italy

Abstract: Normal development of the mammalian central nervous system requires correct tissue patterning, production of the appropriate cell types and establishment of functional neuronal circuits. Transcription factors (TFs) play essential roles in these processes, regulating the expression of target genes responsible for neuronal subtypes specific features. Cell adhesion molecules are key components of neuronal identities that control cell sorting, migration, neurite outgrowth/guidance and synaptogenesis. So far, TFs are known to control neuronal adhesion but not the opposite.

Here, using acute gain- and loss-of-function experiments by *in utero* electroporation in the

developing mouse telencephalon we demonstrate that ectopic expression of Dbx1, a homeodomain TF acting as a cell fate determinant, leads to increased expression of Protocadherin 8 (*Pcdh8*) and cell aggregation, together with the induction of neuronal fate markers *Nurr1* and *Pax6*. These effects were modulated depending on the region and timing of electroporation. Furthermore, we found that *Pcdh8* expression is required for Dbx1-induced fate specification. Surprisingly, *Pcdh8* overexpression also proved sufficient to induce Dbx1 expression as well as a complete reorganisation of the apico-basal and dorso-ventral axes. Finally, we present evidence that these effects are mediated through regulation of the expression of Notch ligands and promotion of cell cycle exit.

Altogether, our work therefore points to cell adhesion molecules as important, yet unexpected, players in the regulation of cell identity and, in particular, *Pcdh8* through its crossregulation with the Dbx1 transcription factor.

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Title: Chromatin remodeler *Arid1a* regulates subplate neuron identity and wiring of cortical connectivity

Authors: ***D. Z. DOYLE**, M. M. LAM, A. QALIEH, Y. QALIEH, A. SOREL, O. H. FUNK, K. Y. KWAN;

Michigan Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: Loss-of-function mutations in chromatin remodeler gene *ARID1A* are a cause of Coffin-Siris syndrome, a developmental disorder characterized by dysgenesis of corpus callosum. Here, we characterize *Arid1a* function during cortical development and find unexpectedly selective roles for *Arid1a* in subplate neurons. Subplate neurons, strategically positioned at the interface of cortical grey and white matter, orchestrate multiple developmental

processes indispensable for neural circuit wiring. We find that pan-cortical deletion of *Arid1a* leads to extensive mistargeting of intracortical axons and agenesis of corpus callosum. Sparse *Arid1a* deletion, however, does not autonomously misroute callosal axons, implicating non-cell autonomous *Arid1a* functions in axon guidance. Supporting this possibility, the ascending axons of thalamocortical neurons, which are not autonomously affected by cortical *Arid1a* deletion, are also disrupted in their pathfinding into cortex and innervation of whisker barrels. Coincident with these miswiring phenotypes, which are reminiscent of subplate ablation, we unbiasedly find a selective loss of subplate neuron gene expression following *Arid1a* deletion. In addition, multiple characteristics of subplate neurons crucial to their wiring functions, including subplate organization, subplate-thalamocortical axon co-fasciculation (“handshake”), and extracellular matrix, are severely disrupted. To empirically test *Arid1a* sufficiency in subplate, we generate a cortical plate deletion of *Arid1a* that spares subplate neurons. In this model, subplate *Arid1a* expression is sufficient for subplate-thalamocortical axon co-fasciculation and extracellular matrix. Consistent with these wiring functions, subplate *Arid1a* sufficiently enables normal callosum formation, thalamocortical axon targeting, and whisker barrel development. Thus, *Arid1a* is a multifunctional regulator of subplate-dependent guidance mechanisms essential to cortical circuit wiring.

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Title: Erk1/2 signaling is required for the maturation of a subset of cortical inhibitory neurons

Authors: *S. KNOWLES¹, M. HOLTER², K. REES³, J. S. MARTINEZ-FUENTES⁴, A. M. STAFFORD⁵, D. VOGT⁶, J. NEWBERN⁷;

²Sch. of Life Sci., ³SOLS, ¹Arizona State Univ., Tempe, AZ; ⁴Boston Univ., Allston, MA;

⁵Pediatrics and Human Develop., ⁶Dept. of Pediatrics and Human Develop., Michigan State Univ., Grand Rapids, MI; ⁷Sch. of Life Sci., Arizona State Univ. - Sch. of Life Sci., Tempe, AZ

Abstract: The canonical RAS/RAF/MEK/ERK1/2 (ERK1/2) pathway is a highly conserved signaling cascade found in nearly all cells which is activated by diverse extracellular signals during development. Dysregulated ERK1/2 signaling is involved in multiple neurodevelopmental syndromes, particularly the RASopathies and select genetic forms of autism spectrum disorder (ASD). Cognitive dysfunction in each of these disorders has been linked to deficits in cortical inhibitory circuits, however the roles of ERK1/2 in the development and basic functions of MGE-derived GABAergic interneurons are poorly understood. Here, we used a conditional genetic approach to specifically delete the principal ERK1/2 components *Mapk3/Erk1* (*Erk1^{-/-}*) and *Mapk1/Erk2* (*Erk2^{fl/fl}*) from MGE derived GABAergic interneurons. We determined that ERK1/2 is not required for the establishment of cortical GABAergic interneuron number or the expression of parvalbumin, a marker for a large subset of interneurons. However, our data reveal that ERK1/2 is necessary for the expression of SST, a neuropeptide expressed in ~30% of cortical interneurons. Due to well established roles of ERK1/2 in activity-dependent development in excitatory neurons, we then tested whether the GABAergic neuron response to in vivo stimulation was altered by loss of ERK1/2. We found that ERK1/2 deleted GABAergic neurons have a significantly attenuated FOSB response following chemogenetic stimulation. Behavioral responses associated with chemogenetic treatment were also reduced in mutant animals. Interestingly, one week of chemogenetic stimulation led to a partial rescue of SST expression in ERK1/2 deleted neurons. Our data highlight the important roles of ERK1/2 and activity in the development and maturation of cortical inhibitory neurons.

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Title: Fate plasticity of interneuron specification

Authors: *M. A. MOSTAJO-RADJI¹, W. R. MANCIA LEON², A. BREEVOORT², L. ZHOU², J. LEHRER¹, M. T. SCHMITZ², Y. PEREZ², T. MUKHTAR², M. G. ANDREWS², J. CHU², F. SULLIVAN¹, D. TEJERA², E. C. CHOY¹, H. E. SCHWEIGER¹, M. F. PAREDES², V. D. JONSSON¹, M. TEODORESCU¹, A. R. KRIEGSTEIN², A. ALVAREZ-BUYLLA², A. A. POLLEN²;

¹Univ. of California Santa Cruz, Santa Cruz, CA; ²Univ. of California San Francisco, San Francisco, CA

Abstract: Competing genetic programs underlie the generation of neuronal subtypes of the mammalian central nervous system. The medial ganglionic eminences (MGEs) give rise to 2 important cortical interneurons (cINs) populations labeled by Somatostatin (SST) and Parvalbumin (PV), which develop at different timelines. To date, the extent to which extrinsic cues influence these identities remains unknown. PV-positive cINs are crucial for regulating cortical circuits due to their strong perisomatic inhibitory targeting, but to date they have been difficult to model in vitro. Here, we investigated the contribution of the environment in shaping and maintaining PV cINs. First, we grafted mouse MGE progenitors onto 2D and 3D mouse and human cortical, MGE and thalamic co-culture models, including dissociated cells, organoids, organotypic and conditioned cultures. We detected different proportions of SST or PV cIN descendants across models. We discovered that grafting onto 3D models of human, but not mouse, corticogenesis leads to the efficient differentiation of PV-positive cINs in a nonautonomous manner. We observed the upregulation of molecular markers of PV maturation, the repression of SST-specific markers and the establishment of perineuronal nets. Furthermore, grafting of lineage-traced postmitotic SST-positive cINs resulted in the upregulation of PV. Altogether, our work unveils an unexpected level of fate plasticity of MGE-derived cINs, whose identities are defined and refined by the environment.

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Program #/Poster #: 433.14

Topic: A.01. Neurogenesis and Gliogenesis

Support: Australian Research Council grant DP200102363
Washington University start-up funding

Title: Nuclear factor one transcription factors regulate the epigenomic landscape of both neurons and glia during cortical development

Authors: ***J. W. C. LIM**^{1,2}, N. A. MUNDELL¹, S. PAL¹, L. A. WILMOTT¹, M. CHING³, J. BUNT⁴, L. J. RICHARDS^{1,2};

¹Dept. of Neurosci., Washington Univ., St Louis, MO; ²Queensland Brain Inst., ³Sch. of Biomed. Sci., The Univ. of Queensland, Brisbane, Australia; ⁴Princess Máxima Ctr. for Pediatric Oncology, Utrecht, Netherlands

Abstract: Cortical development involves the differentiation of both neurons and glia in precise sequential patterns. This process is driven by transcriptional and epigenetic regulatory mechanisms that regulate gene expression in precise spatiotemporal patterns. The nuclear factor one (NFI) transcription factors are disrupted in various human diseases, including brain cancer and a variety of developmental disorders. In mice, NFI proteins are expressed in almost all cell types during development and in the adult cortex. Not only are there multiple *Nfi* gene family members (*Nfia*, *b*, *c* and *x*) but there are also multiple splice variants of each gene, making their biological function quite complex and potentially redundant across family members. It is therefore crucial to understand what core aspects of development are essentially controlled by the NFI transcription factors, but this has been challenging to study in single gene mutants. To address this, we generated an *Emx1*-Cre-driven conditional mouse model to delete *Nfia*, *Nfib* and *Nfix* in cortical progenitors and their progeny (note *Nfic* shows only minimal expression in brain). Histological analyses of *Nfi* triple mutant mice indicated broad defects in brain development and alterations in both neuronal and glial differentiation. To further investigate NFI function in different cell types, we utilised a single-cell multiomics approach to identify both shared and cell type-specific changes in chromatin accessibility and gene expression. We observed changes to chromatin accessibility at distal enhancers that are unique to each cell type. Therefore, our data suggest that histological defects observed in our mouse model are not solely due to defects in the progenitor population that similarly affects both neurons and glia. Further analyses of this large dataset are ongoing to provide a set of core regulatory functions on the NFI gene family.

Disclosures: **J.W.C. Lim:** None. **N.A. Mundell:** None. **S. Pal:** None. **L.A. Wilmott:** None. **M. Ching:** None. **J. Bunt:** None. **L.J. Richards:** None.

Poster

433. Cortical Neurogenesis and Development

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 433.15

Topic: A.01. Neurogenesis and Gliogenesis

Title: Single nucleus RNA sequencing reveals downregulated Wnt signaling in the mouse cortex after prenatal alcohol exposure during neurogenesis

Authors: **D. SAMBO**, C. GOHEL, Q. YUAN, D. GOLDMAN;
Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD

Abstract: Fetal Alcohol Spectrum Disorder (FASD) encompasses the array of conditions associated with prenatal alcohol exposure (PAE), including physical dysmorphism, cognitive deficits, and behavioral issues. While several mechanisms of alcohol teratogenicity have been explored, how specific cell types during neurogenesis in the developing brain may be differentially affected by PAE is poorly understood. In this study, we used single nucleus RNA sequencing (snRNAseq) to investigate whether PAE from neurulation through peak cortical neurogenesis induces cell type specific changes in the developing cortex. Pregnant C57BL/6J dams were treated with 2.5 mg/kg alcohol or saline daily from embryonic day 8 (E8) to E13. On E14, embryo cortices were harvested, and nuclei were extracted and processed for snRNAseq. Unsupervised cluster analysis revealed 25 neuronal cell types, including subtypes of neural epithelial cells (NECs), radial glial cells (RGCs), intermediate progenitor cells (IPCs), projection neurons, and interneurons. Interestingly, only the Wnt-expressing NEC cluster showed a significantly decreased percentage of cells after PAE. None of the cell types showed PAE-induced apoptosis as measured by caspase expression. Cell cycle analysis revealed only a subtype of RGCs expressing the downstream Wnt transcription factor Tcf7l2 had a decreased percentage of cells in the G2/M phase of the cell cycle, suggesting decreased proliferation in this RGC subtype and further implicating the disruption of Wnt signaling in this model. Pseudotime analyses revealed an increased pseudotime score in the IPCs and projection neuron cell types, suggesting increased or premature differentiation of these cells. Biological processes implicated in pathways analysis showed an upregulation of pathways related to synaptic activity and neuronal differentiation and downregulation in pathways related to chromosome structure regulation and regulation of the cell cycle. Several cell types showed a decrease in Wnt-signaling pathways, with several genes related to Wnt signaling being altered by PAE. Overall, these findings implicate a downregulation of Wnt signaling in the developing cortex and increased differentiation of IPCs and projection neurons after PAE. As Wnt-signaling has been shown to promote proliferation and inhibit differentiation at earlier stages in development, the downregulation of Wnt signaling potentially promoted premature neuronal maturation of projection neurons and their intermediate progenitors. These findings provide further insight into the cellular pathogenesis of FASD and deepen our understanding of alcohol teratogenicity.

Disclosures: D. Sambo: None. C. Gohel: None. Q. Yuan: None. D. Goldman: None.

Poster

433. Cortical Neurogenesis and Development

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 433.16

Topic: A.01. Neurogenesis and Gliogenesis

Support: MoE 1-441-122

Title: Effects of prenatal gabapentinoids exposure on human cortical neurons

Authors: *W. ALSANIE¹, M. ALHOMRANI², Y. S. ALTHOBAITI³, H. HABEEBALLAH⁴; ²Collage of applied medical science, ³Pharmacol. and Toxicology, ¹Taif Univ., Taif, Saudi Arabia; ⁴Hlth. and Biomed. Sci., RMIT Univ., Preston, Australia

Abstract: Prenatal substance exposure is a major public health concern associated with many detrimental fetal consequences. Unfortunately, polysubstance use in pregnancy is common. Gabapentinoids are widely used as treatments in psychiatry and neurology; however, they have been increasingly reported as having potential for misuse. Moreover, gabapentinoids can cross the placental barrier. Due to difficulties in accessing fetal brains exposed to gabapentinoids, we used the human embryonic stem cell (hESC) line H9 to generate early, intermediate cortical progenitors and cortical neurons to modulate prenatal gabapentinoid exposure *in vitro*. Since the cortex is responsible for cognition and behavior, we focused on cortical development. We analyzed treated (10uM) and untreated (control) cultures for gene expressions, neurogenesis, and morphogenesis. At the early patterning stage, there was a significant increase in Tbr2+ intermediate progenitors in pregabalin- and gabapentin-treated cultures. In addition, there was a significant increase in the expression of cortical related genes *Pax6*, *Foxg1*, and *Tbr2* in pregabalin-treated cultures, whereas gabapentin significantly increased *Tbr2* expression solely. At the maturation stage, the number of mature cortical neurons was unchanged in pregabalin-treated cultures. At early maturation, gabapentin significantly increased Tbr1+ neurons, but not Ctip2+ neurons. At the genetic level, we screened the effects of pregabalin on different cortical layer related genes. Pregabalin significantly increased expression of *Brn2* without significant effects on other screened genes. Meanwhile, gabapentin did not alter any cortical layer related genes. Regarding morphogenical analysis, both pregabalin and gabapentin significantly decreased neurite length, branches, and neurites of human cortical neurons. Our data also shows that the effects of pregabalin and gabapentin on the morphogenesis of cortical neurons differ based on the presence of maturation factors, such as GDNF and BDNF, suggesting a possible interaction mechanism. Our study demonstrates that exposure to gabapentinoids during early brain development may interfere with the neurogenesis and morphogenesis of various neuronal subpopulations. Currently, we are investigating gabapentinoids' effects on cortical neuron functionality.

Disclosures: W. Alsanie: None. M. Alhomrani: None. Y.S. Althobaiti: None. H. Habeeballah: None.

Poster

433. Cortical Neurogenesis and Development

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 433.17

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant 5R01NS099099-05
NIH grant R35 GM119831

Title: Modeling chromatin state changes across early cortical neurodevelopment.

Authors: *Y. WANG¹, A. R. YPSILANTI², E. MARKENSCOFF-PAPADIMITRIOU³, K. J. LIM², K. CICHEWICZ⁴, R. CATTAPRETA⁵, J. L. RUBENSTEIN⁶, A. S. NORD⁷;

¹UC Davis/NIH NeuroMab Facility, DAVIS, CA; ³Psychiatry, ²UCSF, San Francisco, CA; ⁴UC Davis, Davis, CA; ⁵Univ. of California Davis, Davis, CA; ⁶Nina Ireland Lab. Dev Neurobiol, Univ. of California San Francisco, San Francisco, CA; ⁷Genomics Div., Univ. of California, Davis, Davis, CA

Abstract: In the cerebral cortex, neurons constitute a highly heterogeneous collection of cell types that are characterized by their unique spatial and temporal capabilities to constitute neuronal circuits that, later on, will control high-order functions of the brain. Neuronal dysfunction contributes to neurological disease states and neurodevelopmental disorders (NDDs). A deeper understanding of cortical neuron early development and their integration in cortical circuits through implementation of cell-intrinsic genetic programs and chromatin state in progenitor cells is needed. Regulatory elements (REs) are DNA sequences that, along with the binding of specific transcription factors, regulate gene transcription, and are among the driving factors of cortical neuronal development. These sequences are usually found in genomic portions that generally experience genomic configuration changes in the development process, as they alter between closed and open chromosomal states in different developmental time-points. Here, we hypothesize that, as neural progenitors develop into different types of neurons, there are REs that alter their configurations in a synchronized manner. To detect potential REs and to elucidate initial epigenomic changes, we isolated and FAC sorted neurons and neuronal progenitors at different mouse brain developmental time-points as well as from early postnatal cortical layers 5 and 6. We then sequenced specific DNA regions including loose chromosomal regions via ATAC-seq, and analyzed histone modifications associated with chromatin state, via ChIP-seq looking at H3K4me3, H3K27ac, and H3K27me3 modification marks. Our initial results show temporal changes on epigenomic patterns across prenatal and early postnatal development, and identify synchronized regions with similar patterns of epigenomic changes during development. These preliminary findings are important in setting the foundations to a deeper understanding of mechanisms involved in chromatin state regulation across early cortical neuronal development, processes that are key in the neuropathology of NDDs.

Disclosures: Y. Wang: None. A.R. Ypsilanti: None. E. Markenscoff-Papadimitriou: None. K.J. Lim: None. K. Cichewicz: None. R. Catta-Preta: None. J.L. Rubenstein: None. A.S. Nord: None.

Poster

433. Cortical Neurogenesis and Development

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 433.18

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIMH (K01 MH109747),
NIGMS (DP2 GM137423)
SFARI Pilot Award (615098)

Title: Rbfox proteins coordinate alternative splicing of transcription regulators in the developing neocortex

Authors: *X. ZHANG, X. RUAN, K. HU;
Univ. of Chicago, Chicago, IL

Abstract: Alternative pre-mRNA splicing increases transcript diversity in the mammalian neocortex, and dysregulation of splicing networks has been implicated in neurodevelopmental disorders. The function of splicing regulators remains largely unspecified because of genetic redundancy and compensation between homologous proteins. Here, a comparison of radial glial cells (RGCs) and newly born neurons in the developing mouse neocortex uncovers that transcription regulators are highly enriched for differential splicing, which switches protein isoforms or induces nonsense-mediated mRNA decay. Enriched binding motif and single-cell analyses indicate that *Rbfox3*, together with its homologs *Rbfox1/2*, play pivotal roles in initiating neuron-specific splicing. Ectopic expression of *Rbfox3* in radial glial progenitors induces neuronal splicing events that preferentially affect transcription regulators. We further show that the *Rbfox3* and *Rbfox2* switch the splicing of *Meis2*, RGC-isoform of which promotes *Tgfb3* expression. Surprisingly, *Rbfox3* and *Rbfox2* promote the inclusion of a *Ptbp1* poison exon that decreases *Ptbp1* expression. We utilize multiplexed CRISPR editing to simultaneously ablate *Rbfox1/2/3* and uncover their functions in radial neuronal migration and differential splicing of transcription regulators. Our results indicate that *Rbfox* proteins regulate cortical development by antagonizing *Ptbp1* and coordinating isoform switching of transcription regulators.

Disclosures: X. Zhang: None. X. Ruan: None. K. Hu: None.

Poster

433. Cortical Neurogenesis and Development

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 433.19

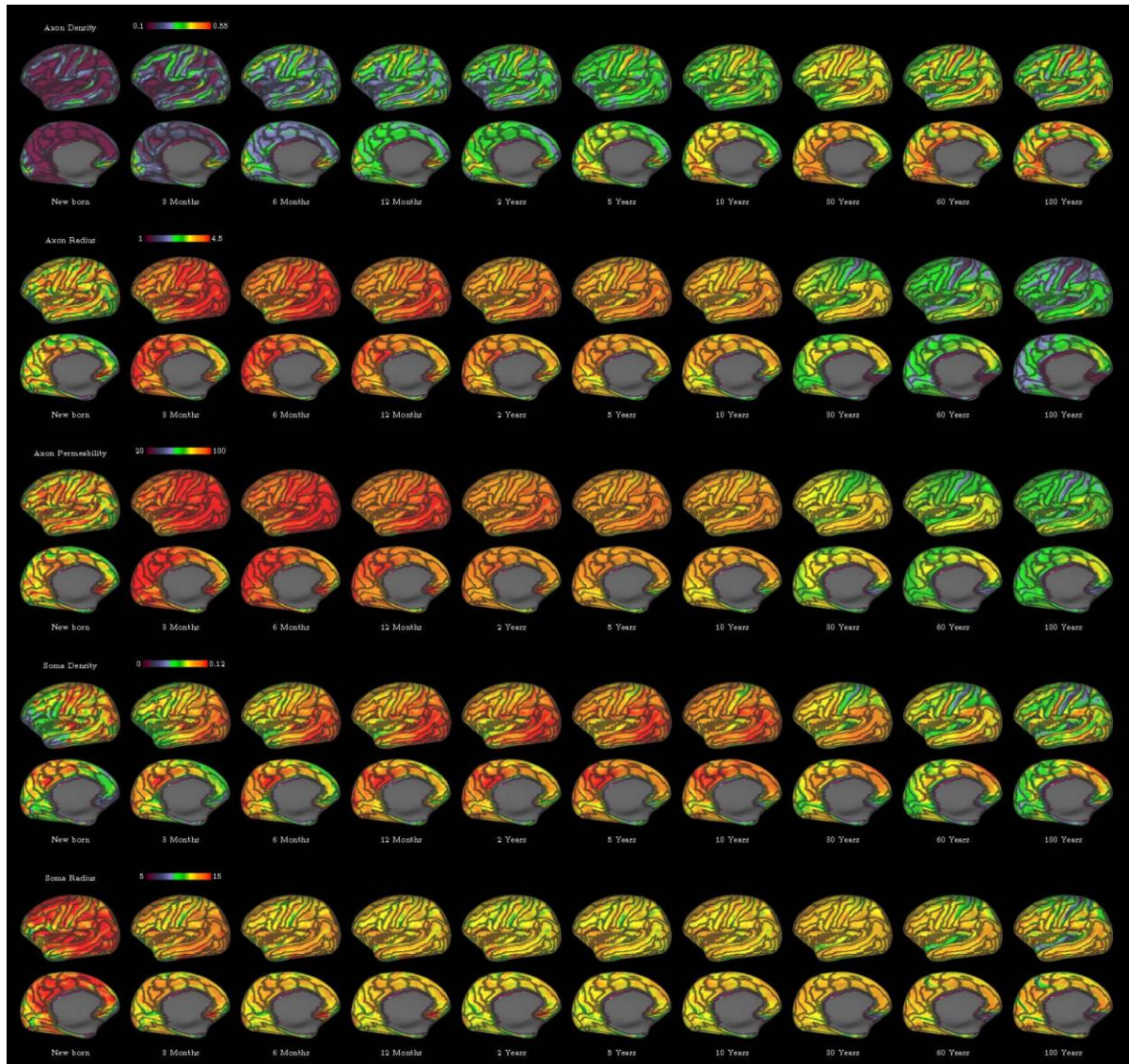
Topic: A.02. Postnatal Neurogenesis

Support: NIH grant MH125479
NIH grant EB008374

Title: Lifespan cortical microstructure development of the human brain

Authors: *K. HUYNH, S. AHMAD, K.-H. THUNG, Z. WU, W. LIN, G. LI, L. WANG, P.-T. YAP;
Univ. of North Carolina Chapel Hill, Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract:



Human brain cortical microstructure undergoes dynamic spatiotemporal changes across the lifespan. However, existing efforts to map such changes are limited to specific age ranges not covering the entire lifespan. Here, we charted the comprehensive developmental curves of cortical microstructure in-vivo using over 1000 diffusion MRI scans of over 800 subjects scanned between 39 weeks gestational age to 100 years chronological age. We used microstructure fingerprinting to quantify cell density, radius, and membrane permeability from multiple datasets from the Lifespan Human Connectome Project. Diffusion-based measurements were then mapped to cortical surfaces, harmonized to remove non-biological inter-site variations, and modeled as a function of individual, age, and gender with a generalized additive mixed model. We found axonal density increases globally from birth, peaks at 30-35 years, and then decreases. Notably, the primary visual cortex, primary sensory cortex, and primary motor cortex develop before other regions and peak between 6-9 years. Cortical axonal radius and membrane permeability increase slightly during the first 6 months of life and then decrease over time,

indicating brain development in terms of myelination. Soma density increases globally and greatly from birth to 10 years and then decreases. The spatial pattern of the soma density map matches the Destrieux cortical atlas, which was semi-manually delineated using ex-vivo cytoarchitecture. Specifically, soma density is higher in the pre-motor, somatosensory, and temporal cortices and lower in the primary motor cortex, in line with the literature. Soma radius shows nonsignificant decline over time. For all indices, there is no significant gender and left-right hemispheric differences. Most measurements change most rapidly during 0-3 months after birth, indicating this is a crucial brain development period. In conclusion, our study provides to date the most comprehensive spatial and temporal mapping of the cortical microstructure developmental changes throughout the entire human lifespan.

Disclosures: **K. Huynh:** None. **S. Ahmad:** None. **K. Thung:** None. **Z. Wu:** None. **W. Lin:** None. **G. Li:** None. **L. Wang:** None. **P. Yap:** None.

Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 434.01

Topic: A.05. Axon and Dendrite Development

Support: MEXT KAKENHI JP20H03346
MEXT KAKENHI JP16H06459
MEXT KAKENHI JP21K18245

Title: Investigating dendritic dynamics of barrel cortex layer 4 neurons in neonatal mouse via high spatiotemporal-resolution in vivo imaging

Authors: ***L. WANG**^{1,2}, **S. NAKAZAWA**¹, **H. MIZUNO**^{3,4}, **T. IWASATO**^{1,2};
¹Natl. Inst. of Genet., Mishima, Japan; ²Dept. of Genet., The Grad. Univ. for Advanced Studies (SOKENDAI), Mishima, Japan; ³Intl. Res. Ctr. for Med. Sci. (IRCMS), ⁴Grad. Sch. of Med. Sci., Kumamoto Univ., Kumamoto, Japan

Abstract: The specific arborization of dendrites determines the inputs the neuron receives, and the proper dendritic projection patterns of cortical neurons are formed and refined during postnatal development in an activity-dependent manner. It is critical to understand the dynamic mechanisms of how dendrites elaborate and refine their morphologies during development. Our laboratory has established two-photon time-lapse in vivo imaging approaches in the neonatal mouse brain to investigate the dynamics of dendritic refinement of cortical neurons (Mizuno et al., Neuron 2014; Nakazawa et al., Nature Commun. 2018). However, the spatiotemporal resolutions of these imaging studies were not sufficiently high, and many details regarding the precise refinement features of cortical neuron dendrites are still largely unexplored. In the present study, we first increased the temporal resolution of the imaging from an 8-hour interval to a 1-hour interval. We then improved the spatial resolution of dendrite morphology imaging by

using a membrane-bound RFP (mRFP) instead of a regular RFP. We used layer 4 neurons of the primary somatosensory cortex (barrel cortex) as the research model. Neurons labeled with the mRFP visualized more precise dendritic morphologies compared to the regular RFP. In-utero electroporation-based Supernova (Mizuno et al., 2014; Luo et al., Sci. Rep. 2016) was used to sparsely label barrel cortex layer 4 neurons with the mRFP. TCA-GFP mice (Mizuno et al., 2014) were used to enable in vivo visualization of the barrel map. Then, we imaged dendrites of the same layer 4 neurons for 8 hours on postnatal day 4, which is during the dendritic refinement. With improved in vivo imaging resolutions, we were able to detect precise changes in individual dendrites including those of short-lived dendritic trees and branches. Our imaging also identified several transient refinement features of L4 neuron dendrites. Our recent findings will be discussed.

Disclosures: L. Wang: None. S. Nakazawa: None. H. Mizuno: None. T. Iwasato: None.

Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 434.02

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS055272
NIH Grant NS090030

Title: The ins and outs of protocadherins: shared and isoform-specific roles for extracellular interactions and intracellular signaling

Authors: C. HANES¹, K. MAH², D. STEFFEN², L. FULLER², C. MARCUCCI², R. BURGESS³, A. GARRETT⁴, *J. WEINER²;

¹Grad. Program in Neurosci., ²Dept. of Biology, Iowa Neurosci. Inst., The Univ. of Iowa, Iowa City, IA; ³The Jackson Lab., Bar Harbor, ME; ⁴Dept. of Pharmacol., Wayne State Univ., Detroit, MI

Abstract: Cell adhesion molecule (CAM) superfamilies are comprised of numerous isoforms that when expressed on the cell surface are able to generate molecular diversity among neurons. Mutations in many CAMs, including members of the cadherin superfamily, are associated with neurodevelopmental disorders. The 3 clustered protocadherin families, particularly the 22 γ -Pcdhs encoded by the *Pcdhg* gene cluster, are critical for normal neurodevelopment and implicated in disease. Each γ -Pcdh is encoded by 4 exons: one long variable exon that encodes 6 extracellular cadherin repeats, a transmembrane domain, and a variable cytoplasmic domain; and 3 short constant exons that encode an intracellular C-terminal domain shared by all 22 isoforms. Using *Pcdhg* mutant mice, we and others have identified many functions of the γ -Pcdhs, including roles in synapse development, neuronal survival, axon patterning, and dendrite arborization. Major unknowns include: the extent to which there is functional redundancy among

isoforms; the role of extracellular *vs.* intracellular interactions; and the coordination of shared *vs.* unique signaling mechanisms. In the present study, we sought to elucidate the constant domain signaling mechanisms. Previous studies indicate that a FAK/PKC/MARCKS signaling pathway mediates dendrite arborization downstream of γ -Pcdhs: γ -Pcdhs bind to and inhibit FAK, which leads to reduced PKC activity and hypophosphorylation of MARCKS, which can then promote arborization. *In vitro* studies indicate that a serine at the C-terminus of the γ -Pcdh constant domain can be phosphorylated by PKC, and that this reduces these CAMs' ability to inhibit FAK. To test this *in vivo*, we created two new lines of mice in which the γ -Pcdh constant domain is mutated so that the 22 γ -Pcdhs cannot be phosphorylated by PKC due to: 1) a point mutation in the target serine; or 2) deletion of a 15-amino acid C-terminal lipid-binding motif that harbors this serine. We find that either mutation enhances γ -Pcdh's ability to promote dendritic arborization, causing MARCKS hypophosphorylation and overgrowth, without any effect on dendritic spines or levels of synaptic proteins. Along with concurrent work from our laboratory, this suggests a model in which arborization depends upon intracellular signaling—both shared (as described here) and isoform-specific (a unique role for the γ C3 isoform in promoting arborization *via* Axin1). In contrast, synaptic roles appear to depend upon common extracellular *cis* interactions with other CAMs including neuroligins.

Disclosures: C. Hanes: None. K. Mah: None. D. Steffen: None. L. Fuller: None. C. Marcucci: None. R. Burgess: None. A. Garrett: None. J. Weiner: None.

Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 434.03

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS084111
NIH Grant NS114914
NIH Grant NS119512

Title: Nir2 regulates early neuronal development via phosphatidylinositol 3,4,5-triphosphate/Akt signaling

Authors: *C.-T. CHIEN, A. GUO, T. LIU, M. SHELLY;
State Univ. of New York, Stony Brook, Stony Brook Univ., Stony Brook, NY

Abstract: Phosphatidylinositol 4, 5-bisphosphate (PI(4,5)P₂) and phosphatidylinositol 3, 4, 5-triphosphate (PI(3,4,5)P₃) are key phosphoinositides at the plasma membrane and are required for activation of various signaling pathways during early neuronal development. In particular, activation of Akt and its downstream effectors are critical during early neuronal development and more specifically, neuronal migration and dendrite development. In the developing brain, neurons are perpetually exposed to extracellular growth factors that trigger the use of PI(4,5)P₂

and PI(3,4,5)P₃, resulting in its rapid depletion. Currently, little is known about how neurons regulate PM PI(4,5)P₂ homeostasis to enable sustained growth factor mediated PI(3,4,5)P₃-dependent Akt signaling during early neuronal development. A group of ER-PM non-vesicular lipid transport proteins present at sites of direct contact between the two membranes have been identified to regulate PM PI(4,5)P₂ homeostasis. Studies using cell lines have revealed that the lipid transport protein, Nir2, is a regulator of PM PI(4,5)P₂ replenishment and Akt signaling upon growth factor stimulation by transporting phosphatidylinositol (PI) from the ER to the PM to generate PI(4,5)P₂ and phosphatidic acid (PA) from the PM to the ER to synthesize PI. In this study, we aim to determine the role of Nir2 in PM PI(3,4,5)P₃ replenishment and subsequent activation of Akt signaling during early neuronal development. We find that shRNA-mediated knockdown of Nir2 in cultured rat hippocampal neurons results in a delay in Akt phosphorylation/activation following bath application of brain derived neurotrophic factor (BDNF), a well-known extracellular cue in the developing cortex that regulates axon and dendrite development. We also use *in utero* electroporation to knockdown Nir2 in the developing rat cortex to observe the role of Nir2 in dendrite development *in vivo*. Cortical pyramidal neurons transfected with Nir2-shRNA show a significant migration delay and severe defects in both apical and basal dendrite development, including the length and branching. Together, these results indicate that localized lipid transport via Nir2 is critical for growth factor mediated-Akt signaling to ultimately regulate dendrite development during early neuronal development.

Disclosures: C. Chien: None. A. Guo: None. T. Liu: None. M. Shelly: None.

Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 434.04

Topic: A.05. Axon and Dendrite Development

Support: MIUR/PRIN Grant 2017T9JNLT

Title: Secreted Semaphorin 3A compromises growth cone elongation and dendritic arborization in human neural progenitors during differentiation

Authors: *G. FERRETTI¹, A. ROMANO², R. SIRABELLA¹, S. SERAFINI¹, T. MAIER³, C. MATRONE¹;

¹Dept. of Neurosci., Univ. of Naples "Federico II", Naples, Italy; ²CEINGE-Advanced Biotechnologies Res. Ctr. s.c.a.r.l., Naples, Italy; ³Paul-Ehrlich Institute, Federal Inst. for Vaccines and Biomedicines, Langen, Germany

Abstract: Semaphorins are repellent guidance cues considered instrumental in many processes that shape the nervous system during development. Particularly, Class 3 Semaphorins (Sema 3) are the only produced as secreted proteins in mammals, exerting both autocrine and paracrine functions (Alto et al. 2017). One of the best characterized subtypes of this class, is Semaphorin

3A (Sema 3A), secreted by surrounding cells to guide migrating neurons in the developing nervous system and vital for normal neuronal pattern organization (Pascual, 2005). Interestingly, increased Sema 3A expression levels have been detected in patients with autism or schizophrenia (Eastwood et al. 2003, Schafer et al. 2019) and polymorphisms in Sema 3A or in Sema 3A receptors, Neuropilin 1 (Npn 1) and Plexin A2 (Plxn A2), have been associated to neurodevelopmental disorders (van der Klaauw et al. 2019). We here investigated whether alterations in Sema 3A expression, during the very early stages of neuronal development affect neuronal growth and/or differentiation thus resulting in neurodevelopmental defects. As experimental model, using human neural progenitors (HNPs) we developed two different approaches consisting in either increasing endogenously Sema 3A levels by transfecting HNPs with Sema 3A cDNA or exposing HNPs to an exogenous source of Sema 3A derived from media of Sema 3A-cDNA transfected human primary microglia. Media from GFP transfected as well as not transfected microglia were used as controls. We found that Sema 3A overexpression affects HNPs morphology causing axonal growth cone retraction and an aberrant arborization of the apical dendrites. In addition, increased levels of Sema 3A activate a neuroinflammatory pathway also affecting neuronal survival. Of note, Sema 3A signaling requires the activation of Fyn Tyrosine Kinase-Cdk5 pathway and involves Npn 1 and Plxn A2 receptors. All together these data suggest that Sema 3A plays as a critical role in HNPs differentiation thus implying that any insults causing Sema 3A overexpression during the early stages of neurodevelopment might compromise HNPs survival, differentiation and connectivity and make neurons more vulnerable to other insults during their lifespan. Future studies will be enquiring about how an increase in Sema 3A signals might lead to neurodevelopmental disorders.

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Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 434.05

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant EY031690
NIH Grant NS055272
NIH Grant NS090030

Title: The role of Protocadherin γ C4 in neuronal survival and self-avoidance in the mouse retina

Authors: *C. M. MCLEOD¹, H. G. LANTHIER¹, J. A. WEINER², R. W. BURGESS³, A. M. GARRETT¹;

¹Pharmacol., Wayne State Univ., Detroit, MI; ²Biol., The Univ. of Iowa, Iowa City, IA; ³The Jackson Lab., Bar Harbor, ME

Abstract: The γ -Protocadherins (γ -Pcdhs) are a family of 22 cell adhesion molecules expressed throughout the central nervous system from the *Pcdhg* gene cluster. Each isoform has distinct protein sequence through the extracellular, transmembrane, and membrane proximal cytoplasmic domains, but share common sequence through the C-terminal cytoplasmic domain. This raises the possibility of isoform-specific roles as well as those common to all isoforms. The γ -Pcdhs play essential roles during neurodevelopment, including the promotion of neuronal survival. Absence of γ -Pcdhs results in excessive cell death of many neuronal subtypes and neonatal lethality in mice. This death is an exacerbation of developmental apoptosis, but the mechanism by which γ -Pcdhs promote survival is unclear. In subtypes that survive without γ -Pcdhs, some (including starburst amacrine cells) exhibit loss of self-avoidance, where neurites from the same neuron fasciculate with each other rather than sampling their receptive field. Evidence suggests that a single γ -Pcdh isoform can support self-avoidance, but if every isoform is capable remains unknown. Expression of isoform γ C4 alone is sufficient for postnatal viability and normal neuronal density in mice. This raises the question if γ C4 is specialized for one function or if it also contributes to other processes such as self-avoidance. Our purpose is to define the role of γ C4 in neuronal survival and self-avoidance in mouse retina. We hypothesize 1) γ C4 is necessary and sufficient for neuronal survival in the retina due to unique protein sequence within the cytoplasmic domain, 2) γ C4 alone does not promote self-avoidance in starburst amacrine cells due to unique protein sequence. Our methods include analyses of γ -Pcdh reduced isoform diversity mutants and transgenic mice *in vivo* with complementary primary culture studies *in vitro*. We measured the density of amacrine and retinal ganglion cell types in the retinas of these mice and found that loss of γ C4 is comparable to loss of all γ -Pcdhs. We are using new transgenic mice and a series of domain swapped and truncated γ C4 constructs to determine the domains of the protein essential for survival. A parallel approach will determine if γ C4 can promote self-avoidance in starburst amacrine cells.

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Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 434.06

Topic: A.05. Axon and Dendrite Development

Support: VA Merit Review Award I01BX001819
NIH/NIAAA R01AA022948
P60AA010760
R01AA029486
U01AA029965

Title: Third trimester-equivalent alcohol exposure accelerates dendrite development in pyramidal CA1 hippocampal neurons in post-natal day 7 mice

Authors: *N. MARGOLIES, J. KARPf, J. HASHIMOTO, M. GUIZZETTI;
Oregon Hlth. & Sci. Univ., portland, OR

Abstract: Fetal alcohol spectrum disorders are characterized by impaired cognitive and behavioral functioning, and imaging studies in humans show altered network connectivity. In the hippocampus, a region associated with learning and memory, changes in the number of synapses and the locations of established connections are correlated with behavioral anomalies. Here, we examine the effects of neonatal alcohol exposure in mice during the equivalent of the third trimester of gestation in humans on the morphology and spine density of hippocampal CA1 pyramidal neurons. C57BL/6J mouse litters were exposed to vaporized ethanol or air for 4 hrs/day from PD2 to PD7 in two experiments (experiment 1 EtOH n = 5, CON n= 4; experiment 2 EtOH n = 6, CON n = 6). Brains and trunk blood were collected after exposure on PD7 where pups reached an average blood ethanol concentration of 40.5 mM (+/- 1.60 mM SEM). Brains from at least 1 male and female per litter were Golgi-Cox stained and traced in NeuroLucida 360. All intact, distinct pyramidal CA1 neurons from Bregma 1.855 to -2.155 were measured. Spines were analyzed in a subset of animals on the secondary segments of apical dendrites. A multilevel linear model was used to account for nesting of multiple neurons per animal, and multiple animals within litter and experiment. In both males and females, ethanol exposure significantly increased complexity, length, number of nodes, number of ends, and terminal orders of apical and basal dendrites in pyramidal CA1 hippocampal neurons. Ethanol also increased the maximum terminal distance of basal dendrites, and the number of basal dendrites in males but not females. Ethanol did not alter spine density on secondary apical segments when spines were normalized to the length of the dendrite. However, we observed that ethanol exposure decreased the diameter of secondary apical segments, resulting in an increase in spine density of secondary branches when normalized to dendrite volume. In conclusion, neonatal ethanol exposure appears to accelerate the development of pyramidal CA1 neuron dendrites in male and female mice. These effects may be in part responsible for altered hippocampal connectivity and cognitive and behavioral dysfunction observed in FASD.

Disclosures: N. Margolies: None. J. Karpf: None. J. Hashimoto: None. M. Guizzetti: None.

Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 434.07

Topic: A.05. Axon and Dendrite Development

Title: A microRNA cluster downstream of the selector gene Fezf2 coordinates cortical neuron fate with subtype-specific dendritic branching and synaptic connectivity

Authors: *L. VAASJO¹, A. IYER², V. B. SITHTHANANDAN², V. LU², R. NAIR³, M. J. GALAZO^{4,5}, S. THARIN^{2,6};

¹Tulane Univ. Grad. Neurosci. Program, New Orleans, LA; ²Dept. of Neurosurg., ³Stanford Ctr.

for Genomics and Personalized Med., Stanford Univ., Stanford, CA; ⁴Neurosci. Program, Tulane Brain Inst., ⁵Dept. of Cell and Mol. Biol., Tulane Univ., New Orleans, LA; ⁶Div. of Neurosurg., Palo Alto Veterans Affairs Hlth. Care Syst., Palo Alto, CA

Abstract: In the cerebral cortex, projection neurons comprise distinct classes of neurons that project to distant regions of the central nervous system. While these classes of neurons develop from the same progenitor pool, they acquire strikingly different inputs and outputs to underpin strikingly different functions. The question of how corticospinal projection neurons -involved in motor function and implicated in paralysis - and callosal projection neurons - involved in cognitive function and implicated in autism - develop represents a fundamental and clinically important question in neurodevelopment. A network of transcription factors, including the selector gene *Fezf2*, is central to specifying cortical projection neuron fates. However, gene regulation up- and down-stream of these transcription factors is not well understood, particularly as it relates to the development of the major inputs to cortical projection neurons. Here we show that the corticospinal-enriched miR-193b~365 microRNA cluster is downstream of *Fezf2* and cooperatively represses the signaling gene *Mapk8* to differentially regulate dendritic morphology and synaptic connectivity in callosal and corticospinal projection neurons. *In vivo* overexpression of miR-193b and miR-365 in callosal projection neurons alters their dendritic branching pattern, dendritic spine density, and spine morphology during postnatal development. These findings indicate that the miR-193b~365 microRNA cluster downstream of *Fezf2* plays a significant role in establishing neuron subtype-specific synaptic connectivity and likely contributes to distinct synaptic plasticity properties across cortical projection neuron classes.

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Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 434.08

Topic: A.05. Axon and Dendrite Development

Support: NIH NIGMS R35GM137921
Presbyterian Health Foundation

Title: Fis1 is a major regulator of dendritic mitochondria morphology in cortical neurons

Authors: K. STRUCINSKA, *T. L. LEWIS, Jr;
Aging & Metabolism, Oklahoma Med. Res. Fndn., Oklahoma City, OK

Abstract: Mitochondria are dynamic organelles known primarily for their ability to produce ATP; however, they play critical roles in a number of important cellular processes including calcium buffering, immune response, lipid biogenesis and apoptosis. Interestingly, pyramidal neurons in the cortex appear to have at least two distinct subpopulations of mitochondria in their

axons and dendrites respectively. We hypothesized that these subpopulations are created and maintained by the regulation of fission and fusion dynamics via four fission receptors on the outer mitochondrial membrane - MFF, FIS1, MIEF1 and MIEF2. We previously reported that loss of MFF function via shRNA mediated knockdown (KD) in cortical mouse neurons resulted in axonal mitochondria elongation leading to reduced presynaptic neurotransmitter release and branching, all while having no effect on dendritic mitochondria. Here we focus on the fission factor FIS1 and its potential role in regulating these neuronal mitochondria subpopulations. Using two unique and validated shRNAs, we analyzed the effects of FIS1 loss on mitochondria morphology, motility and function in cortical neurons of CD-1 mice. We introduced shRNAs into neurons through in/ex utero electroporation (IUE/EUE) to acquire sparse neurons with cell autonomous FIS1 KD. We observed that FIS1 KD *in vitro* and *in vivo* surprisingly reduced dendritic mitochondria length, while having no impact on axonal mitochondria. Interestingly, live imaging of mitochondria labeled with photo-activable GFP revealed higher dendritic mitochondria motility in cells with FIS1 KD, suggesting that FIS1 may also be involved in mitochondria trafficking or anchoring. Finally, the reduction in dendritic mitochondria membrane potential following Antimycin A treatment ($\Delta F/F_0$ of tetramethylrhodamine) is 50% less in Fis1 KD mitochondria arguing for highly reduced complex III dependent OXPHOS. Future experiments will focus on identifying potential interactions with mitochondrial transport and anchoring proteins, as well as determining the impact of altered fission and motility on dendritic mitochondrial function and neuronal activity. Overall, our results demonstrate that mitochondrial fission is regulated by distinct molecular effectors in the axons and dendrites of cortical neurons.

Disclosures: K. Strucinska: None. T.L. Lewis: None.

Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

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Topic: A.05. Axon and Dendrite Development

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Title: Effect of ethanolic *Lippia alba* extracts on the structural plasticity of cortical neurons *in vitro*

Authors: M. VELASQUEZ¹, *J. J. SUTACHAN², G. M. COSTA^{2,1}, S. L. ALBARRACÍN²;
¹Univ. de los Andes, Bogotá, Colombia; ²Pontificia Univ. Javeriana, Bogotá, Colombia

Abstract: The growing prevalence of major depressive disorder, as well as the limited effectiveness of conventionally used antidepressants, has exposed the need to find new

therapeutic alternatives. This exploratory path has included the identification of natural compounds with limited adverse effects and that can enhance neuronal plasticity, an adaptation mechanism that is disrupted in depression. In the present study, the dendritogenic potential of the ethanolic extract of *Lippia alba*, an aromatic plant with a long history of use in traditional medicine, was evaluated. An in vitro model of rat cortical neurons was used and the kinetics of the effect of the plant was evaluated at different time intervals. It was identified that the extract increases the dendritic arborization, without being cytotoxic to the cells. Additionally, it was found that this effect could be mediated by the phosphatidylinositol-3 kinase (PI3K) pathway, which is involved in neuronal proliferation, differentiation, and survival mechanisms. The evidence provided in this study suggests that the plant extract does have antidepressant potential, as the activation of dendritogenesis would be one of the mechanisms through which antidepressants reduce symptoms of mood disorders

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Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 434.10

Topic: A.05. Axon and Dendrite Development

Support: NIH R01 NS086082
Brains & Behavior, Georgia State University
Honeycutt Fellowship, Georgia State University

Title: Cct and the torc1 pathway function to shape dendritic arbors

Authors: *E. N. LOTTES, S. BHATTACHARJEE, B. TETE, F. CIGER, D. N. COX;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Developing neurons rely on three major forms of proteostatic regulation - protein synthesis, maintenance, and degradation - to grow and maintain a dendritic arbor. In *Drosophila melanogaster*, larval multidendritic (md) neurons develop to form a variety of arbor shapes, ranging from simple class I (CI) to complex class IV (CIV) neurons, each dependent on carefully balanced proteostatic processes. One such process is protein maintenance, which is carried out by chaperones that ensure proper conformation of other proteins. While chaperones are particularly important to maintaining neurons, they have been under-studied in dendrites. Chaperonin-containing tailless complex polypeptide-1 (CCT) is an ATP-dependent chaperonin comprised of eight subunits which come together to form a double-ringed complex. CCT is thought to fold anywhere from 1-15% of the cellular proteome. Two of its most notable clients are actin and tubulin - major cytoskeletal components essential to the development and maintenance of dendritic arbors. Using live confocal imaging of larval md neurons, we have

found that knockdown of CCT results in significant decreases in arbor complexity in CIV neurons, appearing at approximately 72 hours after egg lay. Two-channel live imaging of CCT loss-of-function (LOF) CIV neurons has revealed an underlying significant reduction in MTs, but not in F-actin. Though stability of MTs is compromised, polarity of MTs is unchanged in CCT LOF conditions. CCT has recently been shown to fold components of the TOR (Target of Rapamycin) complex 1 (TORC1). TORC1 regulates S6 kinase, and we have found that S6K LOF and overexpression results in CIV dendritic arbor complexity reduction and enhancement, respectively. Preliminary studies of Cullin1 (Cul1), a component of the SCF E3 ubiquitin ligase, reveal that Cul1 LOF results in dendritic hypertrophy and increase in phosphorylated S6K signal, opposite to the hypotrophy and decrease in phosphorylated S6K seen in CCT LOF. Cul1 has been previously linked to negative regulation of TORC1 through inhibition of Akt. Altogether, our work suggests CCT operates as a part of a regulatory network spanning protein synthesis, maintenance, and degradation that collectively cooperate to regulate dendritic growth and elaboration.

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Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

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Program #/Poster #: 434.11

Topic: A.05. Axon and Dendrite Development

Support: NIH R01 HD094809
University of Minnesota LEADR Fellowship

Title: Preventative Methylene Blue Treatment Improves Adenosine 5' Triphosphate Production in Iron Deficient Developing Hippocampal Neurons

Authors: *D. C. MENDEZ, M. K. GEORGIEFF, T. W. BASTIAN;
Pediatrics, Univ. of Minnesota, Minneapolis, MN

Abstract: Iron deficiency (ID) is the most common micronutrient deficiency in the world. When acquired in early life (fetal through toddler stages), ID causes deficits in neurobehavioral outcomes (e.g., hippocampal-mediated learning and memory), which persist in adulthood despite iron repletion. Human neonatal brain development is metabolically demanding, consuming 60% of the body's oxygen. Iron is necessary for mitochondrial enzymes involved in cellular energy production, supporting neurodevelopmental processes such as dendrite growth/branching. Early-life ID impairs mitochondrial adenosine 5' triphosphate (ATP) production and causes permanent reductions in hippocampal neuron dendritic branching complexity, an important determinant of neural circuit formation. Alternative therapies targeting the underlying iron neurobiology, beyond iron repletion, are needed to prevent the long-term neurobehavioral deficits caused by

early-life ID. Methylene blue (MB) is a mitochondrial targeted therapy that can cross the blood-brain-barrier and acts as an alternative electron shuttle in the mitochondrial matrix, increasing ATP production. We hypothesized that MB treatment of iron-deficient neurons would 1) mechanistically show that mitochondrial impairments are a major mechanism driving the neuronal structural impairments caused by early-life ID and 2) show efficacy as a potential alternative therapy to improve these impairments by reversing the decrease in ATP production seen in ID. ID was induced in mouse embryonic day 16 derived hippocampal neuron cultures at 3 days in vitro (DIV) and concomitantly treated with 10nM or 25nM MB. Neuronal ATP concentrations were measured at 7DIV (n=12 per group). ID reduced neuronal ATP concentration by nearly 50%. In support of our hypothesis, MB treatment of iron-deficient neurons increased ATP levels in a dose-dependent manner compared to ID only neurons. Furthermore, 25nM MB restored ATP production to iron-sufficient levels, demonstrating a preventative/protective effect of MB. Ongoing studies will elucidate whether MB treatment is sufficient to improve the mitochondrial motility that is slowed in iron-deficient neurons at 7DIV and neuronal dendrite structural complexity long-term (21DIV). The stimulation of ATP production in ID neurons could hold promise as a novel strategy using MB and other candidate mitochondrial therapies to rescue the neurobehavioral deficits observed in ID. This work also has potential relevance to neuropsychiatric disorders such as schizophrenia where mitochondrial dysfunction plays a large role in its pathology.

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Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 434.12

Topic: A.05. Axon and Dendrite Development

Title: Effects of chronic hypoxia on neurite outgrowth and synaptic development in human cortical organoid

Authors: *W. WU, H. YAO, W. JUAN, G. HADDAD;
UCSD, UCSD, La Jolla, CA

Abstract: Hypoxia in utero is one of the most critical challenges in human fetal life and has been implicated as a major cause for fetal brain injury. The impact of prenatal hypoxia on human brain development and the underlying molecular mechanism are not fully understood. Brain organoids resemble early human brain development and thus, provide a suitable model for studying the impact of hypoxia on fetal brain development. In this study, we investigated in human cortical organoids whether chronic hypoxia affects synaptogenesis. First, we performed immunofluorescence staining to examine whether hypoxia affects neurite outgrowth. We found that prolonged hypoxia insult (5% O₂, 2 weeks) caused prominent morphological alterations in neurite outgrowth with swellings and constrictions in MAP2+ dendrites, suggesting neuronal

injury. Further, we identified synapse density by coimmunostaining for presynaptic marker (SYN1) and postsynaptic marker (PSD95) in the neuronal processes. Quantification of the number of SYN1+/PSD95+ puncta pairs showed a 83% reduction in the number of synaptic contacts in hypoxia-treated organoids (control 0.40 ± 0.09 pairs per $10 \mu\text{m}$ vs. hypoxia 0.07 ± 0.02 pairs per $10 \mu\text{m}$, $n=8-10$ organoids, $p < 0.01$). In support of the morphological changes in synapse, we further used whole-cell patch-clamp techniques to investigate the synaptic transmission in cortical organoids by recording the spontaneous postsynaptic excitatory currents (sEPSCs). Our electrophysiological studies showed that hypoxia induced an 85% reduction in the frequency of sEPSCs (control 1.06 ± 0.28 Hz vs. hypoxia 0.16 ± 0.05 Hz, $n=9-12$, $p < 0.01$) whereas the amplitude of sEPSCs was not significantly changed (control -9.79 ± 0.086 pA vs. hypoxia -8.79 ± 0.63 pA, $n=9-12$, $p > 0.05$). Overall, our studies suggest that hypoxia can significantly decrease synaptogenesis by altering the morphology, density and function of synapse in cortical organoids. Future experiments will explore the signaling pathway(s) that lead to hypoxia-induced impairment of synaptogenesis during fetal brain development.

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Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 434.13

Topic: A.05. Axon and Dendrite Development

Support: NIH KL2
NIH T32GM008629

Title: Initiation vs Elongation: Determining the contribution of Ca_v1 channels to activity-dependent dendritic growth in cerebellar granule neuron

Authors: *A. L. PARKER, B. MEADE, C. HAYES, O. KLEIN, H. WEN, A. J. WILLIAMS; Psychiatry, Univ. of Iowa, Iowa City, IA

Abstract: Dendritic growth is a key component of normal neurodevelopment and dendritic abnormalities have been identified in many neurodevelopmental conditions. Two major processes that comprise dendritic growth are dendritic initiation and dendritic elongation, which are measured in cell culture systems as the number and length of dendrites, respectively. Ca_v1 channels have previously been shown to be involved with global changes in activity-dependent dendritic growth in many types of neurons. However, whether these channels are involved in dendritic initiation or elongation specifically has yet to be shown. In this study, I quantified dendritic initiation and elongation in cerebellar granule neurons and show that Ca_v1 channels contribute to activity-dependent initiation of primary dendrites. I used sparsely GFP-labelled cultured mouse cerebellar granule neurons (CGNs) to measure the number and length of the neurites dendrites for each individual cell. I measured both primary dendrite neurites (directly from

the soma) and secondary neurites dendrites (branching from existing neurites) to determine how each of these types of neuronal structures are impacted. I used KCl to simulate neuronal activity, and I used both an antagonist (Isradipine) and agonist (BAY-K-8644 or BAYK) for Cav1 channels to determine the necessity and sufficiency of Cav1 channels for activity-induced dendritic growth. Each experiment was conducted in triplicate with three samples per group, where each sample was the average of up to eight images (typically representing 8-12 individual neurons per sample). CGN isolations were pooled by litter, so male and female cerebella were mixed in each sample. The concentrations of the antagonist isradipine (20nM) and agonist BAYK (2µM) were chosen based on previous literature in CGNs, not the maximum published. Off target effects have been reported for isradipine but the concentration used is far lower than those reported to have off-target effects (1-2µM), to reduce the likelihood of off target effects. High KCl-induced activity (50mM) is significantly associated with increased dendritic number but and no changes in dendritic length for both primary and secondary dendrites. Therefore, our data shows that KCl-induced activity impacts dendritic initiation, not dendritic elongation in CGNs. Use of a Cav1 antagonist shows that Cav1 channels are specifically involved in the initiation of primary but not secondary dendrites. Interestingly, the Cav1 agonist alone was not sufficient to induce dendritic growth independent of stimulation. Future experiments will test whether BAYK can potentiate the effects of lower levels of stimulation.

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Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 434.14

Topic: A.05. Axon and Dendrite Development

Support: NSF Grant 1855474
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Scott Edward Erickson Endowed Research Award

Title: Rna helicase mov10 regulates dendritic arborization and memory extinction.

Authors: ***T. SHILIKBAY**, A. NAWAZ, S. CEMAN;
Cell and Developmental Biol., Univ. of Illinois, Urbana-Champaign, Urbana, IL

Abstract: MOV10 is an RNA helicase that is involved in post-transcriptional regulation of gene expression. MOV10 participates in the development of the brain, which is demonstrated by its high expression level in early postnatal mice compared to adult brains and the observed abnormalities in *Mov10* morpholino treated *Xenopus laevis* embryos (Skariah et al. 2017; 2018). However, the exact role of MOV10 in neuronal development is unknown. To address this

question, we created a novel mouse model called *Mov10* Deletion by crossing *Emx1-Cre;Mov10^{tm1d(EUCOMM)Wtsi}* and *Mov10^{Gt(IST13267G7sE6)Tigm}* mice. This mouse has a 90% reduction in MOV10 protein level in the hippocampus. *Mov10* Deletion PROX1⁺ granule cells (n=42) have decreased proximal arborization at 5-20 μ m from the soma and increased distal arborization at 105-130 μ m from the soma compared to WT (n=34). *Mov10* Deletion PROX1⁻ pyramidal neurons (n=25) have increased distal arborization at 55-70 μ m, 80-115 μ m, and 125-135 μ m from the soma compared to WT (n=18). These results were obtained by performing Sholl analysis on DIV14 primary hippocampal cultures stained with MAP2 and PROX1 antibodies. We also found that *Mov10* Deletion mice have increased thickness in cortical layer II-IV (268.45 \pm 6.17 μ m, n=5) compared to WT (230.99 \pm 6.50 μ m, n=7) by examining coronal sections of 12-week-old mice stained with hematoxylin and eosin. We conducted behavior tests on *Mov10* Deletion and WT mice, ages 8-13 weeks old. We identified that *Mov10* Deletion mice have enhanced cued memory (40.93 \pm 3.63 % freezing, n=15 vs 29.34 \pm 3.01 % freezing, n=16) and contextual fear memory (31.68 \pm 2.58 % freezing, n=14 vs 20.44 \pm 2.47 % freezing, n=16). Finally, we found that MOV10 depletion affects the transcriptome of hippocampi by performing RNA-sequencing on RNAs isolated from P0 hippocampi of *Mov10* Deletion (n=3) and WT (n=3) pups. Out of 1,088,379,642 total reads across six samples 815,947,858 were mapped to the reference genome spanning 16,995 genes. We found that 1,985 genes and 2,069 genes were significantly downregulated and upregulated in the *Mov10* Deletion hippocampi, respectively. The gene ontology analysis on differentially expressed genes showed that microtubule binding and microtubule motor activity are in the top ten most enriched categories, suggesting that MOV10 regulates the expression of proteins participating in cytoskeleton production. MOV10 is a cofactor of miRNA pathway protein AGO2. Since reduction of miRNA production in brain also leads to dendritic phenotype and enhanced memory (Konopka et al. 2010), we propose that we have identified the cytoskeletal mRNAs that participate in the development of dendritic arbor and memory extinction.

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Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

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Topic: A.05. Axon and Dendrite Development

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Title: Ras-gaps control dendritic development in barrel cortex layer 4 neurons

Authors: *M. S. RAO^{1,2}, H. MIZUNO^{1,2}, T. IWASATO^{3,4}, H. MIZUNO^{1,2};

¹Intl. Res. Ctr. for Med. Sci., Lab. of Multi-dimensional Imaging, Kumamoto Univ., Kumamoto, Japan; ²Grad. Sch. of Med. Sci., Kumamoto Univ., Kumamoto, Japan; ³Lab. of Mammalian Neural Circuits, Natl. Inst. of Genet., Mishima, Japan; ⁴Dept. of Genet., SOKENDAI, Mishima, Japan

Abstract: The cerebral cortex has a complex yet exquisite network of neuronal circuits which is important for advanced brain functional and cognitive purposes. To explore the molecular mechanisms of neuronal circuit formation, the tactile somatosensory pathway that connects the whiskers and cortex of rodents is useful. The rodent somatosensory barrel cortex layer 4 (L4) comprises a unique ‘barrel map’ that corresponds to facial whisker patterns. Thalamocortical (TC) axon terminals are clustered in the barrels, with ring-shaped distributions of neurons in the barrel cortex L4. Dendrites of L4 neurons are oriented toward the barrel center and receive inputs from the TC axon terminals. The neuronal circuit in the barrel cortex L4 is formed during early postnatal development. These traits make the barrel cortex a useful system to study neuronal circuit development. Our focus is Ras-GTPase activating protein (Ras-GAP) which possibly plays a role in the MAPK and PI-3 kinase pathways. We have created knockdown constructs for Ras-GAPs (NF1 and SynGAP) following which we transfected the constructs to the barrel cortex L4 using *in-utero* electroporation. We have analyzed the effects of Ras-GAPs knockdown in the L4 circuit formation by the histological method. We found that proper dendritic orientation towards the barrel center and TC synapse formation were distributed in Ras-GAPs knockdown L4 spiny stellate neurons. These results demonstrate the essential roles of Ras-GAPs in circuit formation in the cerebral cortex and imply that developmental changes in dendrites and synapses in RasGAP KD neurons may be related to cognitive disabilities in RasGAP-deficient individuals, such as patients with neurofibromatosis type 1.

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Poster

434. Dendrite Dynamics: Development, Arborization, and Branching

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Program #/Poster #: 434.16

Topic: A.05. Axon and Dendrite Development

Support: NIH R01 NS086082
Brains & Behavior, Georgia State University
2CI Neurogenomics, Georgia State University
NIH T34 GM131939

Title: Differential roles of ribosomal proteins in regulating cell-type specific dendritic development

Authors: F. CIGER, S. BHATTACHARJEE, E. N. LOTTES, E. A. WILDE, V. VEERANKI, *D. N. COX;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Maintenance of dendritic shape and complexity in neurons is a challenge for the proteostatic network that requires dynamic temporal and spatial regulation. Neurological disorders that exhibit deformities in dendritic architecture show the importance of this challenge. The proteostatic network involves cellular processes that control synthesis, folding, and turnover of proteins to maintain integrity of cells. Ribosomal proteins (RP) play an essential role in these processes and translational regulation of these proteins is required for the proper functioning of the cell. Historically, ribosomes were thought to be homogeneous amongst cells. However, recent studies have shown differences in ribosome composition and introduced a concept of ‘specialized ribosomes’ which differentially regulate active translation of mRNAs. Our transcriptomic analyses demonstrate that RPs are expressed differentially in multi-dendritic neurons of the peripheral nervous system of *Drosophila melanogaster*, where the more complex CIV neurons show higher levels of expression of most RPs compared to simpler CI neurons. To investigate the potential functional significance of ribosomal subunits in dendrite development regulation, we conducted cell-type specific phenotypic screens of a subset of 30 RPs in CI and CIV neurons. Dimensionality reduction analysis showed that in CIVs, disruption of these RPs form 5 clusters depending on the severity of their effects. In CIs in contrast, RP knockdown (KD) formed 3 distinct clusters. KD of select RPs such as *RPL31*, *RPL36* and *RPS15* severely affected morphology in CIVs but did not affect morphology in CIs highlighting the possibility of differential requirements of these RPs in different neuronal subclasses. In addition, KD of *RPL7-like*, *RPL22-like*, and *RPLP0-like* did not affect dendritic morphology in either CI or CIV neurons suggesting that these neurons may show a preferential requirement of certain RP paralogs. We also explored the effects of RP KD on ribosome localization and trafficking using GFP tagged *RpL10Ab*. KD of *RpL7*, *RPL22*, and *RpL36A* lead to severely restricted trafficking into the dendrites in both CI and CIV neurons. KD of *RPL7-like* and *RPL22-like* which did not affect dendritic morphology in either neuronal subclass, did not have any effect. Our data suggests that distinct neuron types have different RP requirements, and certain RPs have more general roles in regulating dendritic morphology. It also suggests that the ribosomal machinery promoting dendritic architecture is cell-type specific. Overall, our study provides novel insights into putative functional roles of specialized ribosomes in dendritogenesis.

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Poster

435. Autism: Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 435.01

Topic: A.07. Developmental Disorders

Support: Startup Grant

Title: *Auts2* is a critical regulator of human neural progenitor cell proliferation and differentiation

Authors: *A. BIEL, R. RUTHERFORD, M. HESTER;
Inst. for Genomic Med., Res. Inst. at Nationwide Children's Hosp., Columbus, OH

Abstract: The Autism Susceptibility Candidate 2 (AUTS2) gene is a master epigenetic regulator of multiple neurodevelopmental genes. AUTS2 variants are associated with multiple neuropsychological conditions, such as autism, intellectual disability, microcephaly, schizophrenia, and epilepsy. Functions of AUTS2 have been extensively studied in zebrafish and mouse models, demonstrating its critical role in controlling neuronal differentiation and neurite morphogenesis. For example, *Auts2* loss leads to reduced neuronal differentiation and increased cell death in differentiating mouse embryonic stem cells. Despite these advancements, mechanisms underlying disease pathology remain elusive, in part due to the lack of model systems which more closely resemble human physiology. Here we investigate the role of AUTS2 in proliferating and differentiating human neural progenitor cells (NPCs). Human NPCs were infected with either a scramble control or AUTS2 shRNA GFP-lentivirus and were then separately differentiated into neurons, astrocytes, and oligodendrocytes under defined conditions. Human NPCs were also maintained under undifferentiated conditions to investigate the role of AUTS2 in maintaining proliferation and stem cell characteristics. An automated high-content confocal imaging system was used to acquire time-lapse images to assess the effect of AUTS2 knockdown in human NPCs under these conditions. Immunofluorescence analysis was used to quantify cell type-specific markers and markers of neurite morphogenesis. We found that AUTS2 loss resulted in increased cell death and decreased differentiation capabilities in both glial and neuronal cell types, suggesting a broader, critical role of AUTS2 in regulating differentiation of human NPCs. Furthermore, cell morphology and neurite morphogenesis were adversely affected in differentiated cell types. Next, we used CRISPR-Cas9 to knock-out (KO) AUTS2 in induced pluripotent stem cells and generated cerebral organoids (COs). COs are three-dimensional *in vitro* models that more accurately recapitulate complex and human-specific features of early brain development compared to traditional two-dimensional cell cultures. AUTS2 KO COs displayed reduced growth compared to wild-type COs and displayed compromised NPC morphology. These data support an essential role for AUTS2 during early human cortical development as well as a specific function of AUTS2 in regulating proliferation of human NPCs, which may underlie AUTS2-associated microcephaly.

Disclosures: A. Biel: None. R. Rutherford: None. M. Hester: None.

Poster

435. Autism: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 435.02

Topic: A.07. Developmental Disorders

Support: Startup fund Uconn Health Center
Eagles Autism Foundation
CT Institute for the Brain and Cognitive Sciences

Title: Role of excessive embryonic neurogenesis in autism spectrum disorder

Authors: *S. SINGH¹, H. KIM², B.-I. BAE³;

¹Neurosci., Univ. of Connecticut Hlth. Ctr., Farmington, CT; ²Neurosci., Uconn Hlth. Ctr., Farmington, CT; ³Neurosci., Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: Role of Excessive Embryonic Neurogenesis in Autism Spectrum

Disorder **Authors** *Sonu Singh, Hyopil Kim, Byoung-il Bae; University of Connecticut (Uconn) Health Center, CT **Abstract** Autism spectrum disorder (ASD) is a highly heritable, heterogeneous neurodevelopmental disorder affecting 1 in 53 children in the US with a male to female prevalence ratio of 3:1. The cerebral cortex, especially the prefrontal cortex that mediates social cognition and language, is oddly enlarged in up to 20% of patients with ASD, who suffer from particularly severe symptoms. However, it is unclear whether macrocephaly (large brain) itself contributes to ASD. Here, we uniquely address the causal relationship between macrocephaly and ASD by investigating a human ASD-associated variant of the *ASPM* (*abnormal spindle-like microcephaly-associated*) gene. *ASPM* is a major determinant of brain size, especially in the prefrontal cortex, and encodes a centrosomal protein that is highly expressed in proliferating cortical progenitors, but not in postmitotic neurons. Human genetic studies have revealed dozens of *de novo* variants in *ASPM* that are associated with ASD, and we found that one such variant, p.Ala1877Thr (p.A1877T), significantly increases protein levels of exogenous *ASPM* in HEK293T cells, suggesting gain-of-function mutation. Using genome editing, we have generated *Aspm* knock-in (KI) mice harboring the orthologous mutation, p.A1845T, and analyzed cortical development in male and female mice. *Aspm* KI mice show excessive embryonic neurogenesis with (a) increased progenitor proliferation and cortical thickness, (b) decreased cell cycle exit, and (c) enlarged ventricle, resulting in macrocephaly between embryonic day 14.5 and postnatal day 10. Interestingly, male KI mice exhibit reduced density of inhibitory synapses as well as impairment in social behavior at postnatal day 50, whereas female KI mice failed to display these phenotypes, suggesting sex-specific ASD-like effects of the *Aspm* point mutation. Collectively, our data indicate that *ASPM*-dependent excessive embryonic neurogenesis is sufficient to elicit at least some ASD-like behaviors in male mice by disturbing cell composition and synaptic connection. Further studies are required to understand molecular and cellular mechanisms by which excessive embryonic neurogenesis disturbs synaptic connections and social behaviors of postnatal mice in a male-specific manner.

Disclosures: S. Singh: None. H. Kim: None. B. Bae: None.

Poster

435. Autism: Cellular Mechanisms

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Topic: A.07. Developmental Disorders

Support: P50 HD093079

Title: Cd99 expression dynamics is associated with brain overgrowth in autism spectrum disorder

Authors: *M. MAMUN, S. CHETTY;

Ctr. for Regenerative Med., Massachusetts Gen. Hosp., Boston, MA

Abstract: Autism spectrum disorder (ASD) is a complex condition characterized by important changes to the brain resulting in impaired behavior. Approximately 15-20% of individuals with ASD have disproportionate megalencephaly (ASD-DM) or enlarged brain relative to body size. An increase in brain size often precedes the first clinical signs of the disorder, yet the underlying mechanisms leading to brain overgrowth remain unknown. Here, we develop cellular models using patient-specific induced pluripotent stem cells (iPSCs) to understand the mechanisms contributing to brain overgrowth in genetic (e.g., 16p11.2 deletion syndrome) and idiopathic forms of autism. RNA-sequencing and transcriptome analyses of neural progenitor cells (NPCs) derived from patient-specific iPSCs show an important role for cancer related pathways (e.g., CD99) in contributing to brain overgrowth in ASD. While basal expression of CD99 is required for the differentiation of NPCs into well-defined functional neurons, overexpression of CD99 can lead to aberrant differentiation of NPCs into immature neurons. Here, we explore the role of CD99 in regulating proliferation and terminal differentiation of NPCs into neurons in genetic and idiopathic forms of ASD with and without brain overgrowth. Furthermore, we show how genes impacted in genetic forms of autism (e.g., 16p11.2 deletion syndrome) directly interact and modulate the expression of CD99 to ultimately regulate cellular proliferation and differentiation. Thus, our study provides a mechanistic link for CD99 overexpression in brain overgrowth in autism and highlights genes and pathways as potential therapeutic targets in future work.

Disclosures: M. Mamun: None. S. Chetty: None.

Poster

435. Autism: Cellular Mechanisms

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Program #/Poster #: 435.04

Topic: A.07. Developmental Disorders

Support: NIMH R01 MH097949-01

Title: Disruption of mTORC2 and AKT1/3 rescues neuronal overgrowth resulting from PTEN loss

Authors: *A. R. GOYETTE¹, K. TARIQ¹, M. PRINA¹, B. SHAFIT-ZAGARDO², B. W. LUIKART¹;

¹Mol. and Systems Biol., Geisel Sch. of Med. at Dartmouth, Hanover, NH; ²Pathology, Albert Einstein Col. of Med., Bronx, NY

Abstract: Prevalence of autism spectrum disorder (ASD) has significantly increased in recent decades; however, molecular mechanisms underlying symptoms remain poorly understood. Dysfunction of phosphatase and tensin homolog (PTEN) – a negative regulator of downstream AKT/mTOR signaling – is among the most common single gene mutations in ASD, causing dramatic neuronal overgrowth. mTORC1 loss completely rescues neuronal hypertrophy due to *Pten* knockout (KO), but mTORC2 loss only partially rescues *Pten* KO overgrowth, suggesting the *Pten* KO phenotype is mTORC1-dependent. In our experiments, we intend to elucidate whether mTORC2 loss can rescue *Pten* KO morphological changes independently of mTORC1 by targeting AKT1/3. We co-injected a mCherry control retrovirus and a GFP-T2A-Cre retrovirus into the neonatal hippocampus (P7) in Rictor and single AKT KO mice (*Pten*^{flx/flx}; *Akt1*^{flx/flx}; *Rictor*^{flx/flx} and *Pten*^{flx/flx}; *Akt3*^{flx/flx}; *Rictor*^{flx/flx}) to compare age-identical control and KO granule neurons in the dentate gyrus of the same animal. Rictor loss caused a decline in AKT phosphorylation (reduced pS472/pS473 intensity), and it did not rescue the morphological effects of phospho-mimetic AKT, suggesting mTORC2 may modulate the *Pten* KO phenotype at least partially through AKT. We are currently performing this experiment in single AKT KO mice (*Pten*^{flx/flx}; *Akt1*^{flx/flx} and *Pten*^{flx/flx}; *Akt3*^{flx/flx}) and double AKT KO mice (*Pten*^{flx/flx}; *Akt1*^{flx/flx}; *Akt3*^{flx/flx}), and we will quantify effects on soma size, spine density, total dendritic length, and migration to characterize the neuronal morphology of each KO.

Disclosures: A.R. Goyette: None. K. Tariq: None. M. Prina: None. B. Shafit-Zagardo: None. B.W. Luikart: None.

Poster

435. Autism: Cellular Mechanisms

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Topic: A.07. Developmental Disorders

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NIMH R01 MH110558
NIMH R01 MH080134
NIMH R01 MH104446
SfN Neuroscience Scholar's Program

Title: Characterizing human iPSC-derived neural progenitor cells in idiopathic autism

Authors: *C. M. AAMODT¹, A. M. SHARMA⁵, E. ARMINGOL¹, S. LINKER⁵, K. PIERCE², E. COURCHESNE³, N. E. LEWIS¹, C. MARCHETTO⁴;
¹Pediatrics, ²Neurosciences, ⁴Anthrop., ³UC San Diego, La Jolla, CA; ⁵Salk Inst., La Jolla, CA

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects social behavior. The vast majority (80-90%) of ASD patients suffer from the idiopathic form of this disorder. Tractable models that directly correlate to patient phenotypes are essential for applying precision medicine to ASD. To address this, our research group has longitudinally phenotyped a cohort of developmentally delayed toddlers who go on to develop ASD, as well as typically developing controls. From a subset of these subjects with brain overgrowth, we generated induced pluripotent stem cell-derived neural progenitor cells (NPCs) and performed single cell RNA sequencing to better understand the mechanisms associated ASD in early prenatal development. Preliminary results showed a shift in neural progenitor subtype distribution between ASD and typically developing subjects. ASD NPCs showed a reduction of S100B+ and NEUROD1+ NPCs, with an increase in VCAM1+, LGALS1+ and RBFOX3+ NPCs. Using cell-cell communication analysis we identified a downregulated cluster enriched in glial markers that aberrantly secretes WNT2B in the ASD subject NPCs. Additionally, we found that genes differentially expressed in ASD NPCs were enriched in microRNA-128 targets and genes regulated by histone seronylation. Ongoing research will further characterize these molecular mechanisms as potential targets for ASD prevention, diagnostics, and therapeutics.

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Poster

435. Autism: Cellular Mechanisms

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Title: Characterization of patient-derived fibroblasts with Fragile X Syndrome, as a model to study Autism Spectrum Disorders.

Authors: O. LORA-MARIN^{1,2}, L. GÓMEZ-VIRGILIO¹, M. SILVA-LUCERO¹, C. TORRES-ROSAS¹, *M. CARDENAS-AGUAYO¹;
¹Physiol., ²Neurosci., UNAM, Sch. of Med., Ciudad de México, Mexico

Abstract: Introduction. Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability and autism. The patients exhibit major behavior disorders such as hyperactivity, impulsiveness, anxiety, poor language, and severe mental disability. It is caused by the absence of the fragile X mental retardation 1 (FMR1) gene product, fragile X mental retardation protein (FMRP), an RNA-binding protein involved in the regulation of translation of a subset of brain mRNA, causing deregulations, especially in postsynaptic neurons. Fibroblasts from FXS patients have significantly elevated rates of basal protein synthesis along with elevated levels of the phosphorylated mechanistic target of rapamycin (p-mTOR), phosphorylated extracellular signal-regulated kinase 1/2, and phosphorylated p70 ribosomal S6 kinase 1 (S6K1). Fibroblasts from FXS patients exhibit an opportunity to study FXS in a low invasive way and may be a useful in vitro model for unraveling the underlying mechanisms FXS and testing the efficacy of potential new therapeutical targets. Proteomics allows studying the protein universe of FXS cells and gives information on the differential expression characteristic of this syndrome. The objective of this project was to analyze the expression profile of patients with FXS and compare it with apparently healthy individuals (AHI). **Methods.** Skin Fibroblasts of FXS patients and AHI were obtained from the Coriell Institute (New Jersey) repository and cultured in Earl MEM salts medium with 15% non-inactivated FBS. The cells were characterized by Western blot and immunofluorescence detection of Vimentin and FMRP. The cells were cultured to reach a monolayer on nine T300 flasks to obtain enough protein for the proteomic analysis. The samples were processed at USAI facility (UNAM Chemistry Faculty) where 2-DE and mass spectrometer shotgun analyses were made. Proteomics results were confirmed using Western blot techniques. **Results:** Proteomics show deregulation in pathways involved in protein recycling such as Ubiquitin C protein, Ubiquitin peptidase 5, and genes related to gene expression. **Conclusion:** FXS Fibroblasts proteomic analysis shows alterations in several pathways related to the pathology, meaning that this tool is useful in the study and proposal of new FXS therapeutic approaches and biomarkers.

Disclosures: O. Lora-Marin: None. L. Gómez-Virgilio: None. M. Silva-Lucero: None. C. Torres-Rosas: None. M. Cardenas-Aguayo: None.

Poster

435. Autism: Cellular Mechanisms

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Topic: A.07. Developmental Disorders

Support: NIH grant MH079407

Title: Aberrant mRNA trafficking and AMPA receptor down-regulation in UBE3A-dependent autism spectrum disorder

Authors: *Y. TIAN, H. QIAO, H. MAN;
Boston Univ., Boston, MA

Abstract: Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental disorders characterized by deficits in social interactions, language development, and the presence of restrictive and repetitive behaviors. The ubiquitin E3 ligase UBE3A is one of the most common genetic factors in ASD etiology, and mice of UBE3A overexpression (UBE3A OX) demonstrate typical autistic behaviors. Consistent with a crucial role of synaptic dysfunction in ASD pathology, research has shown that in UBE3A OX mice the strength of excitatory synaptic activity is impaired, however, mechanisms underlying the synaptopathy remain unknown. We find that AMPA receptor (AMPA) subunit GluA1 is downregulated in the UBE3A OX mice, which is accompanied by a reduction in AMPAR-mediated neuronal activity. Further examinations find that the reduction of GluA1 is not caused by receptor ubiquitination. Surprisingly, measurements on RNA distribution reveal an elevated accumulation of GluA1 mRNA in the nucleus of UBE3A OX neurons, presumably leading to the suppression of GluA1 protein synthesis. Indeed, replenishing the identified mRNA nuclear transport factor rescued the defects in AMPAR mRNA subcellular distribution and receptor protein expression in UBE3A OX neurons. These findings reveal a novel mechanism in AMPAR regulation and provide new insights into the pathobiology in Ube3A ASD.

Disclosures: **Y. Tian:** None. **H. Qiao:** None. **H. Man:** None.

Poster

435. Autism: Cellular Mechanisms

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Program #/Poster #: 435.08

Topic: A.07. Developmental Disorders

Support: NICHD/P50 HD093079

Title: Evaluating the role of CD47 during brain development in autism spectrum disorder using human pluripotent stem cells

Authors: *S. CHEN, S. CHETTY;
Massachusetts Gen. Hosp., Boston, MA

Abstract: Copy number variation (CNV) at the 16p11.2 region has been associated with neurodevelopmental disorders, such as autism spectrum disorder (ASD), intellectual disability (ID), and schizophrenia (SCZ). CNV genes at the 16p chromosome can manifest in opposing head sizes. ASD patients with 16p11.2 deletion tend to acquire macrocephaly with both white and gray matter enlargement, whereas those with 16p11.2 duplication often have microcephaly as well as increased risk for SCZ. Our previous work demonstrated that CD47 (a “don’t eat me” signal) and calreticulin (a pro-phagocytic signal) are overexpressed in neural progenitor cells (NPCs) and oligodendrocyte progenitor cells (OPCs) with 16p11.2 deletion with macrocephaly, leading to suppressed phagocytosis or cellular elimination of unhealthy brain cells. Here, we investigate the role of CD47 further in relation to CNV genes during brain development. Using

CRISPR screening technology, we explore the roles of 29 CNV genes at the 16p11.2 locus in relation to CD47 using normal human induced pluripotent stem cells (hiPSCs). *KCTD13*, *MAPK3*, and *ALDOA* play central roles in neuronal function and network formation by regulating proliferation and apoptosis of NPCs as well as supply of energy. We demonstrate how loss of function of these genes in hiPSCs regulates the CD47 pathway in cortical NPCs. Our findings indicate cellular mechanisms that are relevant for endophenotypes related to ASD. By understanding the underlying mechanisms regulating ASD, these genes may be promising new therapeutic targets for intervention and mitigation of symptoms in ASD.

Disclosures: **S. Chen:** A. Employment/Salary (full or part-time);; Harvard Medical School. **S. Chetty:** A. Employment/Salary (full or part-time);; Harvard Medical School.

Poster

435. Autism: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 435.09

Topic: A.07. Developmental Disorders

Support: NICHD F30 HD103360

Title: Functional characterization of ASD associated *EEF1A2* mutations in human neurons.

Authors: ***M. MOHAMED**, E. KLANN;
New York Univ., New York, NY

Abstract: Protein synthesis is a fundamental process in all living cells and is highly regulated to accommodate the specific needs of each cell. Neurons specifically, require local translation at synapses for crucial functions such as learning, memory, and plasticity. Dysregulated protein synthesis in neurons has been demonstrated to underlie many of syndromic forms of autism such as Fragile X syndrome (FXS), which results from defects in a gene that regulates protein synthesis. Recent studies have shown that Eukaryotic Elongation Factor 1A2 (*EEF1A2*), a protein responsible for GTP-dependent transport of aminoacyl-tRNAs to the elongating ribosome, is mutated in patients with autism, intellectual disability and epilepsy. Elongation Factor 1A has two isoforms, one that is ubiquitously expressed, *EEF1A1*, and another, *EEF1A2* that is expressed only in neurons and myocytes. It is unclear why another isoform is needed in these specific cells; however, it has been found that *EEF1A2* is critical for neuronal survival. A mouse model with a homozygous deletion of mouse *Eef1a2*, has been found to exhibit neuron degeneration, tremors, and loss of muscle bulk after weaning. Here, we use CRISPR-Cas9 to recapitulate patient mutations in human induced pluripotent stem cells followed by differentiation into excitatory cortical neurons. We characterize the effects of the two most common patient mutations in *EEF1A2* on global protein synthesis and neuronal morphology. These mutations are found in or near coding regions for different functional domains of *EEF1A2*. Notably, G70S is in the GTPase domain and E122K is in the tRNA-binding domain.

We find that these mutations alter the rate and net de novo protein synthesis suggesting that cells may not synthesize protein at a sufficient rate to keep with cellular demand. Furthermore, we show that the ASD-associated mutations in EEF1A2 alter neuronal morphology and axonal growth cones indicating that mutated EEF1A2 may exert a dominant negative effect. Further studies will explore RNA-sequencing and Ribosomal profiling in these neurons to determine the misregulated and mistranslated genes that result from these mutations. These studies will help elucidate the role that translation elongation factors may play in neuronal function and activity.

Disclosures: **M. Mohamed:** None. **E. Klann:** None.

Poster

435. Autism: Cellular Mechanisms

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Topic: A.07. Developmental Disorders

Support: DST Grant- DST/INSPIRE Fellowship/2018/IF180201
DBT Grant- BT/IN/Denmark/07/RSM/2015-2016

Title: Multidimensional interaction of Fragile X Mental Retardation Protein (FMRP) with the ribosome controls protein synthesis in neurons

Authors: ***M. N. DSOUZA**^{1,2}, N. GOWDA¹, D. PALAKODETI², R. MUDDASHETTY¹;
¹Ctr. for Brain Research, IISc, Bangalore, India; ²Inst. for Stem Cell Sci. and Regenerative Med., Bangalore, India

Abstract: The Fragile X Mental Retardation Protein (FMRP) is a selective RNA Binding Protein that localizes to both the cytoplasm and the nucleus. The loss of FMRP results in Fragile X Syndrome (FXS). FMRP interacts with ribosomes and regulates the translation of mRNAs essential for synaptic development and plasticity. But the biochemical nature of this interaction is unknown. Here we report that a key feature of FMRP's interaction with ribosomes is through the 2'O methylation of ribosomal RNA. 2'O methylation is the major epi-transcriptome mark on rRNA essential for ribosome assembly and function. We found that FMRP both influences the generation of ribosomes bearing a distinct 2'O-Methylation pattern on its rRNA component and also recognizes that pattern. 2'O Methylation on rRNA is facilitated by a class of snoRNA (C/D box snoRNA) in the nucleus that eventually directs the assembly of the ribosome. Small RNA sequencing revealed that FMRP interacts with a selected set of C/D box snoRNA in the nucleus resulting in the generation of a distinct pattern of 2'O-Methylation in H9 ESCs. Through immunoprecipitation, we also show that FMRP itself recognizes ribosomes with this 2'O methylation signature. This epi-transcriptome pattern on rRNA undergoes a significant change during the differentiation of ESCs to cortical neurons. Importantly, this gradual change in 2'O methylation pattern during differentiation is altered in the absence of FMRP, which may impact neuronal development and contribute to dysregulated protein synthesis observed in FXS. Our

initial observations through in-vitro experiments describe the structural involvement of FMRP's C-terminus in ribosome binding. Through polysome profiling, we report that the intrinsically disordered C-terminus domain of FMRP is sufficient to bind to ribosomes similar to the full-length protein. Furthermore, the C-terminus domain alone is essential and responsible for FMRP-mediated translation repression in rat primary cortical neurons measured through FUNCAT. Additionally, we also describe the importance of phosphorylation of FMRP at Serine-500 in modulating the dynamics of translation regulation by controlling ribosome/polysome association. This is a fundamental mechanism governing the size and number of FMRP-containing puncta as well. We propose that the specific interaction of FMRP with the ribosome and its recognition of 2'O methylation pattern on rRNA play an important role in regulating neuronal protein synthesis.

Disclosures: M.N. DSouza: None. N. Gowda: None. D. Palakodeti: None. R. Muddashetty: None.

Poster

435. Autism: Cellular Mechanisms

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NRF-2020M3E5D9079908
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NRF-2021R1A5A802987612

Title: Organoid models of an autism spectrum disorder patient with a heterozygous DSCAM mutation

Authors: H. KIM¹, Y. XIONG¹, Y.-K. LEE², B.-K. KAANG³, J.-A. LEE², *C.-S. LIM¹;
¹Wonkwang Univ. Sch. of Med., Iksan, Korea, Republic of; ²Hannam Univ., Daejeon, Korea, Republic of; ³Sch. of Biol. Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Mutations in Down syndrome cell adhesion molecule (DSCAM) have been highlighted as a highly penetrant genetic risk factor of autism spectrum disorder (ASD). Our previous study demonstrated that a heterozygous *DSCAM* mutation was associated with down-regulation of NMDA receptor (-R) function in a forebrain-like induced neuronal (iN) cells derived from patient-specific induced pluripotent stem cells (iPSCs). However, the limitations of two-dimensional (2D) iN cells compelled us to further study cellular pathogenesis in a three-dimensional (3D) forebrain organoid model. Here, we generated iPSC-derived human cortical organoids (hCOs), which also showed reduced DSCAM levels. In addition, the reduced DSCAM expression in ASD hCOs was accompanied by reduced mRNA levels of NMDA-R components, which were consistent with the previous results from iN cells. Interestingly, ASD hCOs showed

a significantly smaller organoid size compared to control hCOs while they showed normal developmental trajectories. Now, we are investigating how reduced expression of DSCAM causes size reduction and alters expression levels of NMDA-R components in ASD hCOs. Collectively, these data may reinforce the causal relationship between *DSCAM* mutation and reduced NMDA-R function in ASD pathogenesis.

Disclosures: H. Kim: None. Y. Xiong: None. Y. Lee: None. B. Kaang: None. J. Lee: None. C. Lim: None.

Poster

435. Autism: Cellular Mechanisms

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Topic: A.07. Developmental Disorders

Support: NSF1456818
NIH NS104705
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Title: Glutamate delta 1 receptor regulates autophagy mechanisms in multiple brain regions

Authors: *K. S. NARASIMHAN¹, D. Y. GAWANDE², R. PAVULURI¹, J. M. BHATT⁴, V. KESHERWANI³, S. DRAVID¹;

¹Pharmacol. and Neurosci., ²Dept. of Pharmacol. and Neurosci., ³Creighton Univ., Omaha, NE;

⁴Chem., Univ. of Texas At El Paso, Omaha, NE

Abstract: The GluD1 and GluD2 delta glutamate receptors are one of four types of ionotropic glutamate receptors that play a role in synaptic transmission and learning. Emerging studies indicate that autophagy has essential functions at the synapse both in formation and pruning during development. Impaired autophagy can have a profound impact during neurodevelopment, especially in autism spectrum disorders (ASDs). Earlier, we demonstrated that deletion of GluD1 leads to several molecular and behavioral phenotypes of ASDs including defective spine-pruning, social deficits, and repetitive behavior. Taken together, loss of GluD1 may result in autophagy impairments and may act as a foundation for the progression of ASDs. We tested this hypothesis in constitutive and conditional knockout (KO) models of GluD1. Interestingly, we observed hyperactive Akt-mTOR signaling ($p > 0.05$) in the dorsal striatum (dSTR) and somatosensory cortex (SSC) upon GluD1 deletion. This increase in mTOR signaling cascade further led to the inhibition of ULK-1 and beclin-1 leading to disruption in autophagosome maturation. We also observed an elevated p62 ($p = 0.0126$) and decline in the LC3-II/LC3-I ratio ($p = 0.0063$) in the dSTR and SSC regions. Moreover, we performed an age-dependent (P14, P30, and P60) analysis of p62 and LC3-II/LC3-I ratio in the constitutive GluD1 KO and observed that the autophagy is impaired at P30 and P60 whereas no change was seen in P14.

Thus, there is an age-dependent effect of GluD1 deletion on autophagy relevant to ASDs. Next, we sought to determine whether these autophagic deficits lead to impairment in excitatory transmission. Excitatory elements were increased in number but had immature phenotype based on vGluT1/vGluT2 puncta size, lower AMPA subunit (GluA1) expression, and impaired development switch from predominantly GluN2B to mixed GluN2A/GluN2B subunit expression. Together, these results demonstrate a novel function of GluD1 in the regulation of the autophagy pathway which may underlie ASD phenotypes and are relevant to the genetic association of GluD1 coding, GRID1 gene with autism, and other developmental disorders. Further studies are examining the potential relevance to GluD1 regulation of autophagy mechanisms in distinct brain regions and disease conditions.

Disclosures: **K. S. narasimhan:** None. **D.Y. Gawande:** None. **R. Pavuluri:** None. **J.M. Bhatt:** None. **V. Keshewani:** None. **S. Dravid:** None.

Poster

435. Autism: Cellular Mechanisms

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Title: WITHDRAWN

Poster

435. Autism: Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 435.14

Topic: A.07. Developmental Disorders

Support: AIMS-2-TRIALS 777394
DFG 251293561

Title: Developmental alterations of neuron and glial cell number in a Shank3 deficiency model of Autism Spectrum Disorder

Authors: ***A. PEREZ AREVALO**¹, A.-K. LUTZ¹, M. MALARA¹, T. BOECKERS^{1,2};
¹Inst. for Anat. and Cell Biol., Ulm Univ., Ulm, Germany; ²DZNE, Ulm, Germany

Abstract: Autism Spectrum Disorder (ASD) is a complex and heterogenous neurodevelopmental disorder with a multifactorial background. It is known that both environmental and genetic factors have an impact on the development of ASD. Moreover, the crosstalk between neurons and other glial cells, like microglia, astrocytes and oligodendrocytes during development is

known to be involved. One of the genes that is closely associated with the occurrence of ASD is *Shank3*. SHANK3 is a scaffolding protein located primarily at the postsynaptic density of excitatory synapses, where it crosslinks the actin cytoskeleton with trans-membrane receptors. In a screening approach, we analysed expression patterns as well as the number of neurons and different glial cells during early steps of development in a *Shank3* KO mouse model. To that end we collected brains from E12.5 embryos, performed a multiplex analysis and found that specific cytokines were increased in the *Shank3* KO embryos. Moreover, immunohistochemistry of P0, P7 and P21 mice for Iba1, GFAP and NeuN revealed an increased number of microglia and astrocytes specifically at P0 in *Shank3* KO mice, but no changes in the number of neurons or the total cell density. In addition, immunohistochemistry of P7, P21 and P140 mice for MBP show an upregulation of MBP in the Corpus Callosum at P7 followed by a downregulation at P21 and P140 in *Shank3* KO mice. The close analysis of cell number as well as of specific interactions and communications between glial cells and neurons at early developmental stages can help to elucidate mechanisms that are involved in the development of ASD. These mechanisms might eventually lead to novel entry points for future treatment options.

Disclosures: A. Perez Arevalo: None. A. Lutz: None. M. Malara: None. T. Boeckers: None.

Poster

435. Autism: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 435.15

Topic: A.07. Developmental Disorders

Title: Development and characterization of an in vitro model of SSADH deficiency using patient iPSC-derived neurons to support unbiased screening of novel therapeutic approaches to treatment.

Authors: *W. AFSHAR SABER¹, N. TEANEY², M. SUNDBERG³, H. JUMO², K. D. WINDEN⁴, D. EBRAHIMI-FAKHARI⁵, P. L. PEARL⁵, M. SAHIN⁴;

¹Boston Children's Hosp. Harvard Med. Sch., ²Boston Children's Hosp. - Harvard Med. Sch., Boston, MA; ³Children's Hosp. Boston, Children's Hosp. Boston, Boston, MA; ⁴Boston Children's Hosp., ⁵Boston Children's Hosp., Boston, MA

Abstract: Succinic semialdehyde dehydrogenase (SSADH) deficiency is an autosomal-recessive neurometabolic disorder caused by bi-allelic mutations in the ALDH5A1 gene. It is the most prevalent inherited disorder of GABA metabolism and is characterized by accumulation of two neuromodulators, gamma-aminobutyric acid (GABA) and gamma-hydroxybutyric acid (GHB), in the CNS. Previous studies using rodent models have shown that disruption in GABA signaling can lead to dysregulation of mitochondria numbers, turnover, and function. Over the last 30 years, an expanded understanding of pathophysiology based on the corresponding animal model (*Aldh5a1*^{-/-} mice) has emerged, but effective pharmacotherapy remains elusive. Alternative models and therapies that address the accumulation of GABA and GHB, and their downstream

effects, are needed. In collaboration with the Human Neuron Core at the Boston Children's Hospital, five clinically-phenotyped patients and unaffected sex-matched parents have been consented and recruited from our SSADH deficiency registry. Fibroblasts have been collected for reprogramming. Three iPSC patient lines and sex matched parental controls have been generated at the Harvard Stem Cell Institute. We have established the first *in vitro* model of SSADH Deficiency based on iPSC-derived neurons. We successfully generated GABAergic and excitatory neurons based on transcription factor programming and characterized these models in respect to SSADH deficiency phenotypes such as GABA levels and mitochondria function. Additionally, we performed functional assays to investigate neuronal excitability based on optogenetics and calcium imaging in co-cultures of GABAergic and excitatory neurons to evaluate epileptiform activity in SSADH deficient iPSC-derived neurons and create cell-based models suitable for drug screening.

Disclosures: W. Afshar Saber: None. N. Teaney: None. M. Sundberg: None. H. Jumo: None. K.D. Winden: None. D. Ebrahimi-Fakhari: None. P.L. Pearl: None. M. Sahin: None.

Poster

436. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 436.01

Topic: A.07. Developmental Disorders

Support: NIH Grant RO1 NS057819
Rett syndrome research trust grant

Title: Identifying genetic regulators of MeCP2 protein levels using a pooled CRISPR screen approach

Authors: *A. ANDERSON¹, R. SCHUMAN², J.-P. REVELLI², H. Y. ZOGHBI³;
¹Baylor Col. of Med., ³Howard Hughes Med. Inst., ²Baylor Col. of Med., Houston, TX

Abstract: Methyl-CpG Binding Protein 2 (MeCP2) is a critical regulator of neuronal function. Loss- or gain-of-function mutations in *MECP2* cause severe neurological disorders, Rett Syndrome (RTT) or MECP2 duplication syndrome (MDS), respectively. Several studies in mice show that normalizing MeCP2 levels in the CNS, either by increasing MeCP2 levels in RTT models or decreasing MeCP2 levels in MDS models, can rescue key symptoms of both disorders. In the context of RTT, even boosting mutant MeCP2 levels in mice with hypomorphic mutations can extend survivability and improve neurological symptoms. Therefore, investigating ways to modulate MeCP2 dosage is an important therapeutic strategy. In this study, we aimed to identify druggable genetic targets that can modify MeCP2 protein levels using a high-throughput pooled CRISPR screening approach. To this end, we developed a transgenic, bi-cistronic HEK293T cell line that expresses RFP, MeCP2-eGFP, and CAS9. This clonal cell line enables identifying cells with low or high MeCP2 levels via a fluorescently activated cell sorting (FACS) strategy. We

can then use the CRISPRCloud2 platform to find gRNAs enriched in either low MeCP2 or high MeCP2 groups relative to bulk samples. We performed pooled CRISPR screens using both a whole genome library and a kinome sublibrary. We found numerous hits that either upregulated or downregulated MeCP2 levels. We are currently validating top screen hits in both patient-derived iNeurons and *in vivo* mouse model systems. Moreover, we identified novel genes that regulate MeCP2 expression, such as the top hit BRD4 (bromodomain containing 4 protein). We confirmed this regulation by showing that pharmacologically inhibiting BRD4 with the drug JQ1 significantly lowered endogenous MeCP2 levels. These results provide promising avenues of future research into both the basic biology of MeCP2 regulation and potential therapeutic treatments for MeCP2-related disorders.

Disclosures: **A. Anderson:** None. **R. Schuman:** None. **J. Revelli:** None. **H.Y. Zoghbi:** None.

Poster

436. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 436.02

Topic: A.07. Developmental Disorders

Title: Novel unsupervised machine learning approaches for understanding sleep EEG brain state dynamics in Rett syndrome

Authors: ***C. HUANG**^{1,4}, **A. MAHAT**^{2,4}, **D. GLAZE**^{2,5}, **M. MALETIC-SAVATIC**^{3,4}, **A. BUCKLEY**⁶, **M. J. MCGINLEY**^{1,4,7};

¹Neurosci., ³Pediatrics, ²Baylor Col. of Med., Houston, TX; ⁴Jan and Dan Duncan Neurolog. Res. Inst., Houston, TX; ⁵Texas Children's Hosp., Houston, TX; ⁶NIMH, NIH Clin. Ctr., Bethesda, MD; ⁷Rice Univ., Houston, TX

Abstract: Conventional description of the dynamic sleep process in neurotypical subjects relies on manual scoring of the sleep polysomnography (PSG) into 5 stages (Wake, rapid eye movement (REM), and Non-REM 1-3). However, sleep brain state dynamics may be so significantly altered in some developmental brain disorders (DBD), such as Rett syndrome, that conventional PSG may provide an inaccurate and incomplete model. Redefining this altered ultradian rhythm offers insight into underlying neuropathology in DBD. Here we developed a fully unsupervised Hidden Markov Model (HMM) approach to describe sleep brain activity as continuous transitions between quasi-stable states. We compared differences between the inferred states from healthy subjects and the states from Rett syndrome patients. To validate the model, for a given holdout segment of unlabeled EEG signals, the models trained from healthy and Rett groups were used to predict which group the EEG signals were from. 51 healthy children (age: 8.3±4.7 years) and 38 Rett syndrome girls (age: 11.6±5.4 years) participated in the study. As the model input, we extracted power spectrum from seven frequency bands (in Hz): 0.5-1.5, 1.5-4, 4-6, 6-8, 8-12, 12-14 and 14-16 of electroencephalogram (EEG) from Fp1, F7, C4, O1, T4 leads. Additional features were signal root mean squared (RMS) and per-channel sample

entropy and the EMG RMS. We then employed multivariate Gaussian HMM to infer the hidden states, including initial states probability, the mean activity and covariance matrix and states transition probabilities. For the control group, 15 inferred states described the sleep EEG statistics well. Some states were highly specific to a PSG stage, while some states appeared to reflect within-stage microstructure that was not captured by conventional PSG staging. For the Rett group, the states that were highly specific to the REM stage varied between individuals. And the states highly related with sleep spindles found in control group were not found in the Rett group. The leave-one-out validation showed high accuracy rate when predicting whether a subject is from the healthy group (sensitivity: 96%, precision: 91%) or the Rett syndrome group (sensitivity: 87%, precision: 94%). Altered sleep-state generation and rhythms, as uncovered here in the proposed HMM framework, can help define brain state pathology in Rett syndrome. This approach holds promise for diagnosis as well as evaluation of treatment efficacy, particularly when treatments aim to alter early developmental neuromodulatory brain state aberrancies in DBDs.

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Poster

436. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 436.03

Topic: A.07. Developmental Disorders

Title: Molecular consequences driving neurological deficits caused by changes in MeCP2 levels

Authors: *A. K. ENGSTROM¹, L. L. LAVERY¹, M. DIAS¹, H. YALAMANCHILI¹, Z. LIU¹, H. Y. ZOGHBI²;

¹Baylor Collage of Med., Baylor Collage of Med., Houston, TX; ²Howard Hughes Med. Inst., Baylor Col. of Med., Houston, TX

Abstract: Methyl CpG binding protein (MeCP2), a reader of methylated cytosines, is critical for neuronal function. Levels of MeCP2 are tightly regulated during mammalian brain development and in the mature brain. Loss-of-function mutations and duplications of *MECP2* result in the severe neurodevelopmental disorders, Rett Syndrome (RTT) and *MECP2* Duplication Syndrome (MDS), respectively. Further, the severity of neurological symptoms observed in patients correlates with the degree of change (both increased or decreased) in MeCP2 levels; i.e., mutations that result in complete MeCP2 loss cause much more severe and wide-ranging symptoms than those mutations that retain some amount of MeCP2 protein. This suggests that the mechanism of MeCP2 function is acutely influenced by dosage. However, the molecular consequences of varying MeCP2 levels, which lead to the observed gradient of phenotypes, are poorly understood. Here we utilize an allelic series of mouse models, including one mouse line that lacks MeCP2 completely and four mouse lines that express a gradient of MeCP2 ranging

from 50%-500% of wildtype levels. To identify how changing levels of MeCP2 *in vivo* impacts MeCP2 DNA-binding and resultant gene expression changes, we performed both CUT&RUN and RNAseq on the frontal cortex of mice across the allelic series. Through this analysis we will determine if the rise in MeCP2 levels drives an increase in MeCP2 binding at the same loci, or if it drives ectopic binding to new loci. We will also determine if the gene expression changes occur at the same sets of genes or if new genes are altered as MeCP2 dosage increases. Through systematically interrogating the molecular impact of changing MeCP2 levels, we will elucidate the mechanism of MeCP2 dosage alterations. This will advance our understanding of MeCP2 biology and MECP2-related disease regardless of which model proves to be driving the gradient of neurological phenotypes.

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Poster

436. Rett Syndrome

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Topic: A.07. Developmental Disorders

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Title: Emergence of reverberating bursts in human stem cell derived neuronal networks of Rett Syndrome

Authors: *K. PRADEEPAN^{1,3}, R. S. MOK^{4,7}, F. MCCREADY^{4,7}, W. ZHANG⁸, M. W. SALTER^{5,9}, J. ELLIS^{6,7}, L. MULLER^{2,10}, J. MARTINEZ-TRUJILLO^{1,3,10};

¹Grad. Program in Neuroscience, Schulich Sch. of Med. & Dent., ²Dept. of Applied Mathematics, Western Univ., London, ON, Canada; ³Robarts Res. Inst., London, ON, Canada; ⁴Dept. of Mol. Genet., ⁵Dept. of Physiol., ⁶Mol. Genet., Univ. of Toronto, Toronto, ON, Canada; ⁷Developmental & Stem Cell Biol., ⁸Neurosciences & Mental Hlth., ⁹Neurosci. & Mental Hlth., The Hosp. For Sick Children, Toronto, ON, Canada; ¹⁰Brain and Mind Inst., London, ON, Canada

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder that produces a distinct period of normal infancy followed by neurological regression. RTT is caused by a single heterozygous loss-of-function mutation in the X-linked gene methyl-CpG-binding protein 2 (*MECP2*). Using

human stem cell (hSC)-derived models of RTT, researchers are investigating the reenactment of *MECP2*-mutant altered brain development. In combination with multielectrode arrays (MEA) and electrophysiological analyses, hSC-derived brain development is revealing cellular and network mechanisms involved in RTT. Previous MEA research has found patterns of *in vitro* spontaneous and synchronous activity resembling fundamental features of *in vivo* developing neuronal networks even in the absence of stimuli. Dynamics of neural bursting during *in vivo* network development has been previously reported, such as burst reverberations. Burst reverberations emerge as an initial spike of activity across the network, followed by repeated bursts occurring over hundreds of milliseconds after an initial network burst. Here we analyzed MEA recordings and characterized burst reverberations occurring in hSC-derived networks. hSC-derived networks exhibited bursting patterns that went from sparse firing to asynchronous bursting to synchronous bursting between weeks 2-7 of development. RTT networks exhibited significantly slower network burst frequencies compared to isogenic controls (previously reported). Interestingly, RTT networks began to exhibit burst reverberations in greater proportion compared to wildtype networks – occurring between weeks 4-6. Observations of burst reverberations also marked a transition of the network from a slow to a faster network state in RTT networks. Despite observations of burst reverberations *in vitro* and *in vivo*, mechanisms underlying this oscillatory behaviour remain unclear. We further investigated potential mechanisms by developing a tightly constrained computational modeling framework from single-neurons and networks. Using data from *in vitro* intracellular recordings, we developed a single-neuron model to capture the subthreshold membrane potential and spiking dynamics following standard current injection protocols. We implemented our single neuron models into a spiking network model of an excitatory population using connectivity parameters that resemble biologically observed synapse numbers. Through this, we are currently investigating the interplay of fast excitatory currents and adaptation at multiple timescales that may be underlying the changes in population burst reverberations in hSC RTT networks. Simulations being validated.

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Poster

436. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 436.05

Topic: A.07. Developmental Disorders

Support: NIMH R15MH124042
Postdoctoral fellowship award from Rettsyndrome.org
UTK ORE Graduate Fellowship
UTK BCMB Departmental Fellowships
UTK Chancellor Fellowship

Title: Pose estimation analysis aids in identifying atypical movement kinematics during pup retrieval in a female mouse model for Rett syndrome

Authors: *M. MYKINS, B. BRIDGES, A. JO, J. ELROD, B. Y. LAU, K. KRISHNAN;
Biochem. & Cell. and Mol. Biol., Univ. of Tennessee Knoxville, Knoxville, TN

Abstract: Maternal behavior is a paradigm used to study complex social behavior and sensorimotor integration during pup retrieval in mice. Previous studies report on endpoint measurements of retrieval as a proxy for studying underlying neural circuitry. Little is known about the dynamic sequences and trajectories of retrieval over time, which is an essential first step towards determining dynamic neural circuitries involved in this complex sensorimotor behavior. Previously, we observed an adult female *Mecp2*^{heterozygous} mutant mouse model for Rett syndrome (Het) performs inefficiently at pup retrieval. Systematic frame by frame analysis revealed wild type (WT) adapt a stereotyped search-approach-retrieval pattern, but not Het. Using DeepLabCut, a marker-less pose estimation software, we generated pose and quantified trajectory kinematics across six days of pup retrieval behavior in adult female WT and Het mice. Analysis of movement kinematics identified significant dynamic changes in metrics such as sinuosity and distance travelled by individual mice on different trials during retrieval. Interestingly, Principal component analysis of trajectory profiles revealed two distinct behavioral responders: Het that improve in efficiency over trials similar to WT, and Het that regress and perform worse over trials. This is a novel result of significance to Rett syndrome research, as behavioral regression, a key feature of Rett syndrome and other neurodevelopmental disorders has been difficult to model in preclinical animal studies. These metrics are crucial for probing the distinct and mosaic neural circuitry required for efficient pup retrieval in Het. Pose generated from this study will aid in predicting behavioral sequences with supervised and unsupervised machine learning approaches to identify goal-related movements during pup retrieval.

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Poster

436. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 436.06

Topic: A.07. Developmental Disorders

Support: R01-MH118563

Title: Social ranking is disrupted in Rett syndrome mice

Authors: *C. ACEVEDO-TRIANA, L. POZZO-MILLER;
Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Alterations in social behaviors are prevalent in neurodevelopmental disorders (NDDs). The mPFC is involved in cognitive function, behavioral flexibility, and social behaviors, and is dysfunctional in NDDs due to its protracted postnatal development. Monosynaptic inputs from the ventral hippocampus to the mPFC regulate social memory, and are altered in *Mecp2* KO mice, a model for the NDD Rett syndrome. How dysfunction in this vHIP-mPFC pathway affects the formation of social ranks, which requires intact social memory, is unknown. We first confirmed that *Mecp2* KO mice don't have deficits in odor discrimination, showing the expected preference for social stimuli that is necessary for proper social behaviors, and is typically observed in WT mice. The hierarchy of social ranking of either WT or *Mecp2* KO mice was determined using the standard "tube" test in groups of 3 mice of the same genotype and age (P40) during 6 daily pairwise competitions using a round-robin format, where winners were those that either pushed the opponent or waited for its retreat and exit the tube through the opponent's side. Social ranking was determined by the percentage of these wins, losses, and ties, which allow the classification of mice into dominant (DOM), intermediate (INT), and subordinate (SUB) in each cage. WT mice formed a stable and strong social hierarchy across 6 consecutive days, while *Mecp2* KO mice did not show hierarchical distance between DOM and INT mice. Further behavioral analyses during the "tube" test revealed that dominant behaviors (e.g. pushing, resistance, or chasing) were more prevalent and showed a clear hierarchical pattern in WT mice (DOM>INT>SUB), while *Mecp2* KO mice showed fewer of these dominant behaviors. SUB *Mecp2* KO mice spent more time retreating, while *Mecp2* KO DOM and INT mice spent comparable times retreating. Next, we used the "warm spot" test, where mice are placed in an open arena with a cold floor ($5\pm 2^\circ\text{C}$) and a single warm spot ($37\pm 2^\circ\text{C}$) on a slightly raised platform that accommodates a single mouse. When 3 mice of each genotype are allowed to freely compete for this "warm spot", WT mice in the highest social rank (DOM) spent more time on it than WT mice in lower social ranks (INT, and SUB). On the other hand, the 3 *Mecp2* KO mice spent comparable times on the "warm spot", irrespective of their social ranking, confirming that behaviors required to solve conflicts between different social ranks is altered in Rett mice. We plan to characterize neuronal activity in the mPFC during the "warm spot" using GCaMP8f imaging with miniscopes to gain a deeper understanding of their contribution to altered sociability in Rett mice.

Disclosures: C. Acevedo-Triana: None. L. Pozzo-Miller: None.

Poster

436. Rett Syndrome

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 436.07

Topic: A.07. Developmental Disorders

Support: R01-MH118563

Title: The small-molecule TrkB ligand LM22-A4 improves dendritic spine phenotypes in male and female Rett mice

Authors: *D. MEDEIROS¹, K. AYALA-BAYLON¹, H. EGIDO-BETANCOURT², E. MILLER¹, C. CHAPLEAU¹, H. ROBINSON³, T. YANG⁴, F. LONGO⁴, W. LI¹, L. POZZO-MILLER¹;

¹Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL; ²Wake Forest Univ., Winston-Salem, NC; ³John Hopkins Univ., Baltimore, MD; ⁴Stanford Univ., Palo Alto, CA

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder caused by loss-of-function mutations in the X-linked *MECP2* gene, affecting specifically females with a prevalence of 1:10,000 births. RTT females develop typically until 6-8 months of age, when a regression of acquired sensory-motor and cognitive skills starts, together with the appearance of neuropsychiatric symptoms. MeCP2 is a transcriptional regulator of multiple genes, including brain-derived neurotrophic factor (*Bdnf*), whose levels are lower in postmortem RTT brains and *Mecp2*-deficient mice. Consistently, overexpressing *Bdnf* improves several cellular, network, and behavioral phenotypes in *Mecp2*-deficient neurons and mice. As BDNF has very low blood-brain barrier permeability, a brain-penetrant small molecule ligand of the BDNF receptor TrkB has become a potential therapeutic to improve impaired BDNF signaling in RTT. LM22A-4 improves breathing irregularities and restores spatial learning in female *Mecp2* heterozygous (HET) mice. Here, we confirmed that LM22A-4 increases the phosphorylated TrkB/total TrkB ratio in cultured and *ex vivo* hippocampal slices from male *Mecp2* knockout (KO) mice as much as recombinant BDNF does. In addition, LM22A-4 (11 days *in vitro*) increased dendritic spine density in CA1 pyramidal neurons in slice cultures from P7 *Mecp2* KO mice as much as recombinant BDNF. To evaluate LM22A-4's effects *in vivo*, we used 4-6 month old *Mecp2* HET mice that express GFP-tagged MeCP2 in cells that had silenced the mutant allele by X-chromosome inactivation in their 'mosaic' brain. We dye-loaded CA1 pyramidal neurons of known 'genotypes' (based on MeCP2-GFP expression) in *ex vivo* slices during whole-cell recordings, followed by fixation and confocal microscopy. Surprisingly, mutant neurons lacking MeCP2-GFP showed dendritic spine volume comparable to that in controls, while MeCP2-GFP-expressing neurons show smaller spines, opposite to the phenotype observed in *Mecp2* KO. Consistently, LM22A-4 (i.p. 60 days *in vivo*) had an effect only in MeCP2-GFP-expressing neurons, which improved dendritic spine volumes to control levels. Unexpectedly, we found no differences in social preference and social memory in *Mecp2* HET mice, opposite to the impairments we observed in *Mecp2* KO mice. However, we found that *Mecp2* HET mice engaged in atypical 'digging' behaviors significantly more than controls, which was reduced to WT levels by LM22A-4. Altogether, these data revealed unexpected differences in dendritic spine and social phenotypes in *Mecp2* HET mice, while providing support to the potential usefulness of BDNF-related therapeutic approaches such as the partial TrkB agonist LM22A-4.

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Poster

436. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 436.08

Topic: A.07. Developmental Disorders

Support: NIMH MH70727
NIMH MH064913

Title: Evaluation of the therapeutic potential of ketamine in a mouse model of Rett syndrome

Authors: *M. K. PIAZZA¹, E. T. KAVALALI², J. L. NEUL³, L. M. MONTEGGIA⁴;
²Vanderbilt Brain Inst., ¹Vanderbilt Univ., Nashville, TN; ³Neurosciences, Univ. of California San Diego, La Jolla, CA; ⁴Vanderbilt Brain Inst., Nashville, TN

Abstract: Rett syndrome (RTT) is primarily caused by mutations in the gene methyl-CpG binding protein 2 (MECP2), representing the second leading cause of intellectual disability in females. Multiple lines of investigation have implicated the N-methyl-D-Aspartate receptor (NMDAR) in the pathophysiology of RTT, as dysfunction of MeCP2 alters the development of NMDARs, contributing to an imbalance of inhibitory and excitatory neurotransmission. Interestingly, both genetic manipulation and pharmacological blockade with the NMDAR antagonist ketamine have shown efficacy in reversing electrophysiological deficits, while a small number of studies have demonstrated an ability of systemic ketamine treatment to rescue behavioral symptoms of RTT and extend lifespan in an animal model. However, a thorough characterization of ketamine's modulation of RTT symptoms and its molecular underpinnings are warranted. Our lab has demonstrated that ketamine administration causes desuppression of brain derived neurotrophic factor (BDNF) protein synthesis in the mouse hippocampus, enhancing glutamatergic activity and alleviating depression-like symptoms. As BDNF is similarly down-regulated in animal models of RTT, it is plausible that this mechanism may be convergent between these disparate disorders. We explored this possibility utilizing biochemical and electrophysiological paradigms in an *Mecp2* constitutive knockout mouse model. Indeed, our findings show an up-regulation of hippocampal BDNF protein expression in wild type and *Mecp2* knockout mice following systemic ketamine treatment. Furthermore, electrophysiological examination of field excitatory post-synaptic potentials (fEPSPs) revealed a concomitant potentiation of the fEPSP response in wild type and knockout animals. However, ketamine induced potentiation of fEPSP responses is blunted in animals lacking expression of functional *MECP2* protein. Interestingly, this effect appears to be dynamic across development in *Mecp2* knockout mice. Furthermore, this study demonstrates the effect of ketamine treatment on inhibitory signaling in the hippocampus, providing an important perspective on ketamine's modulation of the balance of excitatory and inhibitory hippocampal neurotransmission in both wild type mice and mice modeling Rett syndrome. Taken together, this study contributes to the growing investigation of ketamine's therapeutic efficacy and putative molecular action in RTT.

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Poster

436. Rett Syndrome

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Program #/Poster #: 436.09

Topic: A.07. Developmental Disorders

Support: IRSF Grant #3515

Title: Exploring nonsense suppression as a treatment for Rett syndrome

Authors: *X. XU¹, K. M. KEELING², M. DU², J. D. ECHOLS², B. BOSTWICK³, C. AUGELLI-SZAFRAN³, M. SIMMONS⁴, Y. YIN⁵, J. MERRITT⁶, J. L. NEUL⁶, Q. CHANG⁵, R. M. COWELL⁴, M. J. SUTO³, D. M. BEDWELL², **L. POZZO-MILLER¹**;

¹Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL; ²Dept. of Biochem. and Mol. Genet., Univ. of Alabama at Birmingham, Birmingham, AL; ³Drug Discovery Div., ⁴Dept. of Neurosci., Southern Res., Birmingham, AL; ⁵Med. Genet. and Neurol., Univ. Wisconsin-Madison, Madison, WI; ⁶Vanderbilt Kennedy Ctr., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Approximately 60% of Rett syndrome individuals (RTT) carry a nonsense mutation in *MECP2*. A nonsense mutation introduces a premature termination codon (PTC) into the *MECP2* mRNA, which reduces MeCP2 protein by terminating translation of the mRNA before a full-length protein is made, and by triggering nonsense-mediated mRNA decay (NMD) to degrade the PTC-containing mRNA, preventing its translation. Our aim is to identify compound(s) that restore full length MeCP2 protein levels by suppressing translation termination at a PTC alone or in conjunction with inhibiting NMD. We generated dual luciferase readthrough reporters expressing R168X, R255X, R270X, and R294X *MECP2* nonsense mutations, commonly found in RTT. After high throughput screen of 165 compounds in 4 reporter cell lines, 11 compounds increased reporter activity, indicating that they suppressed one or more *MECP2* nonsense mutations. Based on their ability to promote readthrough and their medicinal chemistry properties, the top 3 compounds were tested in human neurons derived from induced pluripotent stem cells (iPSCs) obtained from a RTT individual with the R294X mutation, assessed by Western immunoblots for MeCP2. These 3 compounds failed to increase MeCP2 protein levels, in contrast to the expected increase after treatment with gentamycin as an established readthrough positive control. Different classes of compounds have been identified already, which are expected to promote more effective MeCP2 readthrough, either alone or in combination. Here, we evaluated cell body size, dendritic length and branching, dendritic spine density, as well as the density of excitatory synapses in cultured cortical neurons from knock-in mice harboring the *MECP2* R255X mutation as the first step to validate these cellular phenotypes as outcome measures for the effect of new compounds. We expect these combined approaches will identify effective compounds to suppress nonsense mutations and improve RTT symptomatology.

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Poster

436. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 436.10

Topic: A.07. Developmental Disorders

Support: NIH R15MH124042 (KK)
Rettsyndrome.org (BL)

Title: Role of perineuronal nets in the primary somatosensory cortex of adult female mouse model for Rett syndrome

Authors: *B. Y. B. LAU, B. KARTAL, M. MYKINS, B. BRIDGES, J. ELROD, K. KRISHNAN;
Univ. of Tennessee At Knoxville, Univ. of Tennessee At Knoxville, Knoxville, TN

Abstract: MECP2 is a known regulator of synaptic plasticity in sensorycritical periods during early postnatal development. Previously, we have shownthat MECP2 also regulates inhibitory synaptic plasticity in adult auditory andsomatosensory cortices, in the adult female mouse model for Rett syndrome (*Mecp2*-heterozygote, Het). MECP2 appearsto regulate inhibition through parvalbumin+ GABAergic neurons (PV), which aresurrounded by specialized extracellular matrix structures called perineuronalnets (PNNs). We found atypical increased PNN expression in primarysomatosensory cortex (S1), a brain region responsible for processing somatosensoryinformation, of naïve Het¹. We hypothesize that this atypicalincreased PNN expression leads to atypical tactile sensory perception in Hetduring single modality and complex social behaviors. Here, we will test thishypothesis by manipulating cortical PNN expression in Het S1 and assessingtheir tactile and social behaviors. We use DeepLabCut to analyze pup retrievalvideos for pose and trajectory of freely moving female mice, and DataVyu toanalyze whisker interactions during object recognition and texturediscrimination assays. We will present results showing a correlation between decreased PNN expression in S1 and time-dependent “rescue” in tactile sensoryphenotypes in Het (n = 9 mice per genotype and surgical manipulation withrespective controls, with systematic behavioral analysis over six days for allcohorts, and *Kruskal-Wallis test* and *Pearson r* correlation whereappropriate). On average across all days, reducing PNNs “rescues” tactilesensory perception in Het. However, nuanced trial and day-dependent changes inbehaviors suggests that S1 PNNs do not significantly affect tactile sensoryperception in early days of the trials, and have significant impact on laterdays. These findings allow us to speculate about the roles for S1 PNNs in sensorimotorintegration over days, and set the stage for testing with direct optogeneticmanipulations and EEG recordings.
Reference:1. Lau BYB,Layo DE, Emery B, Everett M, Kumar A, Stevenson P, Reynolds KG, Cherosky A,Bowyer SAH, Roth S, Fisher DG, McCord RP and Krishna K. Lateralized expressionof cortical perineuronal nets during maternal experience is dependent on MECP2.*eNeuro*, May/June (2020).

Disclosures: B.Y.B. Lau: None. B. Kartal: None. M. Mykins: None. B. Bridges: None. J. Elrod: None. K. Krishnan: None.

Poster

436. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 436.11

Topic: A.07. Developmental Disorders

Support: MDBR-18-106-CDKL5
Telethon Foundation Grant GGP19045
Associazione Albero di Greta
Associazione Insieme Verso la Cura

Title: Myelination process is affected in CDKL5 deficiency disorder

Authors: S. DEVI¹, D. COMAI¹, R. PIZZO¹, A. GURGONE¹, M. LORENZATI^{1,3}, A. BUFFO^{1,3}, C. SALIO², *M. GIUSTETTO¹;

¹Univ. of Torino, Torino, Italy; ²Univ. of Torino, Grugliasco-Torino, Italy; ³Neurosci. Inst. Cavalieri Ottolenghi, Univ. of Turin, Turin, Italy, Orbassano-Torino, Italy

Abstract: CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental condition without a cure caused by mutations in the cyclin-dependent kinase-like 5 (*CDKL5*) gene and characterized by: early-onset epilepsy, severe cognitive dysfunctions, sensorimotor and intellectual disabilities. *CDKL5* is a serine/threonine kinase highly expressed in forebrain neurons where it participates in the regulation of epigenetic factors, dendritic morphology, and synaptogenesis. Recently, it has been shown that *CDKL5* may modify cytoskeleton dynamics by phosphorylating microtubule-associated proteins. Along with neurons, *CDKL5* is quite highly expressed in myelinating oligodendrocytes (OLs) where its role is still completely unknown. Indeed, MRI imaging of human CDD subjects shows both grey and white matter alterations, indicating that *CDKL5* could play an important role in the formation and/or maintenance of myelin sheath. However, whether and how the loss of *CDKL5* could affect myelin organization has not yet been investigated. To address this issue, we evaluated the post-natal developmental trajectory of myelination in the cerebral cortex of *Cdkl5*-KO mice by analyzing the expression of molecules involved in myelin deposition or axonal injury. This analysis showed a reduction of both myelin basic protein and phospho-neurofilaments expression in both V1 and S1 cortices of mutant mice. Moreover, g-ratio analysis of myelinated axons showed reduced myelin sheath thickness in mutants while nodes of Ranvier length and density were also severely affected in *Cdkl5*-KO mice. Intriguingly, our results point to a crucial role of *CDKL5* in the maturation of OLs as we found that mature OLs were reduced in mutant mice whereas the number of OL precursor cells (OPCs) was not affected. Finally, consistently with animal data, we found severe alterations of the myeloarchitecture in post-mortem primary visual cortex of two CDD patients. In addition, GO analysis of differentially expressed genes showed enrichment for processes associated with myelination and gliogenesis in CDD patients. In conclusion, our data indicate that primary cortical areas in both *Cdkl5*-KO mice and CDD patients exhibit a severe reduction/distortion of

myelination-related mechanisms thus disclosing a novel role of CDKL5 activity, likely of pivotal importance for CDD.

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Poster

436. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 436.12

Topic: A.07. Developmental Disorders

Support: Loulou Foundation (Fink)
UC Davis MIND IDDRC (Fink)

Title: Cdk15 gene reactivation using dual AAV mediated CRISPR/dCas9 epigenetic editing

Authors: *J. A. HALMAI^{1,2,3,4}, J. WALDO^{1,2,3,4}, J. L. CARTER^{1,2,3,4}, C. GONZALEZ^{1,2,3,4}, D. L. CAMERON^{1,2,3,4}, I. VILLEGAS^{1,2,3,4}, A. ADHIKARI^{5,2,4}, N. COPPING^{5,2,4}, J. L. SILVERMAN^{5,2,4}, K. FINK^{1,2,3,4};

¹Dept. of Neurol., ²Ctr. for Interventional Genet., ³Stem Cell Program and Gene Therapy Ctr.,

⁴MIND Inst., ⁵Dept. of Psychiatry & Behavioral Sci., UC Davis Med. Ctr., Sacramento, CA

Abstract: CDKL5 deficiency disorder (CDD) is an infantile epilepsy and X-linked intellectual disability caused by de novo mutations in the CDKL5 gene. In female neurons, one copy of CDKL5 becomes epigenetically silenced by X chromosome inactivation (XCI). In neuronal cells expressing the mutant allele, reactivation of wildtype CDKL5 could present a shift in treatment paradigm by partially or fully restoring disease-associated neurodevelopmental and molecular phenotypes. We have previously demonstrated that targeted CDKL5 reactivation is feasible in human cells using a dCas9 epigenetic editor. Here we have designed an adeno-associated virus (AAV)-mediated intein split dCas9 epi-editor for gene reactivation of mouse Cdk15 in vivo for delivery and rescue experiments. Following intracranial injection of AAV9 we detect widespread split dCas9 expression and virus biodistribution in the brain, in vivo trans-splicing of split dCas9 and significant upregulation of Cdk15. We also assess the on-target mechanism of action by DNA methylation removal from the inactive X chromosome via bisulfite sequencing and genome-wide off-target demethylation. In addition, we demonstrate promoter and polyA optimization to improve AAV size and increase full capsid ratios. Current studies are investigating functional rescue in Cdk15 transgenic animals. This includes rescue of phosphorylation status of Cdk15 kinase targets as well as behavioral deficits. This approach holds great promise for those affected by CDD.

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Poster

437. Animal Models of Autism: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 437.01

Topic: A.07. Developmental Disorders

Support: NIMH 1R01 MH118631-01

Title: Genetic mouse models with developmental social memory dysfunction have atypical perineuronal nets in hippocampal CA2

Authors: *E. J. DIETHORN, S. H. WANG, A. J. ESPINEL, E. GOULD;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Autism Spectrum Disorder (ASD) is a heterogeneous set of neurodevelopmental conditions, with defining characteristics of restrictive interests/repetitive behaviors and social communication/interaction deficits. Individuals with ASD and related conditions like Phelan-McDermid syndrome (PMS) and Fragile X syndrome (FXS) also have difficulty recognizing familiar faces, as well as recognizing emotional expressions. Since social memory is the basis of most social interactions, understanding mechanisms of its dysfunction may suggest therapies to improve quality of life for people with ASD and related conditions. ASD has a strong genetic component and several mouse models based on genes known to be mutated in individuals with related disorders, like PMS and FXS, have been created. Two of these models - *Shank3B* and *Fmr1* knockout (KO) mice - were used to characterize the postnatal onset of social memory dysfunction, investigate the neural underpinnings of this dysfunction, and devise manipulations designed to improve function. Using a direct social interaction test, we found that both *Shank3B* and *Fmr1* KO mice have impaired social memory at postnatal day (P) 14 compared to their control counterparts, who demonstrate emerging preference for a novel conspecific. Previous studies have shown that molecules associated with the extracellular matrix (ECM) are atypical in the brains of people with ASD, and our findings have revealed aberrant postnatal development of perineuronal nets (PNNs), specialized ECM structures, in the hippocampal CA2 region of *Shank3B* KO and *Fmr1* KO pups. Using two histological markers of PNNs, Wisteria floribunda agglutinin and aggrecan, we found that PNN intensity is significantly increased in P14 *Shank3B* KO mice and significantly decreased in P14 *Fmr1* KO mice compared to their controls. We also assessed a marker of glutamatergic (vGLUT2) innervation to the CA2 from the supramammillary nucleus of the hypothalamus, an area known to be involved in social memory, and found increased vGLUT+ innervation in both KO models at P14. Based on these data, we developed methods to surgically diminish PNNs in the CA2 of *Shank3B* KO pups with the degradative enzyme chondroitinase-ABC and enhance PNNs in the CA2 of *Fmr1* KO pups with a matrix metalloproteinase-9 inhibitor during early development to normalize PNN levels to that of controls in an effort to enable healthy development of CA2 afferent input and improve social discrimination abilities.

Disclosures: E.J. Diethorn: None. S.H. Wang: None. A.J. Espinel: None. E. Gould: None.

Poster

437. Animal Models of Autism: Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 437.02

Topic: A.07. Developmental Disorders

Support: Showalter Research Trust
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NIH Grant R01NS117585
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FamilieSCN2A foundation for the Action Potential Grant
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Indiana Spinal Cord & Brain Injury Research Fund

Title: Hippocampal microglial pruning of excessive synapses impairs learning and memory in mice deficient in autism associated *Scn2a* gene

Authors: *J. WU, J. ZHANG, M. EATON, X. CHEN, K. WETTSCHURACK, Z. QUE, M. I. OLIVERO ACOSTA, N. CUI, B. DEMING, Y. ZHAO, Y. YANG;
Medicinal Chem. and Mol. Pharmacol., Purdue Univ., West Lafayette, IN

Abstract: Mutations in voltage-gated sodium channel Nav1.2, encoded by *Scn2a*, is leading monogenic causes of autism, which is often accompanied by severe comorbidities including intellectual disability. *Scn2a*-related autism often results from protein-truncating variants and loss-of-function variants (*Scn2a* deficiency). To model *Scn2a* deficiency-related disorders, we recently established a gene-trap *Scn2a* deficient mouse model. Here we report that Nav1.2-deficient mice display severely impaired learning and memory, which are closely related to hippocampal synaptic transmission of neurons. We observe that the dendritic spine density and miniature excitatory postsynaptic currents (mEPSC) at P30 are decreased, together with impaired post-synaptic function and morphology. Microglia play a key role in regulating synaptic remodeling both in development and in neurological diseases like autism and schizophrenia, by performing many essential functions including pruning excessive spines and shaping spine morphology. Interestingly, we found the phagocytosis of microglia was activated through quantifying the volume of CD68, which represents microglial lysosome, accompanied by an increased microglial synaptic engulfment at P30, but not P5 or P10 in the hippocampal CA1 region of Nav1.2-deficient mice. Meanwhile, we are also testing the use of PLX3397, which is CSF1R specific inhibitor used to eliminate microglia. Extensive treatment with PLX3397 has been shown to result in about 95% elimination of microglia brain-wide. How the ablation of microglia or inhibition of microglial phagocytosis may affect the neural network connection and the learning and memory of Nav1.2-deficient mice are under investigation. Our results will provide insight into how microglia might play a key role in regulating learning and memory in

Nav1.2-deficient mice, revealing a potential cellular mechanism underlying *Scn2a* deficiency-related neurodevelopmental disorders.

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Poster

437. Animal Models of Autism: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 437.03

Topic: A.07. Developmental Disorders

Support: MIND Institute

Title: Molecular validation and behavioral phenotyping of a mouse model of SYNGAP1-related intellectual disability.

Authors: *E. SMITH, T. FENTON, L. BERG, A. ADHIKARI, C. P. CANALES, J. SILVERMAN, A. NORD;
Univ. of California, Davis, Davis, CA

Abstract: Mutations in the gene encoding Synaptic Ras GTPase Activating Protein 1 (*SYNGAP1*) are associated with autism spectrum disorder (ASD) and intellectual disability (ID) in children. *SYNGAP1* has an established role in influencing molecular changes in dendritic spine synaptic functional changes via negative regulation of Ras-GTPase and AMPAR trafficking to the excitatory postsynaptic membrane. Heterozygous mutations of *SYNGAP1* causes a lack of inhibition of Ras-GTPase, resulting in an increase in the presence of mushroom-shaped dendritic spines. This increase can disrupt neuronal growth and maturation during critical periods of neurodevelopment leading to developmental brain disorders and social dysfunctions. Thus, the mechanistic link between *SYNGAP1* mutations and neurodevelopmental disorders is clear. However, treatments for *SYNGAP1* mutations have not been identified. Despite previous characterization of *SYNGAP1* mouse models, there is still no evidence of safe and functional restoration of *SYNGAP1*. To address this, we are using a commercially available *SYNGAP1*-mutant mouse line maintained on a mixed C57BL/6J and 129 genetic background to identify molecular, cellular, and behavioral indices for studies of treatment efficacy. Validation of the mouse model was performed via western blot of whole brains from wildtype and mutant mice. Expression level of *SYNGAP1* in mutant mice was significantly decreased in comparison to wildtype mice. For the behavioral analysis, we performed comprehensive behavioral phenotyping of motor, cognitive, and physiological domains in both sexes using assays relevant to SYNGAP-1 related intellectual disability. We discovered a robust hyperactive phenotype and substantial impairment in the novel object recognition learning and memory assay. We also observed aberrant respiratory patterns in the heterozygous mice when compared to age and sex-

matched wild-type littermates. These deficits display several clinically relevant functional phenotypes to advance preclinical testing of precision gene and stem cell delivered therapeutic candidates for SYNGAP1. Overall, identification of candidate treatments for *SYNGAP1* mutations will speed up progress in bringing epigenetic gene therapy to rare neurodevelopmental disorders.

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Poster

437. Animal Models of Autism: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 437.04

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI, Grant numbers JP17H03989 (H.H.), JP18H02574 (T.N.), JP20H03556 (K.S.), JP20H03391 (A.K.), JP20K21479 (A.K.), JP20H00492 (H.H.), and JP20K21464 (T.N.)
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Title: Intranasal oxytocin administration suppresses social contact-induced neural activity in a POGZ-Q1038R mutant mouse model of autism spectrum disorder

Authors: *K. KITAGAWA¹, M. BABA¹, T. TAKEMOTO¹, M. TANUMA¹, M. HAYASHIDA¹, S. YAMAGUCHI², Y. AGO³, K. SEIRIKI¹, A. HAYATA-TAKANO¹, K. TAKUMA¹, A. KASAI¹, H. HASHIMOTO¹, T. NAKAZAWA⁴;
¹Osaka Univ., Osaka, Japan; ²Gifu Univ., Gifu, Japan; ³Hiroshima Univ., Hiroshima, Japan; ⁴Tokyo Univ. of Agr., Tokyo, Japan

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication. Despite its high prevalence, molecular and cellular pathogenesis of ASD remains largely unclear. Accordingly, no treatment for the core symptoms of ASD has been established, and the development of therapeutic agents is urgently needed. Oxytocin is a neuropeptide known to play an important role in social behavior. Although oxytocin is attracting attention as a potential therapeutic candidate for ASD, the therapeutic efficacy of oxytocin for social deficits remains controversial. We have previously demonstrated

that a mouse model carrying an ASD patient-derived *de novo* mutation in the pogo transposable element derived with zinc finger domain (POGZ-Q1038R mice) showed ASD-like social behavioral deficits. Interestingly, neural activity was particularly increased in the anterior cingulate cortex in POGZ-Q1038R mice, which may be underlying mechanisms for the ASD-like social behavioral deficits. We recently demonstrated that intranasal oxytocin administration improved their social behavioral deficits; however, the molecular mechanism behind the effect of oxytocin administration remains unclear. In this study, to identify brain regions involved in the ameliorating effect of oxytocin for social deficits in POGZ-Q1038R mice, we performed brain-wide imaging using POGZ-Q1038R mice carrying Arc-dVenus transgene, in which activated neurons are labeled with dVenus, a destabilized green fluorescent protein. After the social contact of a POGZ-Q1038R mouse with a wild-type mouse, the distribution of dVenus-positive cells was analyzed to generate a whole-brain neural activity map of POGZ-Q1038R mice. We found that the total number of dVenus-positive cells in POGZ-Q1038R mice was significantly reduced by oxytocin treatment, suggesting that oxytocin suppressed social contact-induced neural activity in multiple brain regions in POGZ-Q1038R mice. In particular, the number of dVenus-positive cells was significantly reduced in the anterior cingulate cortex and posterior parietal cortex, suggesting that suppression of neural activity in these regions may be important for the therapeutic effects of oxytocin in POGZ-Q1038R mice. These findings suggest a neurological mechanism for the pharmacological effects of oxytocin on social impairment, providing insights into the development of new therapeutics for ASD.

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Poster

437. Animal Models of Autism: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 437.05

Topic: A.07. Developmental Disorders

Support: NIH Grant HD084209

Title: Prenatal stress and fluoxetine exposure in BTBR mice alters the phenotype in male and female offspring

Authors: A. L. ARZUAGA¹, P. TENEQEXHI², K. LOPEZ², *M. E. RAGOZZINO²;
²Univ. of Illinois Chicago, ¹Univ. of Illinois Chicago, Chicago, IL

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social and communication impairments as well as restricted and repetitive behaviors (RRBs). The increased prevalence of ASD in recent decades coupled with the heterogeneity of symptom

severity may arise from a complex interplay between prenatal and genetic risk factors that contribute to autism-like behaviors in offspring. In a recent study, we found that prenatal stress and/or the selective serotonin reuptake inhibitor (SSRI), fluoxetine, in C57BL/6J (B6) led to distinct autism-like behaviors in male and female offspring. The present study investigated in the BTBR mouse model of autism whether prenatal stress and/or fluoxetine potentiated autism-like behaviors in male and female offspring. As previously with B6 mice, pregnant BTBR mice were subjected to chronic restraint stress from gestational days 4-18 (one 30-minute session every two days) and/or administered fluoxetine (3 mg/kg/day) on days 8-18. These manipulations during gestation reduced weight gain in pregnancy and litter survivability. A subsequent study reduced restraint stress to one 30-minute session every other day starting on gestation days 4 through 18. The same dose of fluoxetine was used. Offspring were tested as young adults (8-12 weeks of age) on self-grooming, locomotor behavior, spatial and reversal learning and elevated plus-maze. In male BTBR offspring, the combined action of prenatal stress and SSRI exposure did not affect self-grooming, locomotor activity or anxiety, but did impair spatial acquisition and reversal learning. In BTBR female offspring, prenatal exposure to restraint stress and/or an SSRI significantly reduced self-grooming behavior with a concomitant increase of locomotor behavior. Prenatal SSRI exposure alone in BTBR females contributed to reversal learning deficits. The findings suggest that prenatal exposure to stress and/or an SSRI in a mouse model of autism can produce long-term behavioral alterations that are sex specific. The results suggest that prenatal experiences may interact with polygenic factors that contribute to the heterogeneity of symptoms and symptom severity in ASD.

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Poster

437. Animal Models of Autism: Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 437.06

Topic: H.12. Aging and Development

Support: programs in developmental neuroscience and developmental disabilities (PDNDD)

Title: Role of learning-induced rRNA synthesis in Autism spectrum disorder

Authors: *S. PHATARPEKAR¹, J. KIM², C. ZHU², I. OJIMA³, A. I. HERNANDEZ⁴, K. K. CHADMAN⁵;

¹SUNY downstate medical center, SUNY downstate medical center, brooklyn, NY; ²Univ. of New York at Stony Brook, stony brook, NY; ³Univ. of New York at Stony Brook, Stony Brook, NY; ⁴SUNY Downstate Med. Ctr., SUNY Downstate Med. Ctr., Brooklyn, NY; ⁵NYS Inst. Basic Res., NYS Inst. Basic Res., Staten Island, NY

Abstract: Background: Autism spectrum disorder (ASD) is a group of disorders characterized by social communication deficits and increased repetitive behaviors. The molecular pathway underlying the pathology of ASD is still unknown. Decreased neuronal cellular volume (nucleolar and cytoplasmic) has been found in autistic children, adolescents, and adults. This decrease may be caused by nucleolar inactivation that leads to the decline in nucleolar activity and biogenesis of ribosomes, which may underlie the behavioral deficits found in ASD. Methods: The current experiments examine the role of ribosome biogenesis (rRNA synthesis) on behavioral outcomes in an ASD mouse model. The level of rRNA synthesis was compared between BTBR T+ Itpr3tf/J (BTBR) mice and C57BL/6J mice. A stimulator of ribosome biogenesis, 3BDO, a burryl derivative, was examined for effects on rRNA synthesis, protein levels, social and repetitive behaviors, and learning and memory. The Pol I specific inhibitor, CX-5461, was administered via intra-hippocampal injection prior to contextual fear training to block rRNA synthesis to examine the role of de novo rRNA synthesis in memory formation. Results: rRNA synthesis was lower in the ASD mouse model, both in the home cage and following training for contextual fear conditioning. Treatment with 3BDO stimulated rRNA synthesis during contextual fear conditioning. This increase in newly synthesized rRNA was concurrent with improved cognition in the contextual fear conditioning test. The number of marbles buried was reduced but social behavior was not affected following the 3BDO administration. Conclusion: These data suggest that rRNA synthesis plays a vital role in learning and cognition and may be impaired in the ASD mouse model. CX-5461 administration revealed that de novo rRNA synthesis is required for 24 h memory but not for learning. From a therapeutic point of view, these data may be helpful in developing a potential pharmacological treatment for ASD.

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Poster

437. Animal Models of Autism: Behavior

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 437.07

Topic: A.07. Developmental Disorders

Support: Abstrax Tech

Title: Cannabidiol and cannabis terpene blends have acute prosocial effects in the BTBR mouse model of autism spectrum disorder

Authors: J. STABEN, M. KOCH, K. REID, T. GIBBONS, L. GILMAN, J. MUCKERHEIDE, *J. S. KAPLAN;

Behavioral Neurosci. Program, Western Washington Univ., Bellingham, WA

Abstract: Cannabidiol (CBD) is a non-intoxicating phytocannabinoid with increasing popularity due to its purported therapeutic efficacy for numerous off-label conditions including anxiety and autism spectrum disorder (ASD). Those with ASD are commonly deficient in endogenous cannabinoid signaling and GABAergic tone. CBD has a complex pharmacodynamic profile that includes enhancing GABA and endocannabinoid signaling. Thus, there's mechanistic justification for investigating CBD's potential to improve social interaction and related symptoms in ASD. Recent clinical trials in children with ASD support CBD's beneficial effects in numerous comorbid symptoms, but its impact on social behavior is understudied. Here, we tested the prosocial and anxiolytic efficacy of commercially available CBD-rich broad spectrum hemp oils delivered by vaporization and consumed via passive inhalation in the understudied female cohort of the BTBR strain, a common inbred mouse line for preclinical assessment of ASD-like behaviors. We observed product-specific efficacy on prosocial behaviors using the 3-Chamber Test and a different dose-response relationship between prosocial behavior and anxiety-related behavior on the elevated plus maze. These findings suggest that CBD's prosocial effects in the 3-Chamber Test may be independent of effects on anxiety, and furthermore, may be impacted by the composition of cannabinoids or terpenes in each product. While terpenes are responsible for cannabis' unique odor and flavor, they also may directly contribute to the therapeutic effects traditionally ascribed to the cannabinoids. We next tested the effects of the terpene blend found in the common OG Kush cannabis strain and observed prosocial effects without an impact on anxiety-related behavior. These terpenes produced an even more robust prosocial effect when combined with CBD isolate. We observed similar prosocial effects with two other cannabis terpene blends from the Do-Si-Do and Blue Dream strains. Together, our results illustrate the contributions of both CBD and terpenes in cannabis' promising benefits on prosocial behaviors in ASD that may be independent of reductions in anxiety, and we further highlight that specific compositions involving CBD and unique terpene blends may confer enhanced efficacy.

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Poster

437. Animal Models of Autism: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 437.08

Topic: A.07. Developmental Disorders

Title: Preclinical study of deep brain stimulation of the nucleus accumbens for the treatment of severe self injurious behaviours associated with Autism Spectrum Disorder.

Authors: ***K. ZHANG**^{1,2}, **F. VENETUCCI GOUVEIA**¹, **G. M. IBRAHIM**¹;

¹The Hosp. For Sick Children, Toronto, ON, Canada; ²Inst. of Med. Sci., Univ. of Toronto, Toronto, ON, Canada

Abstract: Severe self-injurious behaviour (SIB) is frequently observed in children with Autism Spectrum Disorder (ASD) with limited treatment options. Severe SIB is associated with physical injury, distress, repeated hospitalizations and reduced quality of life for children and their caregivers. The nucleus accumbens (NAcc) is a brain region implicated in reward circuitry and has been shown to be altered in children with ASD. Deep brain stimulation (DBS) is a novel neuromodulatory technique where electrodes are implanted in specific brain structures to modulate local and widespread neuronal activity. DBS of the NAcc presents an opportunity to modulate NAcc-associated circuitry and offers novel means to treat SIB in affected children; however, the neural underpinnings of this treatment are poorly understood, and preclinical models are required to better understand its mechanism of action. The inbred BTBR T+ Itpr3tf/J (BTBR) mouse strain is commonly used for investigations of ASD as these mice spontaneously present behaviours that mimic symptoms of ASD. Notably, they exhibit excessive self-grooming; a behaviour that is comparable to SIB. To explore the effects of NAcc-DBS on SIB and autism-relevant phenotypes, we treated BTBR mice with bilateral chronic high-frequency stimulation of the NAcc. Animals were tested for: I) excessive self-grooming, II) general locomotion and anxiety (open field test), III) socialization (three-chambered social approach test), and IV) repetitive behaviour (marble burying test). The chronic NAcc-DBS treatment reduced levels of repetitive self-grooming, anxiety-like and repetitive behaviours, with no effect on sociability. Our future steps will involve analysis of whole brain high-resolution magnetic resonance imaging and histology, to investigate brain plastic changes and neuronal activity following treatment. These preclinical results with mouse models of ASD will help better understand the use of NAcc-DBS as a therapeutic approach for the treatment of severe SIB.

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Poster

437. Animal Models of Autism: Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 437.09

Topic: A.07. Developmental Disorders

Title: The ameliorating effect of sulforaphane on a mouse model of autism spectrum disorder

Authors: ***S. RIEBESELL**¹, **N. TOUMANIOS**¹, **M. SCHMID**¹, **E. CRAIG**¹, **R. FREEDMAN**¹, **L. A. GABEL**²;

¹Program in Neurosci., ²Dept. of Psychology & Program in Neurosci., Lafayette Col., Easton, PA

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder associated with social interaction deficits and restricted, repetitive behaviors. BTBR T+Itpr3tf/J (BTBR) mice are commonly used as an animal model of ASD since this strain has previously displayed increased repetitive self-grooming and decreased social interactions. The C57BL/6J (C57) strain is the social counterpart to the BTBR strain and functions as the control for the current study.

Experiment 1 attempts to examine the behavior of BTBR mice to determine if they exhibit ASD-like behaviors. Consistent with previous research, we found that BTBR mice (N = 13) displayed significantly higher repetitive behaviors depicted as increased total time spent self-grooming and a higher number of marbles buried during the marble-burying test compared to C57 mice (N = 11). Given the variability in the presence of generalized anxiety in previous studies, we found that the BTBR model did not demonstrate increased general anxiety in the elevated plus and open field tasks compared to C57 mice. We did not find a social deficit during the three-chamber sociability task when the stranger mouse was of the opposite strain to the test mouse. These findings are consistent with previous research indicating that BTBR and C57 mice strongly prefer interacting with a C57 stranger mouse over a BTBR stranger mouse. BTBR mice did not exhibit an impaired working memory using the Y maze test compared to C57 mice. However, BTBR mice exhibited a significantly higher number of total entries of the Y maze and a significantly higher number of total entries into the outer zone of the apparatus during the open field test. The significantly increased repetitive behaviors, total entries of the Y maze, and entries into the outermost zone of the open field apparatus demonstrated by BTBR mice may be indicative of hyperactive or repetitive/compulsive-like behaviors. Experiment 2 tests the ameliorating effects of sulforaphane, an isothiocyanate derived from broccoli sprouts, on atypical behaviors of BTBR mice. Sulforaphane has been found to upregulate genes that protect against oxidative stress, inflammation, and DNA damage, characteristics commonly associated in children with ASD. Of all behavioral measures tested, sulforaphane had a therapeutic effect only on marble-burying behavior. Sulforaphane, administered via a novel and non-invasive technique of oral self-administration, significantly decreased BTBR mice's marble-burying behavior (N = 6). Our study addresses replicable outcomes on sociability measures in BTBR mice and suggests a possible therapeutic agent for stereotypy.

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Poster

437. Animal Models of Autism: Behavior

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Topic: A.07. Developmental Disorders

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MOST 103-2321-B-002-021
MOST 104-2314-B-002-129-MY4
MOST 108-2320-B-002-006-MY3

Title: Odor Perception in a Mouse Model of Autism Spectrum Disorder

Authors: Y.-T. CHEN¹, *Y.-F. CHEN², H.-C. CHANG³, C.-J. CHENG⁴, Y.-H. CHANG⁴, Y.-C. WANG⁵, K.-Y. LEE⁶, C.-H. CHEN⁷, L.-J. LEE⁸, S.-F. GAU⁹;

¹Univ. of Texas Southwestern Med. Ctr., Dallas, TX; ²Chang Gung Mem. Hosp., Keelung, Taiwan; ³Grad. Inst. of Anat. and Cell Biol., ⁴Natl. Taiwan Univ., Taipei, Taiwan; ⁵Chi-Mei Med. Ctr., Tainan, Taiwan; ⁶Chang Gung Mem. Hospital, Keelung, Keelung, Taiwan; ⁷Dept. of Psychiatry, Chang Gung Mem. Hospital-Linkou, Taoyuan, Taiwan; ⁸Anat. and Cell Biol., Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan; ⁹Natl. Taiwan Univ. Hosp., Taipei, Taiwan

Abstract: Smell problems are correlated with numerous disorders including autism spectrum disorder (ASD) which is a neurodevelopmental disorder characterized by deficits in social communication and restricted, repetitive patterns of behavior, interests, or activities. In addition to these symptoms, patients with ASD also suffer from other problems, such as intellectual disability, anxiety, sensory abnormalities, motor difficulties, and aggression. A microdeletion at the 8p23 terminal region had been identified in a Taiwanese boy with ASD and DLGAP2 gene in this deletion region had been speculated as a candidate for the pathogenesis of ASD. *Dlgap2* mutant mice had been generated and characterized. *Dlgap2* is expressed in numerous olfaction-related brain regions in mice. In homozygous *Dlgap2* knockout (*Dlgap2* KO) mice, the *Dlgap2* was abolished in the olfactory bulb; the levels of several synaptic proteins were also affected. The ability of olfactory detection was comparable between wild-type control and *Dlgap2* KO mice, while these mutant mice exhibited greater activities when encountering social odor but seemed not interested in food odors. Here we demonstrated olfactory phenotypes in a mouse model of ASD. Our study will not only advance our knowledge of the olfactory system but also comprehend the pathogenesis of neurodevelopmental disorders such as ASD which may further improve the diagnosis and treatments.

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Poster

437. Animal Models of Autism: Behavior

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MOST 108-2320-B-002-006-MY3

Title: Fbxo25 mutant mice exhibited aberrant hippocampal protein expression and more aggressive behaviors

Authors: *S.-Y. LIN¹, C.-J. CHENG¹, H.-C. CHANG², L.-J. LEE³, S.-F. GAU⁴;
²Grad. Inst. of Anat. and Cell Biol., ¹Natl. Taiwan Univ., Taipei, Taiwan; ³Anat. and Cell Biol.,
Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan; ⁴Natl. Taiwan Univ. Hosp., Taipei, Taiwan

Abstract: A deletion of 2.4Mb at the 8p23.2-pter region had been identified in a Taiwanese boy with autism spectrum disorder. Patients with 8p23.2-pter microdeletion were characterized by developmental delay, intellectual disability, microcephaly, autism spectrum disorder, attention-deficit/hyperactivity disorders, and mildly dysmorphic features. *FBXO25* is located in this region and thus selected as a possible pathological target in 8p23.2-pter microdeletion syndrome. *FBXO25* is a ubiquitin ligase included in a protein complex, SKP1-cullin-F-box, and is involved in protein degradation across the ubiquitin-proteasome system. We generated the *Fbxo25* gene knockout mice to simulate the condition of 8p23.2-pter microdeletion. *Fbxo25* mutant mice exhibited normal behavioral performances in the open field, novel object recognition, and three-chamber social interaction tests. However, in the resident-intruder test, *Fbxo25* mutants displayed more aggressive behaviors with more c-fos-positive nuclei in the medial nucleus of the amygdala, especially the posteroventral subdivision and paraventricular nucleus of the hypothalamus compared with wild-type mice. *Fbxo25* is expressed in the hippocampus while the protein levels of PSD95, CaMKII- α , mGluR5 and ERK were altered in the hippocampus of *Fbxo25* mutant mice. Together, the removal of *Fbxo25* does not influence locomotor activities, short-term recognition memory, and social interaction but aberrant protein expression in the hippocampus. Deletion of *Fbxo25* also affects intruder-induced aggressive behaviors and neuronal activities in aggression-related brain areas. Our study showed the impact of *FBXO25* deficiency in mice which could be established as a novel model of 8p23.2-pter microdeletion.

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Poster

437. Animal Models of Autism: Behavior

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Topic: A.07. Developmental Disorders

Support: JSPS Kakenhi 19KK0211

Title: Fetal blockade of nicotinic acetylcholine transmission causes autism-like impairments of social attachment formation in domestic chicks; recovery by postnatal bumetanide

Authors: *T. MATSUSHIMA^{1,2}, M. MIURA¹, N. PATZKE^{1,3}, N. TOJI¹, K. WADA¹, Y. OGURA⁴, K. J. HOMMA⁵, P. SGADÒ², G. VALLORTIGARA²;

¹Hokkaido University, Grad Sch. of Sci., Hokkaido University, Grad Sch. Sci., Sapporo, Japan; ²Univ. of Trento, Rovereto, Italy; ³Hlth. and Med. Univ. of Potsdam, Potsdam, Germany; ⁴Univ. of Tokyo, Tokyo, Japan; ⁵Teikyo Univ., Teikyo Univ., 2-11-1 Kaga, Itabashi-Ku, Tokyo, Japan

Abstract: Biological motion preference (BM) appears spontaneously in neonatal domestic chicks (Vallortigara et al. 2005) as well as in newborn human babies in a manner associated with the risk of autism spectrum disorder (ASD; Simion et al. 2008, Di Giorgio et al. 2015). The chick's BM is induced by imprinting to any motion pictures, and the imprinting canalizes subsequent formation of social attachment to living organisms such as the mother hen and siblings via thyroid hormone action (Yamaguchi et al. 2012, Miura et al. 2016, 2018, 2020, Lorenzi et al. 2021). Furthermore, the social predisposition is impaired by sodium valproate (VPA) applied to day 14 fetuses (E14; Lorenzi et al. 2017, Sgadò et al. 2018). Here we report another molecular process, namely BM impaired by fetal blockade of nicotinic acetylcholine receptor (nAChR). We initially hypothesized that spontaneous fetal motion is required for BM and searched for a range of chemicals that arrest E14 fetuses. VPA, ketamine, mk801, tubocurarine and selective blockers of nAChR subtypes (MLA for $\alpha 7$, Dh β E for $\alpha 4$), and imidacloprid (neonicotinoid insecticide) effectively arrested fetal motion. Behaviors of the postnatal chicks revealed that fetal treatment of the nAChR blockers impaired the BM preference, while sparing the imprinting memory formation. However, VPA spared BM and selectively impaired imprinting, indicating a double dissociation of pharmacological bases. Although mk801 arrested the fetal motion, it did not impair either BM or imprinting; involvement of NMDA receptor is thus unlikely. In summary, our initial hypothesis (namely, the fetus motion for the development of BM perception) proved incorrect. Instead, transmission via nAChR proved to be critical, beside the enhanced histone acetylation by VPA. However, when applied to neonatal chicks, bumetanide (blocker of NKCC1 chloride co-transporter) rescued both the impaired imprinting by VPA and the impaired BM by nAChR blockade. Despite distinct molecular processes, a common pathogenesis could underlie the autism-like behavioral phenotypes caused by VPA and nAChR blockade. A preprint of this study is available at bioRxiv (<https://www.biorxiv.org/content/10.1101/2022.05.19.492744v1>). The domestic chick is a valid animal model for studying environmental risk chemicals of ASD even though evolutionarily distant from mammals. (1) Construct validity; common causes such as VPA, nAChR, and GABA transmission. (2) Face validity; impaired BM and social attachment formation. (3) Predictive validity; recovery by bumetanide. The responsible brain regions and epigenetic processes should be identified.

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Poster

437. Animal Models of Autism: Behavior

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Topic: A.07. Developmental Disorders

Support: NIDCD R01DC019371

Title: Role of autism spectrum disorder-associated gene, SCN2A, in myelination and auditory processing

Authors: *K. NIP¹, E. GOULD², A. JUNG³, J. KIM⁴;

¹Cell. and Integrative Physiol., Univ. of Texas Hlth. Sci. Center, San Antonio, San Antonio, TX;

²Cell. and Integrative Physiol., Univ. of Texas San Antonio Hlth. Sci. Cen, San Antonio, TX;

³Cell. and Integrative Physiol., Univ. of Texas Hlth. Sci. Ctr. San Antonio, San Antonio, TX;

⁴Cell. and Integrative Physiol., Univ. of Texas Hlth. Sci. Ctr. San Anto, San Antonio, TX

Abstract: Recent studies using neuroimaging and transcriptional profiling have suggested that insufficient myelination, white matter abnormalities, and impaired neural connectivity are associated to neurodevelopmental disorders such as autism spectrum disorder (ASD). *SCN2A* is a well-known ASD-associated gene, encoding the voltage-gated sodium channel Nav1.2. Our previous studies have shown *SCN2A* is expressed in a subpopulation of immature oligodendrocytes (OLs) that display a spiking phenotype. Here, we investigated the role of *SCN2A* in myelination in the auditory nervous system and ASD-related auditory phenotypes in both male and female P21-P25 mice. We used OL-specific conditional knockouts of *SCN2A* (*Scn2a* cKO) in which a *SCN2A* flox line was crossed into tamoxifen inducible OL-specific cre lines (Sox10CreER or PdgfraCreER, intraperitoneal (IP) 40 mg/kg tamoxifen injections given at P4, P6 and P8). Using transmission electron microscopy (TEM), we examined myelin and axonal properties based on axon diameter and *g*-ratio. We found OL-specific *Scn2a* cKO mice have significantly thinner myelin layers around axon bundles and have larger axon calibers, compared with control. In *Scn2a* cKO mice, the brainstem showed a decreased proportion of CC1 positive OLs (a mature OL marker) among Olig2 positive cells compared with control. These results indicate that loss of *SCN2A* in OLs impact OL development and myelination in the auditory brainstem. Notably, in auditory functional testing using *in vivo* auditory brainstem response (ABR), *Scn2a* cKO mice show significantly increased amplitudes in ABR waves, specifically representing synchronous neuronal activities, in the cochlear nucleus (wave II) and superior olivary complex (wave III). In addition, *Scn2a* cKO mice have increased startle responses to loud sound in acoustic startle reflex (ASR) test. Taken together, the results suggest that *Scn2a* cKO mice have a hypersensitivity to sound, which is commonly observed in humans with ASD.

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Poster

437. Animal Models of Autism: Behavior

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Title: Sex- and genotype-specific behavioral phenotypes in a Shank3 (exon 11) transgenic mouse model of Phelan-McDermid syndrome

Authors: *H. F. BAUER, T. M. BÖCKERS, M. SCHÖN;
Inst. for Anat. and Cell Biol., Univ. of Ulm, Ulm, Germany

Abstract: A thorough characterization of mouse models for genetic diseases and/or syndromes is a necessity to better understand the pathophysiology of genetically defined disorders. Moreover, finding suitable testing methods as therapy readouts and reducing animal testing in pre-clinical trials is needed. In this study, we performed a comprehensive behavioral characterization of a *Shank3* mouse model (exon 11 deletion). Heterozygous SHANK3 loss is thought to be the major cause for signs and symptoms of individuals with the Phelan-McDermid syndrome. Those impairments range from profound global developmental delay, muscular hypotonia, language and communication impairments, autism spectrum disorders and several other comorbidities. In a broad series of behavioral experiments, the social and communication behavior, cognition, repetitive behavior, anxiety, and motor skills were investigated in adolescent and in adult male and female mice. Regarding the motor functions, the transgenic knock-out animals showed severe impairments in motoric endurance and coordination, while muscle strength was not altered. The Open Field test indicated that knock-out mice are hypoactive and exhibit elevated anxiety levels; interestingly, this was more pronounced at adult age. Furthermore, the mutant animals displayed strong stereotypic and repetitive behaviors, measured by the time spent self-grooming and in the nestlet shredding test; the social interaction and communication was only slightly altered (ultrasonic vocalization, approaching a new animal). In the Barnes Maze test we found no impairment in spatial orientation and memory, however, transgenic animals needed a longer time for the initial test, which might indicate an impairment in cognitive flexibility. Comparing the sexes, we saw the same phenotype in both male and female, but often with a stronger manifestation in males. Heterozygous knock out mice often showed only slight changes of behavior not significantly different from wild type mice. Taken together, we performed an in depth behavioral characterization of *Shank3* knock-out (exon11 deletion) mice during development and established suitable behavioral tests as readouts for further therapeutic interventions.

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Poster

437. Animal Models of Autism: Behavior

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Program #/Poster #: 437.15

Topic: A.07. Developmental Disorders

Support: Institute for Basic Science (BS-R002-D1)

Title: Cell type-specific deletion of Shank3 exons 14-16 in mice differentially affects synaptic and behavioral phenotypes

Authors: T. YOO¹, *H. CHO¹, J. LEE¹, H. PARK¹, Y.-E. YOO¹, E. YANG², J. KIM², H. KIM², E. KIM¹;

¹IBS, Daejeon, Korea, Republic of; ²Korea Univ., Seoul, Korea, Republic of

Abstract: Shank3 is an excitatory postsynaptic scaffolding protein, which has been implicated with multiple brain disorders, including autism spectrum disorders and Phelan-McDermid syndrome. Many *Shank3*-mutant mice studies have been reported, but it remains largely unclear how cell type-specific *Shank3* deletion affects disease-related phenotypes in *Shank3*-mutant mice. Here, we generated *Shank3*-mutant mice with exon 14-16 deletion to validate cell type-specific roles of Shank3. Excitatory (glutamatergic) and inhibitory (GABAergic) cell type-specific *Shank3*-mutant (exon 14-16) mice displayed distinct synaptic and behavioral phenotypes. Both glutamatergic and GABAergic *Shank3*-mutant (exon 14-16) mice displayed increased social interaction and increased repetitive behaviors, similar to global *Shank3*-mutant (exon 14-16) mice. However, in the case of social communication and movement, only GABAergic *Shank3*-mutant mice recapitulated the phenotypes of global *Shank3*-mutant; decreased social communication and hypoactivity. In addition, the decreased excitatory synaptic transmission in the dorsolateral striatum of global *Shank3*-mutant mice was well recapitulated in GABAergic but not glutamatergic *Shank3*-mutant mice. Our results suggest GABAergic neuron-specific Shank3 deletion has stronger impacts on phenotypes relative to glutamatergic neuron-specific Shank3 deletion.

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Poster

437. Animal Models of Autism: Behavior

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Topic: A.07. Developmental Disorders

Support: Simons Foundation Autism Research Initiative Bridge to Independence Award
385188
NICHD P50 HD103573

Title: Early-life sleep disruption potentiates lasting sex-specific behavioral divergence in a Shank3 autism model mouse

Authors: *G. H. DIERING¹, J. S. LORD¹, S. M. GAY², K. M. SMITH³, K. M. HARPER⁴, V. D. NIKOLOVA⁴, S. S. MOY⁵;

¹Cell Biol. and Physiol., Univ. of North Carolina - Chapel Hill, Chapel Hill, NC; ²Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; ³Cell Biol. and Physiol., ⁴Psychiatry, Univ. of North Carolina Chapel Hill, Chapel Hill, NC; ⁵Psychiatry, Univ. of North Carolina at Chapel Hill Sch. of Med., Chapel Hill, NC

Abstract: Introduction: Patients with autism spectrum disorder (ASD) experience significantly elevated rates of sleep disruption beginning early in life, however the developmental consequences of this disruption are not understood. We examined sleep behavior and the consequences of sleep disruption in developing mice bearing C-terminal truncation mutation in the high-confidence ASD risk gene SHANK3 (Shank3 Δ C). We hypothesized that sleep may be an early sign of developmental divergence, and that the clinically-relevant *Shank3*^{WT/ Δ C} mouse might be at increased risk of deleterious outcomes following early-life sleep disruption. Methods: We recorded sleep behavior in developing *Shank3* ^{Δ C/ Δ C}, *Shank3*^{WT/ Δ C}, and wild type siblings of both sexes using a non-invasive home cage monitoring system. Separately, litters of *Shank3*^{WT/ Δ C} and wild type littermates were exposed to automated mechanical sleep disruption for 7 days prior to weaning (early-life sleep disruption: ELSD) or post-adolescence (PASD) or control (CON) conditions. All groups underwent standard behavioral testing as adults. Results: Male and female *Shank3* ^{Δ C/ Δ C} mice slept significantly less than wild type and *Shank3*^{WT/ Δ C} siblings as early as we could measure, with increasing sleep fragmentation in adolescence. ELSD treatment interacted with genetic vulnerability in *Shank3*^{WT/ Δ C} mice, resulting in lasting and sex-specific changes in behavior, whereas wildtype siblings were resilient to these effects. Male ELSD *Shank3*^{WT/ Δ C} subjects demonstrated significant changes in sociability, sensory processing, and locomotion, while female ELSD *Shank3*^{WT/ Δ C} subjects had a significant reduction in risk aversion. CON *Shank3*^{WT/ Δ C} mice, PASD mice, and all WT mice demonstrated typical behavioral responses. Conclusion: Our study shows that sleep disruption during sensitive periods of early life can interact with underlying genetic vulnerability to drive lasting and sex-specific changes in behavior. As individuals progress through maturation they gain resilience to the lasting effects of sleep disruption. This work highlights developmental sleep disruption as an important vulnerability in ASD susceptibility.

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Poster

437. Animal Models of Autism: Behavior

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Topic: A.07. Developmental Disorders

Support: NS118026

Title: Disruption of circadian rhythms by ambient light during neurodevelopment leads to autistic-like molecular and behavioral alterations in adult mice

Authors: *R. SINGLA, D. LIU, S. S. PATHAK, R. CAO;
Dept. of Biomed. Sci., Univ. of Minnesota, Duluth, MN

Abstract: Although circadian rhythms are thought to be essential for maintaining body health, the effects of chronic circadian disruption during neurodevelopment remain elusive. Here, using the “Short Day” (SD) mouse model, in which an 8 h/8 h light/dark (LD) cycle was applied from embryonic day 1 to postnatal day 42, we investigated the molecular and behavioral changes after circadian disruption in mice. Adult SD mice fully entrained to the 8 h/8 h LD cycle, and the circadian oscillations of the clock proteins, PERIOD1 and PERIOD2, were disrupted in the suprachiasmatic nucleus and the hippocampus of these mice. By RNA-seq widespread changes were identified in the hippocampal transcriptome, which are functionally associated with neurodevelopment, translational control, and autism. By western blotting and immunostaining hyperactivation of the mTOR and MAPK signaling pathways and enhanced global protein synthesis were found in the hippocampi of SD mice. Electrophysiological recording uncovered enhanced excitatory, but attenuated inhibitory, synaptic transmission in the hippocampal CA1 pyramidal neurons. These functional changes at synapses were corroborated by the immature morphology of the dendritic spines in these neurons. Lastly, autistic-like animal behavioral changes, including impaired social interaction and communication, increased repetitive behaviors, and impaired novel object recognition and location memory, were found in SD mice. Together, these results demonstrate molecular, cellular, and behavioral changes in SD mice, all of which resemble autistic-like phenotypes caused by circadian rhythm disruption. The findings highlight a critical role for circadian rhythms in neurodevelopment.

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Poster

437. Animal Models of Autism: Behavior

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Topic: A.07. Developmental Disorders

Support: NSF NCS Foundations Award 1926818
Portland VA Research Foundation

Title: Effects of donepezil on sleep, social behavior, and cognition in a prairie vole model of autism spectrum disorder

Authors: *K. R. GUTOWSKY¹, P. WICKHAM¹, C. J. TINSLEY^{1,2}, N. E. MILMAN^{1,2}, H. CAO³, M. M. LIM^{1,2};

¹Veterans Affairs Portland Hlth. Care Syst., Portland, OR; ²Oregon Hlth. and Sci. Univ., Portland, OR; ³Univ. of California, Irvine, Irvine, CA

Abstract: Autism spectrum disorder (ASD) is characterized by sleep disruption and difficulty with social interactions. Limited research reports that the use of donepezil, an acetylcholinesterase inhibitor, in children with ASD improved their REM (rapid-eye movement) sleep quality, but less is known about its impact on social behavior. Prairie voles (*Microtus ochrogaster*) are a highly social rodent species that forms lifelong pair bonds with a mate. Previous research with prairie voles has shown that early-life sleep disruption (ELSD) during post-natal days 14-21 recapitulates some aspects of ASD in adult prairie voles, including reduced huddling with a pair bonded partner, decreased REM sleep duration, and reduced cognitive flexibility. Daily injections with donepezil (i.p., 0.3 mg/kg) led to increased social huddling behavior compared to saline injected animals. The effects of donepezil on sleep were measured using EEG/EMG electrodes and the effects on cognitive flexibility were measured using cued fear conditioning and extinction. Further understanding of how donepezil can be used to treat autism-like behaviors in a prairie vole model may lead to its usage as an early intervention in young children first showing signs of ASD.

Disclosures: **K.R. Gutowsky:** None. **P. Wickham:** None. **C.J. Tinsley:** None. **N.E. Milman:** None. **H. Cao:** None. **M.M. Lim:** None.

Poster

437. Animal Models of Autism: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 437.19

Topic: A.07. Developmental Disorders

Support: NSF NCS Foundations Award 1926818
Portland VA Research Foundation
ARCS Scholarship

Title: Persistent effects of early life sleep disruption in prairie voles on REM sleep time are sex specific and age dependent

Authors: *N. E. P. MILMAN¹, R. J. OLSON¹, C. E. JONES-TINSLEY^{1,2}, C. WONG², P. T. WICKHAM², K. R. GUTOWSKY², H. CAO³, M. M. LIM^{1,2};

¹Oregon Hlth. and Sci. Univ., Portland, OR; ²VA Portland Hlth. Care Syst., Portland, OR;

³Electrical Engin., Univ. of California Irvine, Irvine, CA

Abstract: In mammals, rapid eye movement (REM) sleep duration is highest in the early postnatal period of life and is hypothesized to play a role in shaping neural circuits that control the development of complex behaviors. Sleep problems in early childhood coincide with features of autism spectrum disorder (ASD) and wakeful brain activity in the first year of life can distinguish ASD outcomes (Verhoeff et al, 2018; Gabard-Durnam et al, 2019). Prairie voles (*Microtus ochrogaster*) are a wild, highly social rodent that serves as a unique model for the study of species-typical social behaviors, including pair bonding as assessed through partner

preference testing. Previous work in our laboratory has found that early life sleep disruption (ELSD) in prairie voles during a sensitive window of postnatal development (P14-21) leads to long lasting changes in social behaviors as well as structural changes in excitatory and inhibitory neural circuits in the brain (Jones et al, 2019; Jones et al, 2021). To characterize persistent changes in sleep architecture following ELSD, we conducted 24 hours of home cage tethered EEG/EMG recordings at two timepoints relevant to ASD pathophysiology, early adolescence (P28-P31) and adulthood (P91-121). EEG/EMG signals were converted to European Data Format before being scored offline for non-REM and REM sleep and Wake in 4-second epochs across the full 24-hour period (SleepSign, Kissei Comtec). Spectral analysis was performed on each 4s epoch by applying a fast Fourier transform (FFT) using a Hanning window to generate a power spectrum at a resolution of 0.98 Hz. Adult voles spent less time in REM sleep than adolescent voles ($p < 0.001$) and female voles spent more time in REM sleep than males ($p < 0.001$). Adult ELSD males spent less time in REM sleep compared to adult Control males ($p = 0.002$), an effect that was not present in females of any age (adolescents: $p = 0.979$; adults: $p = 0.907$). Defining the developmental trajectory of sleep deficits in the prairie vole ELSD model is an important context with which to further study the importance of early life sleep in shaping social behaviors in adulthood.

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Poster

437. Animal Models of Autism: Behavior

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Topic: A.07. Developmental Disorders

Support: NSF NCS Foundations Award 1926818
Portland VA Research Foundation
R01MH126137

Title: Reduced social interest in early life sleep-disrupted prairie voles as revealed by computer vision

Authors: *L. S. BUENO-JUNIOR¹, C. E. JONES-TINSLEY^{2,3}, P. T. WICKHAM^{2,3}, B. O. WATSON¹, M. M. LIM^{2,3};

¹Dept. of Psychiatry, Univ. of Michigan Med. Sch., Ann Arbor, MI; ²Veterans Affairs Portland Healthcare Syst., Portland, OR; ³Dept. of Neurol., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Prairie voles (*Microtus ochrogaster*) are a highly social, monogamous species that shows lifelong pair bonds with opposite sex adult mates. We previously showed that early life sleep disruption (ELSD) in prairie vole pups during the third postnatal week results in sex-specific impairments in pair bonding later in life, as evidenced in males by both reduced

huddling with and preference for female partners, during the laboratory-based partner preference test as a proxy for pair bond formation (Jones et al., 2019, *Sci Adv* 5). However, because this method relies on manual behavioral scoring and arbitrary thresholding of “social zones” in the cage, it provides an incomplete behavioral picture. To improve this, here we validate a method to monitor male-female cohabitation in a temporally and spatially continuous manner. The method involves overhead video recording of male-female pairs for 72 h while they interact through mesh dividers, followed by markerless tracking (DeepLabCut, Nath et al., 2019, *Nat Protoc* 14) and custom analysis. Initial variable derivation from this very rich dataset yielded three variables: male-female distance, body direction relative to the partner, and locomotion speed. All of these are highly uniform across recordings, and with temporal resolution as high as the video frame rate (e.g., 20 Hz). Using this method, we again returned to examine in more detail the effects of ELSD on pair bonding behavior in adult prairie voles. We found changes in male behavior even as early as during the 72 h cohabitation, with Male-ELSD animals less likely to engage in social behavior compared to other groups (Male-Control, Female-ELSD, Female-Control). In addition, we were able to map our variables onto circadian time, revealing ultradian fluctuations in the incidence of social behaviors. Future studies could use this method in conjunction with electrophysiology to examine neural correlates of social deficits in a targeted manner.

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Poster

437. Animal Models of Autism: Behavior

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Program #/Poster #: 437.21

Topic: A.07. Developmental Disorders

Support: NC3Rs PhD studentship (DM: NC/S001522/1)
NIH (mGAP: R24OD021324)

Title: A translational primate model of autism spectrum disorder: phenotypic traits in social and predictive behaviour and patterns of intolerance to mutation in macaque genome

Authors: M. WOODBURY-SMITH^{1,2}, D. MASSEY^{1,3}, B. SLATER¹, C. WITHAM³, S. WELLS^{4,3}, *Y. KIKUCHI¹;

¹Biosci. Institute, Newcastle Univ., Newcastle Upon Tyne, United Kingdom; ²Cumbria, Northumberland, Tyne and Wear NHS Trust, Newcastle upon tyne, United Kingdom; ³Ctr. for Macaques, MRC Harwell Inst., Salisbury, United Kingdom; ⁴Mary Lyon Centre, MRC Harwell Inst., Harwell, United Kingdom

Abstract: Autism Spectrum Disorder (ASD) is a clinically defined early-onset lifelong neurodevelopmental disorder that affects social cognitive and communicative abilities. A recent

'predictive coding' hypothesis has been advanced to explain another facet of ASD: difficulties managing uncertainty. However, to fully investigate this hypothesis, a primate model that integrates gene-brain-behavioural axes of direct relevance to human ASD would be beneficial. To this end, we characterised phenotypic behaviours in juvenile male macaques ($n = 36$) at a UK breeding colony using the recently developed macaque version of the Social Responsiveness Scale Revised, mSRS-R (Feczko et al., 2016; Talbot et al., 2020). We identified a distribution of mSRS that was largely consistent with the data shown by Talbot and colleagues, including identifying low- and high-scoring animals at the ± 1.5 SD thresholds. In parallel, we have developed an automated home cage training procedure to assess macaque predictive ability using the probabilistic audio-visual association task similar to the ones tested on individuals with ASD ($n = 2$). Our pilot data have shown that macaques engage in predictive learning and update their prediction errors based on a Bayesian model similar to humans. Neurobiologically, our previous studies established macaque and human parallels in neurophysiological signals involved in prediction and prediction error signalling (Kikuchi et al., 2017; 2018). Genetically, there is now a robust rhesus reference genome (MMul_10) available which provides a framework for evaluating mutational characteristics among ASD-implicated genes in humans. We used the macaque Genotype and Phenotype (mGAP) resource (v2.0), to examine genetic variations in 18,168 autosomal genes of 2,054 macaques to test whether the gene sets associated with ASD are evolutionary constraint in macaque genomes. After demonstrating that genetic constraint by way of residual variation intolerance scores (RVIS) are correlated across all the genes tested between humans and macaques ($r = 0.42$, $p = 2.2e-16$), we identified greater constraint in genes implicated in ASD (RVIS = -0.32). Furthermore, Gene Set Enrichment analysis has revealed that ASD genes were over-represented among the top 2% of constrained genes ($p < 9.6 \times 10^{-13}$). Despite this, macaques also harboured predicted damaging mutations among ASD genes (823 variants, 76.8%). Our genetics and behavioural findings highlight the importance of this species as a prominent animal model to understand neurobiological underpinnings of ASD.

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Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.01

Topic: A.09. Adolescent Development

Support: Canadian Institute of Health Research (CIHR)
Natural Sciences and Engineering Research Council (NSERC)
Canada First Research Excellence Fund (CFREF) awarded to BrainsCAN at Western University

Title: Adolescent Δ -9-tetrahydrocannabinol exposure in female rats does not lead to long-lasting memory impairments, anxiety, and schizophrenia-related phenotypes

Authors: *M. DE FELICE¹, H. J. SZKUDLAREK¹, T. C. UZUNESER¹, S. R. LAVIOLETTE²;

¹Dept. of Anat. and Cell Biol., ²Dept. of Anat. and Cell Biology; Dept. of Psychiatry; Lawson Hlth. Res. Inst., Univ. of Western Ontario, London, ON, Canada

Abstract: Growing evidence has shown that the pathological outcomes associated to chronic cannabis use can be remarkably different between sexes. In particular, preclinical studies demonstrated that sustained exposure to the main psychoactive component Δ -9-tetrahydrocannabinol (THC) impacts the functionality of the cannabinoid receptors 1 (CB1Rs) in females differently than males, leading to a wide array of detrimental consequences. In fact, adolescent THC exposure has been found to induce sex-specific pathological phenotypes, highlighting a greater susceptibility to emotional dysregulations in females and disruptions of selective memory processing. While our laboratory has previously reported THC-related neuropsychiatric-like symptoms and profound neural abnormalities in male rats, in this study we investigated whether chronic THC exposure during adolescence in females affected their cognitive performance as well as induced anxiety- and/or schizophrenia-like phenotypes. Adolescent female rats were treated from postnatal day (PND) 35 to 45 with increasing doses of THC (PND 35-37 2.5 mg/kg, PND 38-41 5 mg/kg, PND 42-45 10 mg/kg, i.p., twice a day) or vehicle. At adulthood (PND 75), a battery of behavioral tasks was carried out to assess schizophrenia-like manifestations, memory impairments, and anxiety. We report that adolescent female rats chronically exposed to THC, gained weight slower than their vehicle counterparts throughout the drug administration. However, this difference in body weight was fully restored into adulthood. In contrast to previous findings in males, preliminary data suggests that adolescent THC exposure did not affect the locomotor behaviour, neither increased anxiety levels in females. In addition, THC-treated female rats did not exhibit any cognitive impairments in a variety of memory tasks neither showed sensorimotor gating deficits. Taking advantage of in vivo electrophysiological recordings, we are currently examining the neural activity of glutamatergic cells in hippocampus, which has been identified as a brain area profoundly impacted by chronic THC exposure in clinical and preclinical findings. Specifically, we are focusing our investigations on the dorsal and ventral portions of the hippocampus, two sub-regions mainly involved in memory and emotional processing, respectively. Our results provide evidence that the long-term effects of adolescent THC exposure in females are different than those previously observed in males. Whether the stages of female's estrous cycle played a role in our findings, or they are related to sex differences in neurobiological mechanisms require further investigations.

Disclosures: M. De Felice: None. H.J. Szkudlarek: None. T.C. Uzuneser: None. S.R. Laviolette: None.

Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.02

Topic: A.09. Adolescent Development

Title: Adolescent high-fructose corn syrup consumption contributes to protracted dysregulation of adult accumbal neuroinflammation and affective behaviors.

Authors: M. COLON¹, B. PIKE¹, T. ABAH¹, B. FLOYD¹, *J. L. SANTERRE-ANDERSON²;
¹King's Col., Wilkes-Barre, PA; ²Kings Col., Kings Col., Wilkes Barre, PA

Abstract: Adolescence is an important period of development, in which rapid changes and maturation occur in the brain and body. High fructose corn syrup (HFCS) is highly consumed among children and adolescents in the United States, and our lab recently has shown that adolescent HFCS overexposure can cause protracted alterations in adult behaviors and accumbal protein expression. The purpose of this study is to determine whether neuroinflammation may contribute to previously observed behavioral changes. First, the current study aimed to characterize pro-inflammatory cytokines in the nucleus accumbens (NAc), specifically c-reactive protein (CRP) and interleukin-6 (IL-6), to determine both chronic and protracted alterations in both male and female Sprague-Dawley rats. Additionally, this study mechanistically assessed how neuroinflammation modulates HFCS-induced behavior deficits by administering minocycline (MIN) in tandem with HFCS. Minocycline is an antibiotic drug that has been found to inhibit inflammatory factors at the doses utilized in this study. For all studies, rats received either 11% HFCS or water beginning in adolescence, postnatal day 21 (P21). For behavioral testing, MIN or vehicle was also added to drinking bottles. Following adolescence, all rats were allowed to age until adulthood without exposure to added sugars or MIN. Testing and sample collection were conducted when rats reached adulthood (P75). Depressive-like behaviors were observed through immobility time during the forced swim test (FST) and anxiety-like behaviors and impulsivity were observed on an elevated plus maze (EPM). In opposition to the original hypothesis, CRP decreased at P35 and P54 and continued to decrease into adulthood in male rats. No protracted changes in CRP were observed in female rats. Surprisingly, IL-6 decreased at P35 but began to increase at P54 and continued to increase long-term into adulthood. MIN counteracted the depressive-like behaviors induced by adolescent HFCS consumption but was unable to prevent HFCS-dependent changes in impulsivity on the EPM. Ongoing studies are assessing changes in inflammatory proteins and activity following a stress-challenge to further delineate the role of neuroinflammation in HFCS-dependent behavioral deficits.

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Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.03

Topic: A.09. Adolescent Development

Title: Adolescent cannabis vapor exposure affects functional brain connectivity and Pavlovian sign tracking in adult rats

Authors: J. N. SMITH¹, P. MCCUNN¹, H. KAYIR¹, *P. E. MARINHO², J. KHOKHAR^{2,1};
¹Univ. of Guelph, Guelph, ON, Canada; ²The Univ. of Western Ontario, London, ON, Canada

Abstract: There has been an increase in cannabis-derived vaping product use in adolescents. As cannabis legalization becomes more widespread, the availability of compounds with different concentrations of THC and CBD are increasingly available. Even though THC and CBD are reported to have different psychomotor, cognitive, and behaviour effects, there is a lack of studies investigating their effects on brain structure and function throughout development. The aim of this study is to investigate the long-term effects of cannabis exposure during adolescence on brain development through magnetic resonance imaging (MRI) and behavioral tests. Sprague Dawley rats were divided into three groups (7/group) and chronically exposed to either cannabidiol (CBD), D-9-tetrahydrocannabinol (THC), or balanced CBD + THC at post-natal days (PND) 28-42 via a vapor route (using a volcano vaporizer). Blood samples were collected pre-and post-administration on the final day of cannabis exposure (day 14) to check plasma cannabinoid concentration. On day 7 and day 14 of exposure, rats underwent a cannabinoid tetrad test (e.g., locomotor activity, tail-flick analgesia, rectal temperature, and catalepsy). Upon reaching adulthood (PND68 onward), all rats underwent 18 days of Pavlovian sign-tracking followed by diffusion and functional MRI analysis. Results indicated a statistical difference in the plasma cannabinoid concentrations in all groups when comparing pre-and post-exposure samples. In the cannabinoid tetrad test, rats in the CBD group showed a significant increase in locomotor activity immediately following exposure compared to baseline locomotor measurements. Tail flick analgesia showed a significant decrease for both the CBD and CBD + THC exposed groups 2 hours following exposure, compared to baseline measurements during adolescence. When analyzing the results of Pavlovian learning, there were significant differences between all 3 exposure groups in lever pressing (sign tracking) with both THC groups showing a higher propensity to sign track than the CBD exposed group. Finally, regarding MRI data, in the THC-exposed group, network-based statistics revealed a single network with reduced functional connectivity ($p = 0.009$, 28 edges, 28 nodes). The increased propensity to sign-track, which has previously been linked to increased substance use, in the THC-exposed animals as well as reduced functional connectivity highlights the potential long-term effects of adolescent cannabis exposure, and the importance of assessing the impact of different cannabis constituents on these effects.

Disclosures: J.N. Smith: None. P. McCunn: None. H. Kayir: None. P.E. Marinho: None. J. Khokhar: None.

Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.04

Topic: A.09. Adolescent Development

Support: Grinnell College

Title: Acute corticosterone in juvenile male rats increases spine density in the basolateral amygdala but not orbitofrontal cortex

Authors: N. SCHLEGEL, J. XIONG, C. RHOADS, *N. REMPEL-CLOWER;
Grinnell Col., Grinnell, IA

Abstract: Exposure to stress hormone corticosterone (CORT) in rodents alters neuronal morphology in regions implicated in psychiatric disorders, including anxiety. In particular, the basolateral amygdala and orbitofrontal cortex respond to stress or CORT administration with changes in dendritic morphology and spine density. Both acute and chronic stress increase spine density in the basolateral amygdala (BLA), and chronic CORT exposure has been associated with long-lasting dendritic spine loss in the orbitofrontal cortex (OFC). These effects vary based on the developmental period of the exposure and by the duration after exposure or the age at which these changes are measured. In this study, we examined whether acute CORT exposure in male juvenile rats alters spine density in BLA and OFC pyramidal neurons at two later time points near the onset of puberty, and compared this with the effect of acute CORT exposure during mid-adolescence prior to puberty. Male juvenile Sprague-Dawley rats were given 10 mg/kg CORT or saline control injections at postnatal day 28 (P28). Using Golgi-Cox stained material, dendritic spine density was evaluated in pyramidal neurons in the BLA and the lateral OFC at two later ages during mid-adolescence, P41 and P49, ages immediately before and after typical onset of puberty for male rats. CORT or saline control injections were also administered to male rats early in the mid-adolescent developmental period at P36, with spine density evaluated at P49. In a comparison of all three groups, acute CORT increased BLA dendritic spine density. In the OFC, acute CORT administration did not change spine density in either the apical or basal dendrites. In the basal dendrites, spine density increased with age. Our findings suggest that the male juvenile BLA is responsive to brief exposure to high levels of glucocorticoids, whereas the OFC appears to require later or longer exposure to alter dendritic spine density. These results have implications for region-specific stress sensitivity during adolescent development.

Disclosures: N. Schlegel: None. J. Xiong: None. C. Rhoads: None. N. Rempel-Clower: None.

Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.05

Title: WITHDRAWN

Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.06

Topic: A.09. Adolescent Development

Support: NIH Grant R01-MH086507
NIH Grant R01-MH127850

Title: Early-life adversity disrupts prefrontal GABAergic maturation during adolescence

Authors: ***E. ARTUR DE LA VILLARMOIS**¹, **E. FLORES-BARRERA**¹, **H. BRENHOUSE**², **K.-Y. TSENG**¹;

¹Anat. and Cell Biol., Univ. of Illinois at Chicago- Col. of Med., Chicago, IL; ²Psychology, Northeastern Univ., Boston, MA

Abstract: Early life experiences have been known to impact the development and maturation of corticolimbic connectivity and its regulation of cognitive and affective behaviors. For instance, individuals with a history of early life trauma are often at higher risk for anxiety-like disorders in adolescence, which are thought to result from a dysregulation of affective and cognitive processes, and the maturation of the prefrontal cortex (PFC) and associated neural circuits. It is therefore conceivable that early life events contribute to shape the developmental trajectory of PFC maturation during adolescence. To test this idea, we implemented an early-life adversity paradigm (i.e., maternal separation, MS) and assessed to what extent PFC maturation of synaptic activity from postnatal day (P) 30 to adulthood becomes disrupted in both male and female rats. Data revealed that the normal developmental gain of PFC GABAergic synaptic activity was not observed in animals exposed to MS, while AMPA-mediated transmission was not affected. As a result of the selective GABAergic disruption in MS animals, the excitatory-inhibitory (E-I) ratio that normally becomes balanced by P50 remains unbalanced in adulthood (e.g., > 1.0), resembling the level of E-I synaptic activity typically found in the immature PFC of P30-40 rats. Furthermore, transient chemogenetic inhibition of PFC GABA interneurons during this early adolescent period was sufficient to elicit a similar E-I imbalance that endures through adulthood. Collectively, our findings indicate that PFC inhibitory synapses are preferentially susceptible to early life adversity, likely through disruption of an activity dependent mechanism that is needed to enable the GABAergic maturation during adolescence. In turn, a disinhibited PFC state could increase the risk for psychiatric disorders during adolescence with abnormal affective and cognitive responses.

Disclosures: **E. Artur De La Villarmois:** None. **E. Flores-Barrera:** None. **H. Brenhouse:** None. **K. Tseng:** None.

Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.07

Topic: A.09. Adolescent Development

Support: P60AA011605
T32 grant AA007573

Title: Increases in perineuronal net density following adolescent intermittent ethanol (AIE) exposure is region- and sex-specific in adult rats.

Authors: *C. DANNENHOFFER¹, A. GOMEZ-A¹, V. A. MACHT², B. SUTHERLAND¹, R. P. VETRENO³, F. T. CREWS⁴, C. A. BOETTIGER⁵, D. L. ROBINSON⁶;

¹UNC Chapel Hill, Chapel Hill, NC; ²Bowles Ctr. for Alcohol Studies, Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; ³Dept. of Psychiatry, Sch. of Med., ⁴Prof Pharmacol & Psychiat, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; ⁵Psychology & Neurosci., Univ. of North Carolina, Chapel Hill, NC; ⁶Bowles Ctr. for Alcohol Studies, Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: **Increases in perineuronal net density following adolescent intermittent ethanol (AIE) exposure is region- and sex-specific in adult rats.** CA Dannenhoffer, A Gómez-A, VA Macht, EB Sutherland, RP Vetreno, FT Crews, CA Boettiger, DL Robinson
Adolescent intermittent ethanol (AIE) exposure may cause long-term impairments in cognitive flexibility, or the ability to adapt to changing circumstances. One potential mechanism underlying behavioral flexibility deficits is perineuronal net (PNN) density surrounding parvalbumin-positive (PV+) interneurons. PNNs regulate the intrinsic excitability of PV neurons, and PV neurons contribute to neural synchrony across brain regions necessary for flexible behaviors. This study expands our previous findings that AIE exposure increases PNN density around PV+ neurons in males across multiple brain regions in the prefrontal cortex (PFC). The orbitofrontal cortex (OFC) is implicated in reversal learning strategy while the medial PFC (mPFC) mediates set-shifting ability, both of which are distinct types of cognitive flexibility. We have previously found that AIE exposure impairs reversal learning strategy, but not set-shifting ability, in adult rats, and this is mediated in part by reduced frontostriatal connectivity. In the present experiment, tissue was collected from 40 male and female subjects that underwent behavioral flexibility testing after AIE exposure or water control, and co-immunofluorescence was conducted to visualize PV interneurons and PNNs (*Wisteria floribunda* agglutinin; WFA). In the OFC of adult males, we found significant increases in WFA binding expression ($t(1, 17) = 5.537$, $p < 0.001$), and specifically WFA binding surrounding PV neurons ($t(1, 17) = 2.934$, $p < 0.01$). Interestingly, this effect of AIE exposure did not extend to females, and future work will examine potential mechanisms for sex differences. Within the mPFC, we did not find AIE-related changes in WFA binding, or PV expression; however, some sex differences were observed. Taken together, these findings suggest that AIE exposure may produce region-specific changes in PNN and PV co-expression following behavioral flexibility challenges, which corresponds with evidenced reversal learning (OFC-dependent) deficits, but not set-shifting (mPFC-dependent) deficits in male but not female rats. Future directions will include correlations among frontostriatal connectivity, behavior, and WFA binding expression. Overall,

these data will further elucidate cellular and extracellular mechanisms that contribute to behavioral flexibility following adolescent alcohol exposure.

Disclosures: C. Dannenhoffer: None. A. Gomez-A: None. V.A. Macht: None. B. Sutherland: None. R.P. Vetreno: None. F.T. Crews: None. C.A. Boettiger: None. D.L. Robinson: None.

Poster

438. Adolescent Mechanisms of Vulnerability

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Program #/Poster #: 438.08

Topic: A.09. Adolescent Development

Support: NIAAA- F31AA02930
NIAAA-U01AA019972
NIAAA- T32AA025606

Title: Adolescent intermittent ethanol exposure: Sex-specific social impairment and involvement of the prelimbic cortex

Authors: *T. TOWNER¹, E. I. VARLINSKAYA², D. F. WERNER³;
²Dept Psychol, ³Psychology and Behavioral Neurosci., ¹Binghamton Univ., Binghamton, NY

Abstract: Adolescents often consume ethanol in high quantities, referred to as binge and/or high-intensity drinking. Our lab has repeatedly shown that adolescent intermittent ethanol (AIE) exposure in rats leads to sex-specific social impairment. We recently found that neuronal activation of the prelimbic cortex (PrL) positively correlated with social behavior in control males, with this relationship not evident in AIE-exposed males. The current study sought to further assess the contribution of PrL activity to AIE-induced social deficits. First, we aimed to further assess PrL activation following exposure to a social stimulus between male control and AIE-exposed rats. To do so, we exposed male and female cFos-LacZ rats to water (control) or ethanol (4 g/kg, 25% w/v) via intragastric gavage every other day between postnatal day (P) 25 and 45 (total 11 exposures). In adulthood (P70), rats were socially tested, and brain tissue collected for assessment of social stimulus-induced neuronal activation through the cFos proxy β -galactosidase (β -gal). We found that PrL β -gal expression was elevated in socially tested rats relative to home cage controls regardless of sex, however differences in social stimulus-induced β -gal expression between controls and AIE-exposed rats were evident only in males. More specifically, AIE-exposed males had lower PrL neuronal activation than their water-exposed counterparts. The second goal of this study was to determine the functional role of the PrL in AIE-induced social deficits through chemical genetic inactivation of this region. Male and female cFos-LacZ rats exposed to water or AIE underwent PrL cannulation surgery in adulthood. Following an initial social interaction, we inactivated recently stimulated neurons in the PrL through injection of the prodrug Daun02. In cFos-LacZ rats, Daun02 is converted to

daunorubicin in cells expressing β -gal (cFos proxy) leading to the inactivation of only recently activated cells. Preliminary results suggest that inactivation of PrL neurons previously activated by a social stimulus led to alterations that were dependent upon the specific social behavior assessed. Collectively, these data highlight the contribution of the PrL to social deficits induced by AIE, however more information is needed to gain insight into the specific mechanisms within the PrL that are disrupted by AIE exposure.

Disclosures: T. Towner: None. E.I. Varlinskaya: None. D.F. Werner: None.

Poster

438. Adolescent Mechanisms of Vulnerability

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Program #/Poster #: 438.09

Topic: A.09. Adolescent Development

Support: NIH Grant OD011132
NIMH Grant MH117103

Title: Developmental isolation imperils social value-based decision-making later in life

Authors: *Y. GARCIA-SIFUENTES^{1,3}, A. ALLEN^{2,3}, H. GIL-HENN⁴, S. L. GOURLEY^{1,3}; ¹Pediatrics, Neurosci. Program, ²Pediatrics, Emory Univ., Atlanta, GA; ³Emory Natl. Primate Res. Ctr., Atlanta, GA; ⁴The Azrieli Fac. of Med., Bar-Ilan Univ., Safed 1311502, Israel

Abstract: Social interactions during adolescence are crucial for proper neurodevelopment and behavior. Accordingly, social isolation during adolescence leads to lasting behavioral consequences. However, how developmental isolation impacts decision-making behavior influenced by social experiences has yet to be comprehensively examined, and underlying mechanistic factors remain unclear. Here, mice were isolated from postnatal day (P) 31-56 and then reintegrated into social groups. We then trained mice to nose poke in operant conditioning chambers for two equally-preferred foods. Then, one food was paired with a novel conspecific, and the other with a novel object. Control mice later responded more for the conspecific-associated food, but this preference was ablated in mice that had experienced adolescent isolation. This pattern suggests that social isolation during adolescence obscures the ability of social experience to incentivize instrumental choice behavior, and that these failures in adaptive choice present despite normalization of the social milieu. In separate mice, we over-expressed the stress-related protein, Proline-rich tyrosine kinase 2 (Pyk2), in the basolateral amygdala, also ablating social incentivization of future choice. These findings generate new leads by which to understand how social isolation during adolescence can derail the ability of social experience to incentivize choice behavior later in life.

Disclosures: Y. Garcia-Sifuentes: None. A. Allen: None. H. Gil-Henn: None. S.L. Gourley: None.

Poster

438. Adolescent Mechanisms of Vulnerability

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.10

Topic: A.09. Adolescent Development

Support: NIH Grant DA036657

Title: Understanding the long-lasting effects of adolescent cocaine exposure on behavioral inhibition in adulthood

Authors: *D. TRAN¹, R. BATISTA-BRITO¹, L. L. SJULSON^{1,2};

¹Neurosci., Albert Einstein Col. of Med. Dominick P. Purpura Dept. of Neurosci., Bronx, NY;

²Psychiatry, Albert Einstein Col. of Med., Bronx, NY

Abstract: Lack of regulation of reward-seeking is a central feature of impulsivity and substance use disorder. The medial prefrontal cortex (mPFC) which matures during adolescence is thought to regulate reward-seeking in part via its projections to nucleus accumbens core and shell (NAcC and NAcS, respectively). Despite multiple studies showing that adolescent cocaine exposure might permanently disrupt mPFC pyramidal activity, contributing to dysregulated reward-seeking behavior in adulthood, it remains unknown whether the behavioral dysregulation caused by adolescent cocaine exposure is due to the disruption of NAcC- and NAcS-projecting prefrontal neurons. In order to address this question, we injected naive wild-type mice with either cocaine or saline during their adolescence. Once they had reached adulthood, we performed a projection-specific anatomical tracing experiment using different retrobeads in combination with c-fos staining to determine whether adolescent cocaine exposure modulates these projection-specific neurons. Consistent with previous studies, our data indicates that NAcC- and NAcS-projecting pyramidal neurons are located within layer 5 of mPFC. We evaluated c-fos differential expression in NAcS- and NAcC-projecting pyramidal neurons, in cocaine-injected animals compared to controls in order to test if adolescent cocaine exposure modulates projection-specific mPFC pyramidal activity differentially. Moreover, we trained both cocaine-injected and saline-injected mice to perform a head-fixed go/no-go task, a behavioral paradigm commonly used to assess the level of behavioral inhibition, and we tested the hypothesis that cocaine-injected mice might have deficits in behavioral inhibition. We will then employ a calcium imaging technique to simultaneously record neuronal activities of NAcC- and NAcS-projecting prefrontal neurons while these animals are performing the go/no-go task. We hypothesize that cocaine-exposed mice have decreased behavioral inhibitory control due to less inhibited NAcS-projecting neurons and more activated NAcC-projecting neurons during reward-seeking behavior. These experimental designs will allow us to elucidate the projection-specific prefrontal mechanism of reward-seeking dysregulation caused by adolescent cocaine exposure.

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Poster

438. Adolescent Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: TRDRP T30FT0967 to VL
DP1 DA039658 and R01 DA051831 to CDF
F31 DA050436 to YS

Title: Cognitive differences in male and female adolescent rats following prenatal exposure to nicotine and THC

Authors: *V. LALLAI¹, L. MANCA¹, Y. SHERAFAT², C. D. FOWLER¹;

¹Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA; ²Col. of liberal art and science, Iowa, IA

Abstract: Exposure to nicotine-containing smoke during pregnancy remains a substantial problem worldwide. Indeed, with the recent escalation in the use of e-cigarettes and the legalization of cannabis, it has become essential to better understand the effects of co-exposure to nicotine and cannabinoids during early stages of development. This study sought to examine the effects of prenatal nicotine and/or THC exposure on cognitive behaviors in male and female offspring. Mothers were exposed to vaporized nicotine, edible THC, or respective vehicle solutions during pregnancy. Following parturition, the mothers were monitored for ten days to examine the potential effects of prior drug exposure on maternal behavior. Thereafter, adolescent offspring were subsequently tested in the prepulse inhibition test, object recognition task, and suppressed feeding task. We found that prenatal nicotine vape exposure resulted in a decreased baseline startle reactivity in adolescent male and female rats, and in females, enhanced sensorimotor gating in the prepulse inhibition test. Further, prenatal nicotine and THC co-exposure resulted in significant deficits in the prepulse inhibition test in males. Deficits in short-term memory were also found in males prenatally exposed to THC, either alone or with nicotine co-exposure, and in females exposed to THC alone. Finally, in males, a modest increase in anxiety-associated behaviors was found with THC or nicotine exposure for the latency to approach novel palatable food. Collectively, our results show that exposure to nicotine, THC, or co-exposure during the early stages of in utero development led to long-term behavioral outcomes in adolescence. These findings have important translational implications given the continued use of nicotine and THC-containing products by pregnant women around the world, and thus, these findings can be applied to support health care and policy efforts limiting nicotine use and THC during pregnancy. This work was supported by grants from the Tobacco-Related Disease Research Program (TRDRP T30FT0967 to VL) and NIH National Institute on Drug Abuse (DP1 DA039658 and R01 DA051831 to CDF, and F31 DA050436 to YS).

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Poster

438. Adolescent Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: NSERC Discovery Grant RG203596-13

Title: Mitigating effects of sex and CB1 antagonism on socioemotional responses and GR and CB1-R expression following a 10-day heterotypic stress exposure in adolescent rats

Authors: *E. PERSON¹, E. DENICOLAIS², V. BOISVERT², H. PLAMONDON³;
¹Psychology, ²Univ. of Ottawa, Ottawa, ON, Canada; ³Dept Psychol, Univ. Ottawa, Ottawa, ON, Canada

Abstract: Recreational drugs of the cannabinoid family have been legalized in many states and countries in the last decade. Notably, research has supported an important role of the endocannabinoid system in regulating anxiety and stress responses, in part through regulation of HPA activity. Although adolescents are among common users of cannabinoids, the role of the endocannabinoid in regulating socioemotional responses has been underexplored in this age group. This study examined the effects of a 10-day heterotypic stress exposure during the adolescence (peri-pubescent) period and its impact on CB1 receptors in mediating socioemotional behaviors and hippocampal glucocorticoid receptor expression. Female and male Wistar rats (N = 64; aged postnatal day [PND] 30) were randomly assigned to exposure to heterotypic stress exposure or a control condition. The 10-day heterotypic stress paradigm alternated between 30 min of restraint stress on even numbered days, and 15 min of forced swim on odd numbered days. Over 10-days (from PND30-40), the experimental groups were exposed to the stressor, while control rats received gentle handling. Afterwards, rats were social interaction and preference was tested on PND42, and fear conditioning in the Y-Maze passive avoidance test (YMPAT) on PND44. One hour prior each behavioral test, half the stress and no stress male and female rats were systemically injected with the CB1 antagonist AM251 (1mg/kg;ip) or saline. Rat brain were collected two days following the last behavioral test and drug injection. Hippocampal and hypothalamic paraventricular nucleus Glucocorticoid receptors (GR) expression was determined along hippocampal Cannabinoid 1 receptor (CB1) expression. Both the sex of the animal and AM251 had a significant effect on sociability, but not on social recognition. AM251 had a significant anxiolytic effect in the Y-Maze. Surprisingly, stress exposure had no impact on GR-ir expression. However, an interaction between AM251 and sex indicated a higher density of GR in the CA1 in AM251-treated females, while AM251-treated males showed elevated CA1-CB1 receptor expression. In sum, whereas stress exposure had no significant effects on brain and behavioral responses, treatment with the cannabinoid receptor antagonist AM251 indicated sex-specific impact on behavioral and neurochemical expression. Together, our findings support a role of the cannabinoid system in regulating socioemotional

behaviors, as well as a differential role of the cannabinoid system in pubescent male and female rats.

Disclosures: E. Person: None. E. Denicolais: None. V. Boisvert: None. H. Plamondon: None.

Poster

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Topic: A.09. Adolescent Development

Support: NIH-R37HD083217

Title: Parental presence modifies novelty induced hippocampus activity across children and rodents

Authors: *R. M. ZANCA^{1,2}, S. STANCIU^{1,3}, B. CALLAGHAN⁴, P. A. SERRANO⁵, N. TOTTENHAM⁶, R. M. SULLIVAN^{1,2};

¹Child & Adolescent Psychiatry, NYU Langone Hlth., New York, NY; ²Nathan Kline Inst., Orangeburg, NY; ³Univ. of Bordeaux, Bordeaux, France; ⁴UCLA, Los Angeles, CA; ⁵Dept of Psychology, Hunter College, CUNY, New York, NY; ⁶Dept. of Psychology, Columbia University, New York, NY

Abstract: Across altricial species, early life experience with the caregiver provides the foundation for neurobehavioral developments. Yet, our understanding of the neurobiology of this relationship remains elusive. Here, capitalizing on the specialized prosocial behaviors evoked by parental presence in altricial species, we compare the hippocampal response to a parent vs. a stranger in children and rodents during early life and again as this parent-infant relationship wanes. With a focus on the hippocampus, a brain area important for social information and memory, we question whether parental presence alters hippocampal activity during exposure to a novel environment. The human data were obtained by placing children between the ages of 5-10 years old and peri-adolescents between the ages of 11-16 years old in an fMRI. Hippocampal activity was measured while pictures of their parent or stranger were presented on a screen in the scanner. For the rodent data, we used infant rat pups during a similar age of robust attachment (12 to 18-days old) and again as pups begin adolescence (23-26-days old) and living independently. The neurobehavioral response to either the mother vs. a stranger (diet-dependent maternal odor altered) or just their odors were presented over 55min and brains were harvested for assessment of neural activity (autoradiography, ¹⁴C 2-DG), 2) and western blot assessment of synaptic D1 dopaminergic receptors (DIR), a neurotransmitter identified as altered by the parent in rodents. For children, a comparison of estimated marginal means of the hippocampal response of children to parental cue compared to stranger showed a robust hippocampal activity decrease, which waned in adolescents. For the rodent data, young pups also showed a robust increased

hippocampal response to the mother compared to a stranger across hippocampal subareas, which waned with maturation. The rodent data also showed significantly increased synaptic D1R expression to the mother. Cross-species analysis illustrated the robust impact of maternal cues on infant neural responses within the hippocampus, a brain area important for emotional regulation and learning. While child and rodent results were opposite, these results suggest that parental cues can serve as a gate for infant information processing of their environment, potentially in a species-specific manner.

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Poster

438. Adolescent Mechanisms of Vulnerability

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Program #/Poster #: 438.14

Topic: A.09. Adolescent Development

Title: Adolescent exposure to low-dose drinking water bisphenol-A alters weight gain, anxiety, and decreases dendritic spine density in the dentate gyrus

Authors: ***R. E. BOWMAN**¹, **O. CHAPONIS**¹, **T. REGALADO**¹, **M. FRANKFURT**²;
¹Sacred Heart Univ., Sacred Heart Univ., Fairfield, CT; ²Hofstra Northwell Sch. of Nursing and Physician Assistant Studies, Hempstead, NY

Abstract: Bisphenol-A (BPA) is an endocrine disruptor that modulates estrogenic, androgenic, and antiandrogenic effects. BPA is extensively used in the manufacturing of hard plastics and detectable levels of BPA have been reported in body fluids of humans and animals indicating that BPA exposure is ubiquitous and has potential health hazards. We have previously examined the effects of BPA exposure during adolescence using a dose of 40/ μ g/kg/day which is lower than what is considered acceptable by the United States Environmental Protection Agency (1993). This dose, delivered via subcutaneous injections during postnatal days 38-49 alters anxiety and cognitive functioning in male and female rats and leads to morphological changes in dendritic spine density. In the current study, effects of low-dose (equivalent of 40/ μ g/kg/day) BPA administered via drinking water to intact adolescent female Sprague-Dawley rats (n=16) was examined. Rats received BPA-treated or control drinking water during postnatal days (PND) 38-49 and were weighed regularly. Both groups were transitioned to non-BPA water on the afternoon of PND 49 and behavior was tested on PND days 49-58. Subjects were sacrificed on PND 59 and brains were processed using standard Golgi techniques. Anxiety was measured on the Elevated Plus Maze and Open Field Results indicate that BPA exposure significantly decreased weight gain across time and increased anxiety as measured on the Elevated Plus Maze. There were no group differences between BPA-treated and control subjects on either the object placement or object recognition tasks. Furthermore, while no group differences in hippocampal CA dendritic spine densities were observed there was a significant decrease in spine density in

dentate gyrus of BPA exposed rats compared to controls. Thus, these results suggest that route of administration appears to have differential effects on physiological and cognitive measures.

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Poster

438. Adolescent Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: The Natural Sciences and Engineering Research Council of Canada (NSERC)
The Canadian Institutes of Health Research (CIHR)
Canada First Research Excellence Fund (CFREF) for BrainsCAN at Western

Title: Investigating the long-term neurodevelopmental effects of adolescent edible Δ^9 -tetrahydrocannabinol (THC) consumption

Authors: M. V. DEVUONO¹, M. H. SARIKAHYA¹, H. J. SZLUDLAREK¹, M. DE FELICE¹, S. R. LAVIOLETTE²;

¹Anat. and Cell Biology, Schulich Sch. of Med. and Dent. Lawson Hlth. Res. Inst., ²Anat. and Cell Biology, Psychiatry, Schulich Sch. of Medicine, Lawson Hlth. Res. Inst., Univ. of Western Ontario, London, ON, Canada

Abstract: Adolescent cannabis use is associated with an increased risk of neuropsychiatric illness later in life. Exposure to the psychoactive component of cannabis, Δ^9 -tetrahydrocannabinol (THC), during this critical period of development can disrupt normal endocannabinoid system function resulting in long-term consequences. The underlying mechanisms of the neurodevelopmental effects of THC are undetermined. Previous research has shown in male rats that injection of high doses of THC during adolescence produces long-term behavioural impairments and promotes hyperactivity of ventral tegmental area (VTA) dopamine neurons and prefrontal cortex (PFC) glutamatergic neurons in adulthood. To expand on this past work and improve face validity, the effects of adolescent edible THC consumption, a popular method of cannabis use by humans, were explored in both male and female subjects. A low and high-dose edible THC regimen was used since high potency cannabis is linked to greater psychiatric risk, and THC is known to produce dose-dependent responses. Adolescent male and female Sprague Dawley rats were given edibles containing increasing doses of THC (1-5 mg/kg) in Nutella® either once (low-dose) or twice (high-dose) daily from postnatal day 35-45. Thirty days after THC exposure, adult rats were administered a battery of behavioural tasks to assess the effects of adolescent THC on anxiety-like behaviour and cognition. One edible/day produced an anxiolytic effect in males and impaired social recognition memory in females. The higher dose regimen (2 edibles/day) produced an anxiety phenotype in males but not females and

impaired temporal order object recognition in both males and females. In vivo electrophysiology was then used to determine changes in PFC glutamatergic and VTA dopaminergic activity to investigate the underlying mechanism of the behavioural changes. Preliminary data suggest that low-dose edible altered neuronal firing in the PFC and VTA of females. However, the high dose of THC increased bursting activity in males' PFC and VTA. The effects of edible THC on oscillatory states in the PFC are currently being investigated. Changes in fatty acid profiles and molecular markers of synaptic plasticity and GABAergic function in the PFC are also being investigated in ongoing experiments to further explore the mechanism of the long-term effect of adolescent edible THC. Overall, edible THC consumption during adolescence produces sex and dose-dependent behavioural effects in adulthood. Changes in neuronal function in the PFC produced by adolescent THC may partially mediate these effects.

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Poster

438. Adolescent Mechanisms of Vulnerability

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.16

Topic: A.09. Adolescent Development

Title: Hormonal contraceptive exposure during adolescence impacts the prefrontal cortex and HPA axis response of female rats.

Authors: *R. GILFARB, M. STEWART, A. RAJESH, S. RANADE, C. DYE, K. M. LENZ, B. LEUNER;
Ohio State Univ., Columbus, OH

Abstract: Hormonal contraceptives (HCs) are synthetic hormones that disrupt endogenous hormone levels. HCs are commonly used during adolescence, which is a period of dramatic neurodevelopment, despite their unknown effects on brain maturation. As prefrontal cortex (PFC) development continues throughout adolescence and can be influenced by hormones, we hypothesized that the PFC may be impacted by adolescent HC exposure. Intact female Sprague-Dawley rats were given daily subcutaneous injections of either vehicle or ethinyl estradiol + levonorgestrel (HC) for 22-23 d beginning on postnatal day 35, thus spanning the duration of adolescence. Using daily vaginal lavage, it was validated that HC treatment disrupted estrous cycling. qPCR analysis showed that HC treatment reduced relative PFC expression of PSD95 (excitatory synapse marker), but not gephyrin (inhibitory synapse marker), suggesting that HC administration during adolescence may preferentially diminish excitatory synapses. As HC use is associated with hypothalamic-pituitary-adrenal (HPA) axis dysregulation in adults and the PFC is involved in HPA axis negative feedback, ELISA was used to quantify corticosterone (CORT) levels following acute restraint stress. HC-treated rats had higher CORT at baseline and 60 min after stress cessation but exhibited a similar increase in CORT 30 min after restraint initiation.

Adrenal weights were also increased in HC-treated females. These data suggest that adolescent HC exposure impacts the HPA axis and excitatory synapses in the PFC, which could have implications for PFC function.

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Poster

438. Adolescent Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: National Sciences and Engineering Research Council (NSERC) of Canada (RG203596-13)

Title: Impact of Adolescent Exposure to Omega-3 or High-Fat Diet on Adulthood Glucocorticoid Receptor and TrkB Expression

Authors: ***M. POITRAS**, A. MORIN, J. RAYMOND, H. PLAMONDON;
Sch. of Psychology, Univ. of Ottawa, Ottawa, ON, Canada

Abstract: **BACKGROUND:** Dietary supplementation is known to affect neuroplasticity, homeostasis, and stress responses. Recent studies support omega 3 polyunsaturated fatty acid supplementation to play a role in regulating brain-derived neurotrophic factor (BDNF) and associated tyrosine kinase B (TrkB) receptors in the brain, while high-fat diets are known to increase the stress response, impacting glucocorticoid receptor (GR) levels. Since most studies assessing such supplementations have focused on younger developmental periods (i.e. perinatal) or adulthood, the goals of this study were to assess changes in TrkB and GR expression in mesolimbic and hippocampal regions following adolescent supplementation. **METHODS:** 24 adolescent male Wistar rats were supplemented from postnatal day (PND) 28 to 47 using either: 1) Hydrogenated vegetable fat (HVF) as high-fat diet; 2) Menhaden fish oil (FO) as a high source of omega-3; or 3) Soybean oil (CSO) as control, balanced diet. Whole brains were extracted on PND 51 and immunofluorescence was used to detect TrkB receptor expression in the CA1, CA3, and dentate gyrus of the hippocampus, and GR expression in the nucleus accumbens shell (NAcS) and core (NAcC). **RESULTS:** Findings indicate decreased levels of TrkB in the CA1 of FO-supplemented rats compared to the CSO-fed group. HVF groups had higher GR expression in the NAcS than other groups. **CONCLUSIONS:** Our results present an interesting contrast with previous studies which found increased TrkB expression in hippocampal regions following omega-3 supplementation. These effects could be linked to the developmental stage during supplementation and highlight the marked effects of diet on maturing brain function.

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Poster

438. Adolescent Mechanisms of Vulnerability

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Program #/Poster #: 438.18

Topic: A.09. Adolescent Development

Title: Adolescent nicotine exposure affects sociability in adults rats in a time-dependent manner

Authors: ***R. BARNET**, I. CROPLEY;
Col. William & Mary, Williamsburg, VA

Abstract: The current study used the three-chamber sociability test in rats, a task that measures anxiety as a reduction in normal prosocial behavior, in order to examine the long-term effects of adolescent nicotine on social anxiety in adulthood. Male and female Sprague-Dawley rats received twice-daily intraperitoneal injections from postnatal day 28-42 (PD28-42), an adolescent period of the rat. Rats received injections of either saline, 0.15 mg/kg nicotine, or 0.40 mg/kg nicotine. In early adulthood at approximately PD60, animals were tested for anxiety in the three-chamber sociability paradigm during a 10-min test session. Time and sex dependent effects were observed. Male rats exposed to 0.40 mg/kg nicotine during adolescence were found to exhibit a biphasic sociability preference during the test in which they had elevated sociability in the first five-minutes of the test session and inhibited sociability in the second five-minutes of the test session. Female rats' sociability behavior was independent of drug condition. The biphasic effect in males may be the result of an interaction between two separate effects of adolescent nicotine exposure, an immediate cognitive deficit that increased social behavior by impairing habituation to novel social partners during the early part of the test session, followed by a delayed anxiogenic effect produced by the long-lasting impact of adolescent nicotine on the fear system.

Disclosures: **R. Barnet:** None. **I. Cropley:** None.

Poster

438. Adolescent Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: NIH Grant AA13023

Title: A ferret model of "binge" alcohol consumption during adolescence

Authors: *M. E. PRUITT, D. KEUM, K. J. PULTORAK, A. TARASIEWICZ, A. E. MEDINA;
Pediatrics, Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Binge drinking is defined as a drinking pattern that results in a blood alcohol concentration of 0.08 g/dL or higher in a 2-hour period. Binge drinking rates are often highest in adolescents. Persistent adolescent binge drinking has unique consequences and can produce social, cognitive, and executive function deficits. Most of what we know about the mechanisms underlying the effects of alcohol exposure during adolescence comes from rodent studies, but these are limited. In rodents, adolescence is very short, only lasting 2-4 weeks, which complicates the study of effects of long-term alcohol consumption targeted to a specific phase of adolescence. Here, we tested a binge drinking paradigm of alcohol consumption in ferrets, a gyrencephalic species whose adolescence spans several months. Each week ferrets (6 males, 6 females) received a bottle of 5% alcohol for 24 hours (no water available). Following this “binge” alcohol only day, ferrets had ad lib access to both water and 5% alcohol (2-bottle choice) for the remaining days of the week. This cycle was repeated for 6-9 weeks during the last half of ferret adolescence. Average alcohol consumption during the entire drinking period was statistically higher ($p < 0.001$) in males (1.4 ± 0.1 g/kg) than in females (0.7 ± 0.1 g/kg). On binge days alone, males (2.1 ± 0.1 g/kg) and females (1.8 ± 0.1 g/kg) consumed similar amounts of alcohol on average. On choice days, males (1.3 ± 0.1 g/kg) consumed a significantly higher ($p < 0.001$) amount of alcohol than females (0.5 ± 0.1 g/kg). On choice days, male consumption mildly increased from the first to last week (1.2 ± 0.5 g/kg to 1.6 ± 0.1 g/kg), but female consumption decreased (0.9 ± 0.3 g/kg to 0.2 ± 0.0 g/kg). On binge days alcohol consumption increased in males (1.7 ± 0.3 g/kg to 2.8 ± 0.4 g/kg) and females (1.3 ± 0.3 g/kg to 1.8 ± 0.0 g/kg) from the first to last week. Within the males, half ($n = 3$) consumed high levels of alcohol during binge (2.6 ± 0.2 g/kg), choice (2.1 ± 0.1 g/kg), and overall (2.2 ± 0.1 g/kg), and half ($n = 3$) consumed low levels of alcohol during binge (1.5 ± 0.1 g/kg), choice (0.2 ± 0.1 g/kg), and overall (0.4 ± 0.1 g/kg). High drinkers consumed significantly more than low drinkers overall ($p < 0.001$), on binge days ($p < 0.001$), and on choice days ($p < 0.001$). Whole cell patch clamp showed that pyramidal neurons in the mPFC of high drinkers exhibited increased excitability, since they needed less current to produce maximal firing than low drinkers. Our findings show for the first time that ferrets can drink voluntarily at physiological doses. Furthermore, alcohol consumption patterns were different in males and females. Within the male group, animals were split between “high” and “low” drinkers.

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Poster

438. Adolescent Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: U01 AA019972
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Title: Ethanol-induced neuronal activation of the amygdala in adolescent rats: Impact of age, sex, and prior history of ethanol exposure

Authors: *H. J. COLEMAN¹, T. T. TOWNER², K. M. PAPASTRAT⁴, S. VILLARREAL¹, E. I. VARLINSKAYA², D. F. WERNER³;

²Psychology, ³Psychology and Behavioral Neurosci., ¹Binghamton Univ., Binghamton, NY;

⁴Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The adolescent developmental period is marked by substantial reorganization of the brain that includes synaptic pruning, increases in white matter, and changes of neurotransmitter systems. Adolescents not only initiate alcohol use but tend to drink more per occasion than adults. Cognitive deficits have been demonstrated in young adults following heavy adolescent alcohol use, and similar findings have been reported in animal models, including sex-specific effects of adolescent intermittent ethanol exposure (AIE). AIE has been shown to impact multiple brain regions, including the amygdala. However, neuronal activation in the amygdala during AIE as well as acute effects of ethanol on the amygdala at different adolescent periods (early versus late adolescence) has not been investigated. The present study was designed to assess patterns of neuronal activation of the central (CeA) and basolateral (BLA) portions of the amygdala in adolescent male and female rats in response to ethanol. Experimental subjects were transgenic cFos-LacZ rats acutely challenged with either 0 (water) or 4 g/kg ethanol given via intragastric gavage (IG) on postnatal day (P) 25 or P45. In addition, P45 rats with a previous history of AIE (10 prior exposures to 0 or 4 g/kg ethanol given IG every other day from P25) were included as a chronic exposure condition. As a marker for neuronal activation, we measured β -galactosidase (β -gal) which is a proxy for c-Fos expression in these animals. In the CeA, both males and females demonstrated lower number of β -gal positive (β -gal+) cells at P45 regardless of acute challenge. Similarly, in both males and females, ethanol administration increased the number of β -gal+ cells, regardless of age. In both sexes, subjects previously exposed to AIE had reduced β -gal expression following ethanol administration than subjects acutely challenged with ethanol. In P25 and P45 males, BLA β -gal expression was elevated following water, but not ethanol administration relative to home cage controls. In early adolescent females tested at P25, neuronal activation of the BLA was not affected by water or ethanol. In contrast, P45 females had more β -gal+ cells in the BLA following IG water and ethanol than home cage controls. Chronic exposure did not affect responsiveness of the BLA in males, with females having less β -gal+ cells in the BLA following chronic exposure, regardless of exposure condition (water or ethanol). These data suggest that the CeA, but not BLA is sensitive to ethanol in both sexes, whereas the BLA of adolescent females, but not males, is sensitive to manipulations per se.

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Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.21

Topic: A.09. Adolescent Development

Support: NIH-NCCIH Grant K99AT010903

Title: Exposure to blue light at night during adolescence induces neuroplasticity in the amygdala affecting emotional responses in mice

Authors: S. GOSS¹, A. NILSSON¹, S. BARNES¹, D. DULCIS¹, *A. PORCU^{2,1};

¹Univ. of California San Diego, La Jolla, CA; ²Univ. of South Carolina, Univ. of South Carolina, Columbia, SC

Abstract: Adolescence is the developmental time during which a number of psychiatric disorders emerge, including anxiety and mood disorders. Adolescence also reflects a “sensitive window” during which circuit maturation is highly responsive to environmental stimuli. In modern societies, adolescents are increasingly subjected to irregular environmental lighting, as 80% of youths in the USA reported using computers, smartphones, and tablets at night-time. Altered environmental light is associated with impaired mood and an increased risk of psychiatric disorders. The aim of this study was to test whether exposure to blue light at night affects neuroplasticity in the amygdala regulating emotional responses to reveal whether prolonged blue light exposure at night represents an early risk factor for developing psychiatric disorders. We developed a new light paradigm to mimic human adolescent light exposure in mice: a prolonged light phase (19hrs/day) with light appearing during the biological night phase of the mice. Adolescent mice were exposed to either such light protocol or to control conditions (12hrs/day) for 4 weeks, then tested for anxiety-like behavior and social interaction. Immunofluorescence, chemogenetic approach and fiber photometry were used to establish the effect of blue light on neuroplasticity in the amygdala and emotional responses. We found that exposure to the blue light protocol increased anxiety-like responses and induced a significant difference in the number of GABAergic and somatostatin neurons in the amygdala in adolescent mice. *In vivo* Ca²⁺ recording revealed altered somatostatin activity in the amygdala associated with impaired social behavior in adolescent mice exposed to blue light protocol. Chemogenetic inhibition of amygdala GABAergic neurons interfered with light-mediated GABA/somatostatin plasticity and reduced anxiety states in adolescent mice. Our data indicate that prolonged blue light exposure induces neuroplasticity in the amygdala regulating anxiety-like behaviors during adolescence, revealing a new mechanism by which light might affect mental health in human adolescents.

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Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.22

Topic: A.09. Adolescent Development

Support: NSFC Grant U1805263
FJNU Grant Z0205109

Title: Effects of early experiences on behavioral development: An experiment based on the "Human-Rat Interaction Paradigm"

Authors: *B. YIN¹, X. WU^{2,1}, D. YU¹, H. LI^{2,3};

¹Sch. of Psychology, Fujian Normal Univ., Fuzhou, China; ²Sch. of Psychology, Shenzhen Univ., Shenzhen, China; ³Sch. of Psychology, South China Normal Univ., Guangzhou, China

Abstract: This study aims to investigate the causal relationship between early experiences and later behavioral development based on a novel experimental model termed the “human-rat interaction paradigm” (HRIP). 36 one-month-old male SD rats were randomized as subjects. Based on the HRIP, the experimental groups (Positive Early Experiences (PEE) / Negative Early Experiences (NEE)) and the control group were intervened for 3 weeks, and the effects of the early experience intervention on their behavioral development were tested through a battery of behavioral paradigms. We found that: 1) The characteristics indicators in the process of HRIP suggest that the intervention became fairly effective and stable after two weeks. 2) The PEE group showed least anxiety-like behavior throughout the O-maze test, the open field test and the novelty-suppressed feeding test, and showed least hesitation to adapt to and utilize the new learning device. 3) During the learning tests, the PEE group showed most rule-breaking exploratory behavior in the integrative-learning maze; while the majority of the NEE group learned to open the gate during the early stage of a procedural learning test, the firmness of their long-term memory was subpar in the new object recognition test; the control group was overall passive during the whole series of learning tests. 4) During the social interaction tests, the PEE group showed the most interests while the NEE group showed the most aversion towards the toy rat. While all groups preferred a real rat to a toy rat, both PEE and NEE groups (but not control) showed clear preference to interacting with a stranger rat. Moreover, during the “empathy” tests, when there were no food pellets around, all three groups of rats generally would open the gate to rescue the entrapped rat; however, when there were food pellets to be shared with the entrapped rat, both PEE and NEE groups were less likely to open the gate, and the PEE group ate more steadily than the NEE group. When their entrapped counterpart was only able to access the food pellet through the subjects’ active sharing, the NEE group showed much more frequent behavior of feeding interruption and vigilant sniffing. 4) During the social competition tests, the control group had the highest success rate in the tube test, while the PEE group had the highest success rate in the food competition test. At the same time, the degree of social rank differentiation was smallest in the control group and largest in the PEE group. The success rate of the NEE group was overall the lowest during the inter-group social competition tests. We concluded that early experiences shaped by the HRIP paradigm have profound impacts on behavioral development in rats.

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Poster

438. Adolescent Mechanisms of Vulnerability

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.23

Topic: A.09. Adolescent Development

Support: NIH Grant DP1 DA046537
NIH Grant K01 DA039308
NIH Grant T32 DA007237

Title: Adolescent morphine exposure perturbs object recognition memory in female but not male rats

Authors: *C. TOMAS BALTAZAR¹, A. TOUSSAINT², D. ZEID², M. WIMMER²;
¹Temple Univ. Undergraduate Neurosci. Program, Temple Univ. Undergraduate Neurosci. Program, Philadelphia, PA; ²Temple Univ., Philadelphia, PA

Abstract: Although the impacts of drug exposure on cognition and behavior within the lifespan of the individual are well studied, much less is known about how parental drug exposure can impact offspring. We previously found that first generation (F1) female offspring of male rats exposed to morphine via self-administration exhibited impaired object recognition learning. Importantly, first exposure to opioids often occurs during the sensitive developmental period of adolescence. Adolescent opioid exposure has been shown to impair adulthood learning in both rodent and human models. These findings separately establish the roles of paternal opioid use and early life opioid exposure in impairment of adulthood cognition; however, it is unknown whether these risk factors can compound to affect cognitive function in the F1 generation - e.g., in children of opioid-dependent parents exposed to opioids following sports-related injury. To model this “double” exposure in rats whose fathers self-administered either morphine (morphine-sired F1) or saline (saline-sired F1), morphine was administered in an escalating dose (4mg/kg to 8mg/kg, S.C.) over 5 days to all tested F1 male and female rats during adolescence (PND37-42). F1 rats were then tested in a novel object recognition assay (NOR) in adulthood (\geq PND60). Both morphine- and saline-sired F1 males treated with morphine during adolescence showed typical object recognition memory. In contrast, adolescent morphine-treated F1 females from both sire exposure groups showed impairments in object recognition memory. Comparison with data from a prior F1 NOR experiment, which found normal recognition memory in morphine-naïve saline-sired females, suggested that adolescent morphine exposure alone produces object recognition deficits similar to those induced by paternal morphine exposure in females. Future experiments will include a no-adolescent-morphine control to test this hypothesis more specifically. In sum, our findings suggest female-specific sensitivity to morphine’s effects on recognition learning, whether the exposure was indirect, through parental use, or direct, through adolescent

administration. This points to potential overlap between mechanisms underlying impacts of intergenerational and developmental morphine exposure.

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Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

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Program #/Poster #: 438.24

Topic: A.09. Adolescent Development

Support: NIH-NINDS R01 NS104829-01

Title: The role of KIBRA in circuit-level function in the developing mouse brain

Authors: *R. PENDRY¹, L. QUIGLEY², J. SCARIA³, L. J. VOLK⁴, B. E. PFEIFFER⁵;
¹UT Southwestern Med. Ctr., Dallas, TX; ²Neurosci., UT Southwestern Med. Ctr., DALLAS, TX; ³Texas Tech. Univ. Hlth. Sci. Ctr., Dallas, TX; ⁴Neurosci., Southwestern Med. Ctr., Dallas, TX; ⁵Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Cognitive disorders manifest in part as dysfunctional neural networks, and accumulating evidence suggests that network-level dysfunction may underlie symptoms of such disorders, with these anomalies sometimes arising in an age-dependent manner. Loss of KIBRA, a synaptic protein involved in AMPAR trafficking that is important for plasticity across species, is detrimental to plasticity in adult but not juvenile mice, suggesting a loss in plasticity that is age-dependent. Studying age-dependent changes to *in vivo* brain function in mice during juvenile and early adolescent stages is more difficult than doing so in adults due to technical, surgical, and behavioral hurdles that hinder our ability to analyze network-level changes in real time during this crucial period. Here, we have developed and implanted novel 16-tetrode micro-drives in juvenile male and female WT and KIBRA-KO mice, supporting *in vivo* electrophysiological recordings in awake, freely behaving mice as young as p19. We have performed chronic simultaneous recordings from bilateral dorsal hippocampal areas CA1 and CA3 as well as bilateral anterior cingulate cortex from p19 through p60, allowing us to quantify specific circuit-level changes associated with normal brain development as well as alterations arising from loss of KIBRA.

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Poster

438. Adolescent Mechanisms of Vulnerability

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.25

Topic: A.09. Adolescent Development

Support: Startup funds

Title: Effects of acid-sensing ion channels on conditioned fear memory are age-dependent

Authors: ***H. JANOUSCHEK**, A. BERNS, M. JONES, A. TOWNSEND, A. K. EAGEN, D. R. LANGBEHN, R. J. TAUGHER-HEBL, H. STEVENS, J. A. WEMMIE;
Univ. of Iowa, Iowa City, IA

Abstract: Circuits underlying Pavlovian fear conditioning have been suggested to undergo marked changes during brain development. For example, changes in a number of molecular mechanisms underlying synaptic plasticity and neuron morphology have been reported between postnatal days (PNDs) 17 and 30. In adult mice, acid-sensing ion channels (ASICs) are activated during synaptic transmission in the basolateral amygdala and are critical for synaptic plasticity and for Pavlovian fear conditioning. It is not known whether ASICs contribute to fear conditioning at earlier ages, or during brain development. To explore this possibility, we tested ASIC1A protein expression in the brain and found ASIC1A was abundantly expressed as early as embryonic day 14, raising the possibility that ASIC1A might play an important role in brain development. We next tested Pavlovian fear conditioning in wild-type versus *Asic1a*^{-/-} mice at different ages, ranging from infancy to adulthood. We conditioned separate groups of mice on PND 17, 23, 29, 42, and 83. We then tested cue-evoked freezing behavior 24 hours after training, and compared effects of ASIC1A disruption at each age and across development using mixed effects linear regression analyses. Surprisingly, infant *Asic1a*^{-/-} mice trained on PND 17 exhibited normal cue-evoked fear memory on PND18, which did not differ significantly from wild-types. However, when *Asic1a*^{-/-} mice were trained on PND29, cue-evoked fear memory on PND30 was significantly impaired. This age-related impairment in cue-evoked fear memory continued to grow in *Asic1a*^{-/-} mice trained at older ages. Together these data provide the first evidence for ASIC1A-dependent effects on brain development. The emergence of cue-evoked memory deficits in *Asic1a*^{-/-} mice between PND18 and PND30 correlates with extensive developmental alterations during this time window. The increasing deficit in fear memory with age in *Asic1a*^{-/-} mice suggests that ASIC1A is increasingly important for brain maturation and/or function. Future studies will be needed to pinpoint specifically which developmental changes in fear circuit structure and/or function might be affected by ASIC1A disruption.

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Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.26

Topic: A.09. Adolescent Development

Support: NSERC RGPIN 05570 2014
Mitacs Accelerate Grant IT 23872

Title: Probiotic supplementation decreases peripheral and hippocampal glucose and increases L-lactate concentrations in sleep-disrupted pubertal CD-1 mice.

Authors: *M. MURACK, A. K. KADAMANI, O. H. TRAYNOR, A. GUINDON-RIOPEL, Y. PATEL, C. MESSIER, N. ISMAIL;
Sch. of Psychology, Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Puberty is a critical period of development characterized by sexual maturation, increased metabolic disorder development, and prevalent sleep disturbances. Pubertal maturation increases energy demand yet can impair glucose tolerance, increasing peripheral blood glucose. Greater peripheral blood glucose is also observed following chronic sleep disruption resulting in sleep disrupted adolescents displaying higher risks of developing glucose-related metabolic disorders. Lactic acid probiotics increase cellular glucose uptake while producing alternate energy sources like L-lactate. We investigated whether probiotics improve peripheral and hippocampal glucose and L-lactate concentrations in chronically sleep disrupted pubertal mice. Three-week-old CD-1 male and female mice underwent bilateral cannula implantation in the dorsal hippocampus. After 1 week of recovery, mice received 2-week ad libitum access to either water, a *Lactocaseibacillus rhamnosus* (Lacidofil) mixture, or a *Lactobacillus helveticus* (Cerebiome) mixture. Mice were sleep disrupted during the first 4 hours of their rest for 8 consecutive days prior to testing. Metabolites were analyzed in the hippocampus using biosensors. Then, peripheral metabolites were analyzed following a glucose tolerance tests. Lacidofil and Cerebiome reduced peripheral blood glucose concentration and increased peripheral blood lactate concentration 30 mins following the tolerance test in both sexes. Lacidofil decreased extracellular hippocampal glucose and reduced weight change in male and female mice. Lacidofil and Cerebiome also increased extracellular hippocampal L-lactate concentration. Male mice displayed greater increases in hippocampal and peripheral glucose concentrations than female mice regardless of probiotic treatment. Our results show that lactic acid probiotic treatment may improve peripheral and central cellular energy acquisition in chronically sleep-disrupted pubertal groups.

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Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.27

Topic: A.09. Adolescent Development

Support: NSERC 2020-04302
Mitacs Grant IT16285

Title: Lps- and antibiotic-induced neuroinflammation and depression-like and anxiety-like behaviors are attenuated by probiotic consumption

Authors: ***K. B. SMITH**¹, A. ABDERAOUF BOUTELDJA², S. AL SHARANI¹, C. HAY³, S. MCRAE¹, J. ZHONG¹, N. NAGHMEH⁴, T. A. TOMPKINS⁴, N. ISMAIL¹;

¹Social Sci., Univ. of Ottawa, Ottawa, ON, Canada; ²McGill Univ., Montreal, QC, Canada;

³Univ. du Québec à Montréal, Montreal, QC, Canada; ⁴Lallemand Animal Nutr., Montreal, QC, Canada

Abstract: Puberty is a period of extensive sex-dependent brain reorganizing and remodeling and it is sensitive to exposure to stress. The bacterial endotoxin lipopolysaccharide (LPS) causes gut dysbiosis, and HPA axis and immune activation, and neuroinflammation. Neuroinflammation has been linked to mental disorders such as depression and anxiety, yet it remains unclear whether gut dysbiosis causes neuroinflammation and negative behavioral outcomes. We examined the enduring effects of pubertal gut dysbiosis, and the ability of probiotics to mitigate these outcomes. Male and female mice received saline or antibiotic treatment for 7 days, as of 5 weeks of age while concurrently consuming either probiotics or a placebo for 14 days. Mice were treated with either saline or LPS at 6 weeks of age. At 10 weeks of age, in adulthood, mice were exposed to the open field test, elevated plus maze, tail suspension test, and forced swim test. We examined microglia and glucocorticoid receptor (GR) expression post-mortem using immunohistochemistry. LPS-treated mice displayed greater anxiety- and depression-like behaviors, greater neuroinflammation within the hippocampus and infralimbic medial prefrontal cortex (mPFC). Additionally, LPS treatment decreased GR expression within the paraventricular nucleus (PVN) of the hypothalamus, but not in the amygdala. Probiotic treatment mitigated anxiety and depression-like behaviors, reduced the number of microglia in the hippocampus and infralimbic region of the mPFC, as well prevented the reduction of GR expression in the PVN. Our results illustrate not only the enduring negative consequences of pubertal immune stress, but also show that the gut microbiome mediates these effects. This data suggests that probiotics are a preventative measure for the enduring consequences of pubertal stress.

Disclosures: **K.B. Smith:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Lallemand Animal Nutrition. **A. Abderaouf Bouteldja:** None. **S. Al Sharani:** None. **C. Hay:** None. **S. McRae:** None. **J. Zhong:** None. **N. Naghmeh:** A. Employment/Salary (full or part-time); Lallemand Animal Nutrition. **T.A. Tompkins:** A. Employment/Salary (full or part-time); Lallemand Animal Nutrition. **N. Ismail:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Lallemand Animal Nutrition.

Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.28

Topic: A.09. Adolescent Development

Support: NSERC Grant 2020-04302

Title: Acute effects of pubertal microbial dysbiosis on peripheral cytokine concentrations in male and female CD-1 mice

Authors: ***P. ESPOSITO**¹, M. GANDELMAN², C. RODRIGUEZ¹, J. LIANG¹, N. ISMAIL¹;
¹Sch. of Psychology, ²Translational and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Microbial dysbiosis during critical periods of development (i.e., puberty) has enduring effects on immune responsiveness and contributes to various pathologies such as Alzheimer's disease, Parkinson's disease, anxiety, and depression. However, the mechanisms underlying the effects of gut dysbiosis on immune responsiveness remain unclear. Therefore, the objective of the current study was to examine the acute effects of pubertal microbial dysbiosis (induced by antimicrobial and LPS treatments) on immune responsiveness in male and female CD-1 mice. A total of 80 (40 male and 40 female) CD-1 mice were used in this experiment. At five weeks of age, male and female CD-1 mice were treated with a combination of antimicrobial agents or water, twice a day, for seven days. At six weeks of age, the mice received an intraperitoneal injection of LPS or saline, and were euthanized 8 hours later. Following euthanasia, blood samples were collected for plasma extraction and analysis with multiplex bead-based Luminex immunoassay. Plasma concentrations of cytokines granulocyte-macrophage-colony-stimulating-factor (GM-CSF), interleukin-2 (IL-2), interleukin-23 (IL-23), interleukin-12p70 (IL-12p70), interleukin-17A (IL-17A), and interleukin-10 (IL-10) were analyzed. The results indicated that pubertal microbial dysbiosis altered plasma cytokine concentrations in a sex-dependent manner. More specifically, pubertal female mice showed greater concentrations of anti-inflammatory cytokines (i.e., IL2, GM-CSF, IL10) while pubertal male mice showed greater concentrations of inflammatory cytokines (i.e., IL12 (p70), IL23) in the plasma. Overall, these findings suggest that pubertal female mice may have a more adaptive response to gut dysbiosis in comparison to pubertal male mice, indicating that males may be more susceptible to the detrimental effects of pubertal microbial dysbiosis on immune responsiveness.

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Poster

438. Adolescent Mechanisms of Vulnerability

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Program #/Poster #: 438.29

Topic: F.03. Stress and the Brain

Support: NIDA Grant DA044980
Biological Sciences Initiative (BSI) Grant at CU Boulder
Undergraduate Research Opportunity Program (UROP) Grant at CU Boulder

Title: Impact of Low-Dose Voluntary Alcohol Consumption During Adolescence on Fear Extinction and Renewal in Adult Long Evans Rats

Authors: *M. VANBAELINGHEM¹, T. DOLATI¹, J. WESTERMAN³, M. MARTIN¹, J. GRIZZELL^{4,1}, M. SADDORIS²;

¹Psychology and Neurosci., ²Psychology & Neurosci., Univ. of Colorado, Boulder, CO; ³Psychology and Neurosci., Univ. of Colorado Boulder Dept. of Psychology and Neurosci., Boulder, CO; ⁴Neurosci. and Behavioral Biol., Emory Univ., Atlanta, GA

Abstract: Life experiences during adolescence substantially direct developmental trajectories, some biasing maladaptive responses to adversity in adulthood by altering limbic system structural/functional organization. For example, previous studies illustrate that adolescent ethanol (EtOH) exposure disrupts neural circuits within the ventromedial prefrontal cortex (vmPFC), basolateral amygdala (BLA), and ventral hippocampus (vHipp), which together mediate learning, decision-making, and emotion regulation in normal and threatening conditions. Pilot data in our laboratory support that voluntary low-dose EtOH provided daily (but not necessarily consumed daily) from postnatal days 28-42 can enhance single unit neuronal firing rates in the vmPFC and bias maladaptive behavioral responses in risky (reward+threat) conditions. Here, we test the hypothesis that male and female Long Evans rats similarly consuming low-dose EtOH (e.g. 0.1mg/kg/day) during adolescence via the “Drinking-in-the-Dark” paradigm alters (vHipp-) vmPFC-BLA-dependent fear learning processes in adulthood. Specifically, we predicted that EtOH would disrupt the expression of fear (e.g. freezing in response to a shock-paired tone) during fear extinction and renewal trials using an “ABC” paradigm. Briefly, rats were trained to associate a mild shock with a predictive tone-light cue to acquire fear conditioning in a novel Context A and then placed into novel Contexts B and C wherein they were exposed to the tone-light cues alone for cue extinction and fear renewal testing one and two days later, respectively. Freezing behavior was quantified using a combination of live and video-tracking approaches. Contrary to our predictions, alcohol exposure during adolescence resulted in decreased freezing during fear extinction and renewal in males and, to a lesser degree, females. Females also froze less than males in all conditions, likely due to increased ambulation during cue presentations. Taken together, our study confirms that adolescent alcohol disrupts fear learning in adulthood, regardless of biological sex, but future studies are needed to determine whether the observed reductions in fear are due to adaptive extinction learning or maladaptive failures of fear encoding.

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Poster

438. Adolescent Mechanisms of Vulnerability

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 438.30

Topic: F.03. Stress and the Brain

Support: NIDA Grant DA044980
Biological Sciences Initiative (BSI) Grant at CU Boulder
Undergraduate Research Opportunity Program (UROP) Grant at CU Boulder

Title: Characterizing behavioral models of experience-dependent resilience to social or non-social stress using Long Evans rats

Authors: ***J. GRIZZELL**^{1,2}, J. WESTERMAN², M. MARTIN², T. DOLATI², M. VANBAELINGHEM², G. W. COSTANZA-CHAVEZ², M. V. BARATTA², M. SADDORIS³; ¹Neurosci. and Behavioral Biol., Emory Univ., Atlanta, GA; ²Psychology and Neurosci., ³Psychology & Neurosci., Univ. of Colorado, Boulder, Boulder, CO

Abstract: Learning to control a stressor putatively produces resilience, but most observations in rodents are drawn from models optimized for male Sprague Dawley (SD-M) rats. For example, SD-M trained to terminate a tail-shock (ES) display reduced stress-induced social avoidance and threat-induced freezing when compared to counterparts without control (IS). Importantly, ES females do not differ from IS SD-F, thus challenging elucidation of sex-linked mediators of controllability-conferred resilience. Given that pilot data suggested both male and female Long Evans rats (LE-MF) fail to display ES-mediated resilience, the current study further characterized stressor controllability-mediated resilience in LE-MF using nonsocial (standard ESIS) or social (dominance/social conflict) models. We predicted that stress-induced social avoidance and prolonged expressions of fear would be greatest in uncontrollable-stress conditions (IS; subordinated), whereas controllable stress exposure (ES; dominant) would render behavioral patterns more closely mimicking unstressed controls.

Based on the established Syrian hamster model of dominance-conferred resilience, we adapted the rat-optimized, Warm Spot (WS) task into a daily, experience-dependent, social-conflict learning task. Briefly, sex-, age-, and weight-matched dyads of LE-MF were placed (10-min/day) for two weeks in a modified WS testing arena set atop dry ice (-20C) with a warmed respite (+30C) large enough for one rat. Subsets of LE-MF dyads were housed individually or co-housed prior to/throughout WS conflict. Following the 14th day of WS-mediated conflict, all rats were exposed (alone) in the WS arena but without a respite present for 10 minutes in a "No Spot"/inescapable stress exposure. Following ESIS (nonsocial) or WS (social) stressor controllability training, LE-MF were tested for social avoidance and altered fear conditioning via the juvenile social exploration, social interaction, and/or standard fear extinction paradigms. Results indicate key differences in model-, strain-, and sex-conferred resilience. While controllability in standard ESIS failed to reliably attenuate stress-induced social avoidance, dominant LE-MF in the WS avoided a neutral, novel social target less than IS and subordinate counterparts. On the other hand, ES LE-M and dominant LE-MF displayed more rapid fear extinction. Together, LE-MF respond to ESIS differently than SD-M but experience-dependent social stressor controllability produces resilient-like behaviors.

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Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.01

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH-NIMH: 1R01MH117149-01

Title: Molecular mechanism of KIBRA regulated AMPAR trafficking

Authors: *X. SHAO, L. VOLK;
UT Southwestern Med. Ctr., Dallas, TX

Abstract: Synaptic plasticity is a cellular correlate of learning and memory. Activity-induced changes in AMPA trafficking is a conserved expression mechanism for synaptic plasticity and is highly regulated by complexes of AMPAR-binding proteins. The human memory-associated protein KIBRA has been shown regulate AMPA receptor trafficking and synaptic plasticity, but how KIBRA interacts with and regulates AMPA receptor trafficking remains unclear. Interestingly, we found that KIBRA interacts with the AMPA receptor subunit GluA2 through PICK1, a key regulator of AMPA receptor trafficking. KIBRA promotes PICK1 aggregation and changes GluA2 localization in heterologous cells. To identify the structural determinants of KIBRA-PICK1-AMPA complexes we investigated interactions and cellular localization of different combinations of KIBRA and PICK1 domain mutants. We find that the PICK1 BAR domain is responsible for the interaction between KIBRA and PICK1 and is important for KIBRA-PICK1-GluA2 complex formation. In addition, multiple KIBRA coiled-coil domains are important for KIBRA and PICK1 co-aggregation. These findings suggest that KIBRA regulates AMPA receptor trafficking through coiled-coil domain mediated interaction with PICK1. Ongoing studies are examining the synaptic function of this protein complex.

Disclosures: X. Shao: None. L. Volk: None.

Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.02

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NSFC Grant 81870886

Title: Calcium permeable AMPA receptors in the ventrolateral orbital cortex modulate anxiodepressive-like behavior in the rat with neuropathic pain.

Authors: *Y. WANG, H. CHEN, Y. LIU, Y. YANG, J. ZHOU, X. YUAN, F. HUO;
Xi'an Jiaotong Univ., Xi'an, China

Abstract: It has been generally accepted that patients with chronic neuropathic pain induced by nerve injury usually present with comorbid emotional disorders such as depression and anxiety, which can aggravate chronic neuropathic pain. Recent studies have reported that the ventrolateral orbital cortex (VLO), as a high cortex center, regulates anxiodepressive-like behaviors in mice with chronic pain, but the detailed molecular mechanisms of this regulation are still poorly understood. In this research, we demonstrated that anxiodepressive-like behaviors are induced in rats of chronic neuropathic pain four weeks after spared nerve injury (SNI). We showed that the expression level of GluA1 subunits of AMPA-like glutamate receptors increases significantly in the VLO among SNI rats, which leads to the formation of calcium-permeable AMPA receptors (CPARs). In addition, pharmacologic activation of these CPARs in the VLO of SNI rats produced both antianxiodepressive and antiallodynia effects. However, pharmacological delivery of CPAR antagonist eliminates the effects of CPAR agonist but does not affect the anxiodepressive-like behaviors and allodynia alone. These results indicated that glutamatergic transmission through CPAR in VLO is a novel molecular mechanism modulating the comorbidity of chronic pain and depression and anxiety. Therefore, CPARs may be a promising therapeutic target for developing new treatments for patients with chronic pain and mood disorders. More generally, this research highlights the prospects of glutamate transmission changes due to the receptor subunit modifications and changes in pain states and its potential role in the synaptic plasticity underlying the comorbidity of chronic pain and mood disorders.

Disclosures: Y. Wang: None. H. Chen: None. Y. Liu: None. Y. Yang: None. J. Zhou: None. X. Yuan: None. F. Huo: None.

Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.03

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Colorado State University

Title: Ketamine's rapid antidepressant action is mediated by Ca²⁺-Permeable AMPA receptor expression in the hippocampus

Authors: *A. ZAYTSEVA, I. SCHMIDT, E. BOUCKOVA, M. WILES, S. KIM, M. WUSTRU; Colorado State Univ. Molecular, Cell. & Integrative Neurosciences, Colorado State Univ. Molecular, Cell. & Integrative Neurosciences, Fort Collins, CO

Abstract: Ketamine's rapid antidepressant action is mediated by Ca²⁺-Permeable AMPA receptor expression in the hippocampus. Depression is a complicated mental illness, the causes of which are not entirely known yet. Depression affects the brain through chemical imbalances regulated by inadequate neurotransmitter release and decreased neuronal activity in the hippocampus. Recently, ketamine has become a center of interest due to its rapid antidepressant effect. Ketamine is known to act at the glutamatergic synapse to increase excitatory synaptic communication. However, ketamine is an antagonist for NMDA receptors (NMDARs), which are essential for Ca²⁺ influx to the post-synaptic cell and neuronal Ca²⁺ signaling. The question then is how ketamine increases excitatory neuronal activity while simultaneously blocking NMDARs. Postmortem studies have reported reductions in the mRNA expression levels of AMPA receptor (AMPA) subunit GluA1 and GluA3, but not GluA2, in the hippocampus of patients with depression, suggesting that subtype specific AMPAR decrease in the hippocampus is implicated in depression. This suggests that subtype specific activation of AMPARs is crucial for ketamine's antidepressant actions. Our new data using cultured mouse hippocampal neurons reveal that a one-hour treatment of low dose ketamine significantly decrease Ca²⁺-dependent phosphatase calcineurin activity, which increases phosphorylation of serine 831 (S831) and serine 845 (S845) in GluA1, critical for GluA1-containing AMPAR trafficking, and subsequently induces synaptic expression of Ca²⁺-Permeable, GluA2-lacking, and GluA1-containing AMPAR (CP-AMPA). Moreover, a low dose of ketamine in mice significantly increases *in vivo* synaptic GluA1 levels, but not GluA2, in the hippocampus. Most importantly, ketamine treatment induces antidepressant effects in male mice, which is completely abolished by specifically blocking CP-AMPA. This suggests that when ketamine is administered, CP-AMPA-mediated Ca²⁺ influx can compensate for downregulated NMDA-dependent Ca²⁺ signaling in the hippocampus, which ultimately leads to antidepressant effects.

Disclosures: A. Zaytseva: None. I. Schmidt: None. E. Bouckova: None. M. Wiles: None. S. Kim: None. M. Wustrau: None.

Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.04

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Susceptibility to hyperthermic-induced status epilepticus is altered in the GluA1 receptor knock-out mouse

Authors: *A. LABUZ, H. SUN, S. JOSHI, H. P. GOODKIN;
Neurol., Univ. of Virginia Sch. of Med., Charlottesville, VA

Abstract: The prolonged seizures of status epilepticus (SE) occur due to the failure of seizure termination mechanisms or the initiation of changes that could sustain seizures for longer periods. Febrile SE accounts for 25-30% of pediatric SE episodes. The mechanisms that could sustain these seizures for prolonged periods in a developing brain are unclear. We evaluated the plasticity of AMPA receptors (AMPA) during hyperthermia-induced SE (hSE). GluA1 are the most abundantly-expressed AMPAR subunits in the hippocampi of P10 and older animals. Hence, we evaluated whether deleting the GluA1 subunit expression could alter susceptibility to hSE. hSE was induced in 16 or 17-day-old (P16/17) wild-type C57Bl6 or littermate mice with a global deletion of GluA1 subunits (GluA1KO) of either sex. Bilateral cortical and hippocampal electrodes were implanted to record seizures using continuous video EEG. To induce the seizure, the pups were placed in a hyperthermia chamber with seizure commencing when core and brain temperature reached approx. 43°C. The AMPAR-mediated synaptic currents were recorded from hippocampal CA1 neurons of C57Bl6 animals in hSE and normothermia littermate controls using standard voltage-clamp techniques. The AMPAR synaptic currents of CA1 neurons were potentiated in hSE animals. The AMPAR-mediated sEPSCs recorded from animals in hSE were of larger amplitude (hSE 26 ± 1 pA, n=6 neurons/3 animals vs controls 22 ± 1 pA, n=6 neurons/3 animals, p=0.049, students t-test); other properties of sEPSCs were similar in hSE and control animals. Next, we evaluated whether the hSE was altered in GluA1KO mice. Spike-wave discharges with amplitude at least twice that at the baseline and frequency higher than 2 Hz marked the onset of seizures following hyperthermia exposure. The occurrence of these discharges for 5 min or longer marked the onset of hSE. All the WT mice (n=5) experienced hSE with a duration of 28 ± 10 min (n=5). In contrast, only one of the 5 GluA1KO mice experienced hSE (p=0.048 Fisher's exact test) which lasted for 9 min. These studies revealed the potentiation of AMPAR-mediated glutamatergic transmission of hippocampal CA1 neurons of animals in hSE. Mice lacking the GluA1 subunit expression were resistant to induction of hSE. Thus, potentiation of AMPAR-mediated glutamatergic transmission is a factor underlying the prolonged seizures of SE in young animals. Additional studies evaluating the effect of hyperthermia on the plasticity of AMPAR-mediated neurotransmission and surface membrane trafficking of these receptors could provide additional insights into the mechanisms regulating the SE-associated plasticity of these receptors.

Disclosures: A. Labuz: None. H. Sun: None. S. Joshi: None. H.P. Goodkin: None.

Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.05

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH NINDS F99 NS124126 (NC)
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Dr. W. Burghardt Turner Dissertation Fellowship (NC)
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Title: Dysfunctional biogenesis of AMPA receptor disease-associated variants

Authors: *N. CERTAIN¹, L. P. WOLLMUTH²;
²Neurobiol & Behavior, ¹Stony Brook Univ., Stony Brook, NY

Abstract: AMPA receptors (AMPA receptors) contribute to the majority of fast excitatory neurotransmission in the brain. Controlling the number and subunit composition of AMPARs determines the overall strength of synaptic plasticity, a cellular component of learning and memory processes. *De novo* variants throughout the AMPAR structure have been reported in patients exhibiting intellectual disabilities and other neurodevelopmental abnormalities including seizures, movement disorders, and autism spectrum disorders. Recent evaluations of AMPAR disease-associated variants have mainly focused on disruption to functional receptor properties such as channel gating. Here, we address the efficacy of receptor assembly in AMPAR disease-associated variants using blue native-PAGE and immunocytochemistry. AMPAR assembly is structurally driven by the transmembrane domain which facilitates tetramer formation and enhances stability. Within the transmembrane domain is the eukaryotic-specific M4 segment which is required for dimer to tetramer transition. To study AMPAR assembly deficits, we evaluated human AMPAR variants that show decreased surface expression and localize within the M4 segment. We found that AMPAR variants concentrated in the M4 segment impact the efficiency of receptor assembly. We also observed a high incidence of disrupted receptor assembly of the X-linked *GRIA3* gene. We evaluated AMPAR variants co-expressed with various auxiliary subunits to also measure changes to auxiliary subunit function. We conclude that AMPAR assembly is affected by *de novo* *GRIA2* and *GRIA3* variants within the transmembrane M4 segment, a critical component of the tetramerization process. Overall, AMPAR disease-associated variants are valuable for understanding the pathogenic mechanisms in neurodevelopmental diseases.

Disclosures: N. Certain: None. L.P. Wollmuth: None.

Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.06

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: MH043784
MH122943

Title: Altered expression of excitatory and inhibitory ionotropic receptor subunit across the cortical visuospatial working memory network in schizophrenia

Authors: *K. SCHOONOVER, S. J. DIENEL, H. H. BAZMI, N. E. MILLER, D. A. LEWIS; Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Introduction: Working memory dysfunction in individuals with schizophrenia is thought to reflect altered excitatory and inhibitory neurotransmission across multiple regions of the cortical visuospatial working memory (vsWM) network. However, key ionotropic glutamatergic and GABAergic receptor subunits have only been studied in the dorsolateral prefrontal cortex (BA46). **Methods:** Using qPCR on total gray matter homogenate samples from BA46, posterior parietal cortex (BA7), and primary (BA17) and association (BA18) visual cortices, we quantified transcript levels of critical subunits for excitatory N-methyl-D-aspartate receptors (NMDARs), excitatory alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA), and inhibitory GABA_A receptors (GABRAs) in 20 matched pairs of schizophrenia (SZ) and unaffected comparison (UC) subjects. **Results:** In UC subjects, AMPAR and NMDAR levels generally exhibited opposite rostral-to-caudal gradients, with AMPAR GRIA1 and GRIA2 expression highest in BA46 and NMDAR GRIN1 and GRIN2A expression highest in BA17; however, the regional pattern of NMDAR GRIN2B expression was like that of AMPARs. GABRA5 and GABRA1 levels were highest in BA46 and BA17, respectively. In SZ subjects, levels of all transcripts (except GRIN2B and GABRA5) were lower in caudal regions, with no differences in BA46. **Conclusions:** Our analyses of transcript levels across regions of the cortical vsWM network revealed distinct regional patterns of ionotropic glutamatergic and GABAergic receptor subunits in UC subjects, suggesting that balances between excitation and inhibition are achieved in a region-specific manner. In SZ subjects, the distinct alterations in excitatory and inhibitory receptor transcripts across regions suggests differential contributions of each region to impaired WM performance in the illness.

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Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.07

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NS111414

Title: Interrogating synaptic physiology using GCaMP8f-based quantal analysis and electrophysiology at the *Drosophila* neuromuscular junction

Authors: *J. CHEN¹, Y. HAN², K. HE³, D. K. DICKMAN⁴;

¹Univ. of South California, Univ. of South California, Los Angeles, CA; ²USC, ³Home, USC, Los Angeles, CA; ⁴USC, USC, La Crescenta, CA

Abstract: Electrophysiology offers excellent resolution to define the functional changes during neurotransmission at synapse. Recent advances in genetically encoded calcium indicators (GECIs) now approach electrophysiological resolution. Here, we express the latest and fastest GECIs at the Drosophila neuromuscular junction (NMJ). Specifically, we targeted GCaMP8f and the red shifted RGECO1a to postsynaptic compartments (SynapGCaMP8f and SynapRGECO1a), to determine fundamental properties of synaptic physiology by optical quantal analysis, benchmarked to electrophysiology. We have combined simultaneous electrophysiological recordings and calcium imaging to 1) directly compare the sensitivity and resolution of quantal imaging to electrophysiology; 2) investigate quantal content obtained by current clamp and two-electrode voltage clamp electrophysiology vs direct optical quantal analysis; and 3) determine how these properties are distributed between motor neuron subtypes and how they change in mutants that induce plasticity at the Drosophila NMJ. Preliminary data suggests that while RGECO does not yet approach the sensitivity of electrophysiology, quantal calcium imaging by SynapGCaMP8f is capable of capturing nearly all the synaptic events that conventional electrophysiology does. We will present additional work comparing quantal content accuracy between electrical and optical approach and the pros and cons of each approach in understanding basal synaptic physiology and how these properties change following plasticity. Our work suggests that, with a few caveats, quantal imaging using GCaMP8f can be as sensitive as electrical approaches and reveals important insights that cannot be obtained through conventional electrophysiology.

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Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.08

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant MH119347

Title: Identification of endocytic signals in the proteins of the SynDIG/PRRT family

Authors: D. SPECA, C.-W. HE, C. MEYER, E. SCOTT, R. CANTUA PINA, *E. DIAZ; Pharmacol., Univ. Of California Davis, Davis, CA

Abstract: The transmembrane protein Synapse Differentiation Induced Gene 4 (SD4), also known as proline-rich transmembrane protein 1 (PRRT1), has recently been identified as an auxiliary factor of the AMPA-type glutamate receptor (AMPA) necessary for maintaining extra-synaptic pools of GluA1, a subunit of AMPARs. However, how SD4 establishes and

maintains these pools is unclear. Previous studies suggested that endocytic machinery is important for maintaining a pool of mobile surface AMPARs, and that proteins associated with such cellular machinery are critical for proper protein trafficking and internalization. Additionally, SD4 co-localizes with GluA1 and resides in early and recycling endosomes. Therefore, identifying the sorting signal targeting SD4 to these organelles is essential to elucidate the role of SD4 in GluA1 trafficking. In this study, we report that SD4 possesses a YxxΦ sorting motif, 178-YVPV-181, responsible for binding to the AP-2 complex cargo-sorting subunit μ 2. This motif appears critical for proper SD4 internalization, as SD4 mutant 178-AVPA-181 (SD4 AVPA) induces aberrant SD4 accumulation at the plasma-membrane of heterologous cells and primary rat hippocampal neurons and does not bind to μ 2. Furthermore, we found that similar motifs exist in other proteins of the SynDIG/PRRT family. In conclusion, we identify a sorting signal in SD4 important for understanding the SD4-dependent regulatory mechanism of GluA1 trafficking.

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Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.09

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Functional characterization of TARPs in the context of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor modulation

Authors: *J. LEIFELD, A. K. VIRK, E. KARAKURT, M. HOLLMANN;
Biochem. I - Receptor Biochem., Ruhr Univ. Bochum, Bochum, Germany

Abstract: AMPA-type glutamate receptors (AMPARs) mediate the majority of rapid neurotransmission and are key players of synaptic function. Transmembrane AMPA receptor regulatory proteins (TARPs) enhance AMPAR-mediated currents by affecting gating, pharmacological properties, and transport. Although the structure of AMPAR-TARP complexes has been elucidated, it is still unclear how TARPs exert their functions. Currently, electrostatic interactions between the receptor and the first extracellular loop (Ex1) of TARPs are thought to be responsible for the TARP-specific effect. To elucidate the mechanism of TARP function, we generated TARP mutants and subsequently characterized them electrophysiologically in co-expression with the AMPAR GluA2(Q)flip using the two-electrode voltage-clamp technique in the *Xenopus laevis*-oocyte expression system (all tested constructs from rat). Interestingly, eliminating the charge of amino acids in Ex1 of type-1a TARP γ 2 resulted in increased TARP action. Normalization to wild-type γ 2 (n=44 in total, data was normalized to results from each experiment with a minimum of n=3) resulted in a mean of 180% and SEM of 33 with respect to full agonist glutamate (glu)-induced currents and 108% \pm 33 with respect to partial agonist kainate

(KA)-induced currents (2 experiments, n=7). Therefore, we decided to test mutants in which each secondary structural element in the two extracellular γ 2 loops, Ex1 and Ex2, was eliminated individually. These are: The β sheets β 1 to β 5, the α 1 helix, the loops β 1- β 2, β 4-TM2, and TM3- β 5, and the conserved GLWR motif. Preliminary data from 3 independent experiments suggest that α 1 is most important for TARP function, as elimination of this motif most strongly reduced the TARP-typical effect (46% \pm 18 for glu, 34% \pm 16 for KA, n=12). Deletion of β 2 also strongly reduced overall TARP function (60% \pm 17 for glu, 45% \pm 13 for KA, n=12). Interestingly, motifs of Ex1, including most notably β 4-TM2, appear primarily to be responsible for TARP-typical enhancement of partial agonist efficacy (71% \pm 28 for glu, 43% \pm 18 for KA, n=10). In contrast, the two structural motifs of Ex2, β 5 and TM3- β 5, may be more responsible for potentiation of full agonists (68% \pm 25 for glu, 84% \pm 26 for KA, n=12, and 56% \pm 18 for glu, 88% \pm 17 for KA, n=11, respectively). The results obtained so far provide novel insights into the structure-function relationship of TARPs and AMPARs: electrostatic interactions are not responsible for γ 2 mechanism of action. Instead, α 1 and β 2 are essential for modulatory function and possibly potentiate AMPAR-mediated currents induced by partial and full agonists in a co-working manner with motifs from Ex1 and Ex2, respectively.

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Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.10

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: DFG Grant SE 774/6
DFG Grant STE 552/5

Title: Auxiliary subunits control function and distribution of AMPA receptor complexes in hippocampal NG2 glia during development

Authors: *G. SEIFERT, S. HARDT, D. TASCIO, S. PASSLICK, R. JABS, C. STEINHÄUSER;

Inst. of Cell. Neurosciences, Univ. of Bonn, Bonn, Germany

Abstract: NG2 cells are equipped with AMPA and GABA_A receptors and receive direct synaptic input from glutamatergic and GABAergic neurons. We combined functional and molecular techniques to analyse properties and expression of auxiliary subunits of AMPA receptors, so-called transmembrane AMPA receptor regulatory proteins (TARPs) and cornichons (CNIHs) in NG2 cells of the juvenile and adult mouse hippocampus. To identify Ca²⁺ permeable AMPA receptors, polyamine derivatives (Naspm, IEM-1460) were applied intra- or extracellularly. NG2 glia from the adult hippocampus were more sensitive to the polyamine derivatives, indicating developmental upregulation of Ca²⁺ permeable AMPA receptors. Molecular analysis revealed

frequent expression of TARPs $\gamma 4$, $\gamma 7$, $\gamma 8$, and CNIH-2 in NG2 glia, but they were all downregulated in adulthood. The altered expression of TARP $\gamma 8$ prompted us to test its specific antagonist, JNJ55511118. JNJ blocked responses insensitive to Nasp and IEM-1460, indicating that TARP $\gamma 8$ is mainly associated with Ca^{2+} impermeable AMPA receptors. Thus, at the adult stage, NG2 glia express a mosaic of Ca^{2+} permeable and Ca^{2+} impermeable receptors. The polyamine derivatives also inhibited postsynaptic AMPA receptor responses in NG2 glia. Ca^{2+} permeable AMPA receptors might contribute to the regulation of proliferation and differentiation of NG2 glia. Further pharmacological investigation and/or genetic deletion of individual subunits may help clarifying specific roles of glial AMPA receptor/TARP complexes in neural signaling. Supported by DFG (SE 774/6, STE 552/5).

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Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.11

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Arising evidence of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor modulation by tetraspanins

Authors: *A. BECIC, A. STRUSS, M. HOLLMANN;
Dept. of Biochem. I - Receptor Biochem., Ruhr Univ. Bochum, Bochum, Germany

Abstract: Glutamate receptors play an important role in numerous cognitive processes. Being a subfamily of ionotropic glutamate receptors, AMPA receptors are functionally modulated by transmembrane AMPA receptor regulatory proteins (TARPs). Based on the structural similarity of TARPs and proteins from the tetraspanin family, as well as emerging data of tetraspanin importance in synapses, tetraspanins Tspan3, Tspan7, Tspan13, Tspan28, and Tspan30 were investigated for their potential modulatory effects on AMPA receptors. Experiments were performed using the *Xenopus* oocyte heterologous expression system and two-electrode voltage clamp electrophysiology. Tetraspanin cDNAs, isolated from human brain tissue were in vitro-transcribed to obtain cRNAs that were injected into oocytes. Whole-cell glutamate-activated steady-state current amplitudes of eight different AMPA receptor subunit combinations were recorded and compared to currents in oocytes where additionally tetraspanin cRNAs were expressed. Further, tetraspanins and glutamate receptors were tagged with green (mEGFP) and red (mCherry) fluorescent proteins to observe their subcellular localization in the oocyte using confocal microscopy. Current amplitudes of receptors recorded under two-electrode voltage clamp were normalized and potentiation factors calculated for receptors co-expressed with tetraspanins. We found that Tspan28 increases glutamate-activated currents of GluA4(Q)flip by 2.1-fold ($n=21$; SEM ± 0.29), and Tspan30 does so by 2.4-fold ($n=25$; SEM ± 0.23).

Additionally, kainate-activated currents for the same receptor subunit showed approximately two times stronger modulatory effects, with the potentiation factor of Tspan28 being 3,7 (n=21; SEM \pm 0.37) and that of Tspan30 being 3.9 (n=25; SEM \pm 0.5). On the other hand, Tspan28 decreases the glutamate-induced currents of GluA1(Q)flip up to 3.5-fold (n=17; SEM \pm 0.06) and of GluA2(Q)i+GluA2(R)flip up to 2.6-fold (n=15; SEM \pm 0.05). Furthermore, Tspan7 increases the currents of GluA1(Q)flip to 2.8-fold (n=31; SEM \pm 0.5). Confocal microscopy of AMPA receptor subunits tagged with mCherry showed that the subunits GluA3(Q)flip and GluA4(Q)flip have lower membrane expression than all other homo- and heteromeric subunit combinations. Further, colocalization of the GluA1(Q)flip subunit with Tspan7 was confirmed, while in case of co-expression with Tspan28, no colocalization was seen. Our electrophysiological data demonstrates that the investigated tetraspanins clearly modulate AMPA receptor amplitudes, while confocal microscopy results confirm colocalization of some tetraspanins and receptors in the plasma membrane.

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Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.12

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Characterization of claudins as modulatory proteins for heteromeric alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors

Authors: *J. SHAUKAT, M. HOLLMANN;
Biochem. I- Receptor Biochem., Ruhr Univ. Bochum, Bochum, Germany

Abstract: AMPA receptors (AMPA) mediate the majority of fast synaptic transmission in the central nervous system. They are transmembrane proteins that assemble into homo- or heterotetramers comprising four distinct subunits: GluA1 to GluA4. Phenomena such as alternative splicing and RNA editing contribute to the further diversity of receptor subunit combinations, which ultimately result in an increased multiplicity of channel architectures and properties. In the last decade, many proteins have been identified to interact with and modulate the functions of ionotropic glutamate receptors. The investigation of new potential auxiliary subunits is therefore very interesting. It has been identified in our lab that certain members of the claudin protein family, previously known only to be involved in tight junction formation, are able to interact with homomeric AMPARs and alter their current amplitudes (Simon Haering, 2016). However, AMPARs in vivo mostly form heteromers. Therefore, the role of claudins need to be further clarified as a potential modulator for heteromeric AMPARs. Here, we have investigated their effects on receptor steady-state current amplitudes and channel properties such as desensitization. cRNAs of AMPARs and claudins from *Rattus norvegicus* were injected into *Xenopus laevis* oocytes and electrophysiological measurements using the two-electrode voltage

clamp method were performed to compare the modulation of homomeric and heteromeric receptors. Dependency of modulation on the concentration of receptor was also tested by injecting oocytes with different amount of receptor cRNA. Our data indicate that in some of the heteromeric receptor subunit combinations higher potentiation (26.4 ± 5.79 -fold, $n = 10$) is observable in comparison to homomeric subunits (4.1 ± 1.9 -fold $n = 5$) when coexpressed with previously identified modulatory claudins. However, some of the investigated claudins elicit higher potentiation when the expression level of the receptor is low and obtained currents are moderate in size. Whereas, in some of the heteromeric AMPARs, increased potentiation (16.2 ± 2.3 -fold $n = 8$) was observed when desensitization is inhibited. Our results illustrate that the current amplitudes of heteromeric AMPARs are distinctly affected by several of the investigated claudins and depend on the level of expressed receptor or subunit combination. The latter finding hints at the importance of a defined stoichiometric expression of claudins and AMPARs.

Disclosures: J. Shaukat: None. M. Hollmann: None.

Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.13

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: R00 DA039791 to JJP
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Title: Hiv-1 tat and opiate use induce synaptic instability in dorsal striatum

Authors: *T. RODRIGUEZ^{1,2}, A. N. QRAREYA³, J. GOMEZ², F. ABDUL WALI², S. DILAWARI², M. AGUILAR², J. J. PARIS^{3,4}, P. A. SERRANO^{1,2};
¹Biochem., The Grad. Ctr. CUNY, New York, NY; ²Psychology, Hunter Col., New York, NY;
³Dept. of BioMolecular Sci., ⁴Res. Inst. of Pharmaceut. Sci., Univ. of Mississippi, University, MS

Abstract: The human immunodeficiency virus 1 (HIV-1) protein, transactivator of transcription (Tat) is a potent mediator in the progression of HIV-1 associated neurocognitive disorders (HAND). The neurotoxic effects of Tat on HAND are further exacerbated by opiate drug use and abuse. Females may be particularly vulnerable to these effects given known interactions for estrogens to potentiate drug seeking and reward. HIV-1 Tat promotes neuronal cell dysfunction and death by disrupting intracellular calcium (Ca^{2+}) homeostasis. However, the molecular mechanisms underlying the complex interactions between HIV-1 Tat and opiate drug abuse in the female brain remains to be elucidated. We hypothesize that the complex interaction between HIV-1 Tat and opiates has effects on both α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate receptor (NMDA) receptors, which

are known to play a role in cognition and memory. The NMDAR subunit GluN2B is known to enhance Ca^{2+} influx and excitotoxicity. Consequently, excessive Ca^{2+} levels lead to the dysregulation of AMPAR trafficking and subunit composition resulting in synaptodendritic damage and cognitive decline. We evaluated HIV-1 Tat +/- ovariectomized (ovx) transgenic mice using an unbiased morphine conditioned place preference (CPP) task followed by western blot analyses of the dorsal striatum. The HIV-1 Tat+ ovx transgenic mice significantly preferred the morphine-conditioned place. In the dorsal striatum we show a significant decrease in GluA2, the Ca^{2+} impermeable subunit important for anchoring synaptic AMPAR and limiting Ca^{2+} influx. We also show a significant decrease in GluA1, a subunit that plays a role in promoting AMPAR retention on the post synaptic membrane. Interestingly, there is a decrease in the NR1 subunit in dorsal striatum, an obligatory subunit required for the proper function of NMDAR. The downregulation of NR1 has been associated with the excessive activation of the NMDAR. These results suggest that the dysregulation of AMPAR trafficking may be driven by an increase in Ca^{2+} via the NMDAR subunits, leading to the disruption of the AMPAR GluA1/2 subunit expression pattern in the dorsal striatum. Therefore, the increased sensitivity for morphine on the CPP task may be attributed to the dysregulation of AMPAR subunit composition observed in the dorsal striatum of HIV-1 Tat+ ovx transgenic mice.

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Poster

439. AMPA Receptors: Trafficking, Gating, and Accessory Proteins

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 439.14

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Intramural Program of NICHD, Z01 HD008914

Title: Modulation of glutamate receptors by the auxiliary protein Neto

Authors: *T. HAN, W.-C. HSIEH, C. I. RAMOS, M. SERPE;
Eunice Kennedy Shiver Natl. Inst. of Child Hlth. and Human Develop., NIH, Bethesda, MD

Abstract: Formation of functional synapses during development and their fine-tuning during plasticity and homeostasis relies on ion channels and their accessory proteins, which control where, when and how the channels function. *Drosophila* neuromuscular junction (NMJ) is an excellent system to dissect the function of glutamatergic synapses. The fly NMJ relies entirely on kainate-type glutamate receptors and uses at least 6 different subunits: 5 of them are utilized in the muscle to form two types of postsynaptic receptors, type A and -B, which contain either GluRIIA or GluRIIB subunits, plus GluRIIC, GluRIID and GluRIIE; the 6th subunit, KaiRID, is part of a presynaptic autoreceptor. The function of all these receptors depends on Neto (Neuropillin and Tollod-like), an auxiliary protein conserved from worms to humans.

Drosophila neto codes for two isoforms, Neto- α and Neto- β , which share the extracellular and transmembrane domains but have distinct intracellular parts. Neto- β is the predominant isoform at the NMJ and functions in the muscle to recruit glutamate receptor and other postsynaptic components. Neto- α functions primarily in motor neurons to ensure normal basal neurotransmission. Here we are using outside-out patch-clamp recordings and fast ligand application to study the biophysical properties of *Drosophila* NMJ receptors reconstituted in HEK293 cells. We have previously showed that Neto is critical for the functional reconstitution of postsynaptic iGluRs (type-A and type-B receptors) in *Xenopus* oocytes. Here we report that Neto is absolutely required for the function of type-A and type-B receptors, but not for the homotetrameric KaiRID receptor. The two Neto isoforms, Neto- α and Neto- β , differentially modulate the gating properties of these channels: For example, postsynaptic receptors in complexes with Neto- β have decreased desensitization rates than when with Neto- α . In contrast, only Neto- α decreases the desensitization rate for KaiRID homotetramer channels; Neto- β has no significant effect. Pre-treatment with Concanavalin A increases all single channel open times, whereas extracellular philanthotoxin blocks the channels to various extents. These channels are differentially regulated by addition of intracellular polyamines (spermine) at physiological concentration, which changes the rectification profiles for each channel. These studies demonstrate that Neto is not only required for the function of the NMJ channels but also increases the repertoire of the receptor properties.

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Poster

440. Sodium Channel Dysfunction in Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 440.01

Topic: B.03. Ion Channels

Title: In vitro characterization of a 4-phenyl-2-(pyrrolidinyl)nicotinamide derivative as a potent and selective Nav1.1 positive modulator and its role in E/I balance

Authors: S. HOBSON¹, K. HUBERLE¹, A. OMRANI¹, Z. HART¹, R. LIPINSKI², *J. R. NICHOLSON³, H. J. DRAHEIM¹;

¹CNS Dis. Res., ²Medicinal Chem., Boehringer Ingelheim Pharma, Biberach an der Riss, Germany; ³CNS Dis. Res., Boehringer Ingelheim Pharma GmbH & Co.KG, Biberach an der Riss, Germany

Abstract: Nav1.1 channels (encoded by SCN1A) play a crucial role in the synaptic excitation of parvalbumin (PV)-positive interneurons and thus, in the excitation/inhibition (E/I) balance in CNS circuits. Therefore, we wanted to explore if selective Nav1.1 positive modulators could have therapeutic utility, e.g., in treating CNS disorders such as epilepsy, schizophrenia, autism, and neurodegenerative diseases with known E/I imbalances. Recently, a Nav1.1 positive modulator has been published (compound 4; Ref.1) that was claimed to be both potent and

selective. To explore the potential of Nav1.1 positive modulators, we further investigated this compound to elucidate its influence on the electrophysiological properties of Nav1.1, such as channel opening and closing as well as the specificity of influence on PV-positive interneurons. Potency at hNav1.1 and selectivity vs. other hNav channels were confirmed and extended to action on mouse Nav1.1 that was determined to be in the same range regarding potency as well as efficacy. Interestingly, to consolidate the site of action of compound 4, we demonstrated that its binding site is located extracellularly but does not functionally interfere with the action of either TTX or another Nav1.1 modulating compound (Ref.2). For functionality of compound 4 on Nav1.1 receptors in native tissue, we tested the effects of compound 4 on both pyramidal neurons and PV-positive interneurons in brain slices of the mouse prefrontal cortex. Upon application of compound 4, a shift in both threshold and firing frequency could be observed in PV interneurons while compound 4 was not showing significant effects on pyramidal neuron excitability. This confirms the involvement of Nav1.1 in mediating the excitation of this specific neuronal population and underlines its potential involvement in regulating the E/I balance in CNS circuitry. Taken together, our data not only confirm the utility of this compound as a potent and selective Nav1.1 positive modulator, but more importantly, highlight the PV interneuron specificity of this novel mechanism. These studies enable the further investigation of the therapeutic potential of Nav1.1 positive modulators in neurological indications. References: 1) Miyazaki et al. (2019) Discovery of novel 4-phenyl-2-(pyrrolidinyl)nicotinamide derivatives as potent Nav1.1 activators. *Bioorg Med Chem Lett.*29(6):815-820). 2) Frederiksen K et al. (2017) A small molecule activator of Nav1.1 channels increases fast-spiking interneuron excitability and GABAergic transmission in vitro and has anti-convulsive effects in vivo. *Eur J Neurosci* 46, 1887-1896.

Disclosures: **S. Hobson:** A. Employment/Salary (full or part-time);; Boehringer-Ingelheim Pharma GmbH & Co. KG. **K. Huberle:** A. Employment/Salary (full or part-time);; Boehringer-Ingelheim Pharma GmbH & Co. KG. **A. Omrani:** A. Employment/Salary (full or part-time);; Boehringer-Ingelheim Pharma GmbH & Co. KG. **Z. Hart:** A. Employment/Salary (full or part-time);; Boehringer-Ingelheim Pharma GmbH & Co. KG. **R. Lipinski:** A. Employment/Salary (full or part-time);; Boehringer-Ingelheim Pharma GmbH & Co. KG. **J.R. Nicholson:** A. Employment/Salary (full or part-time);; Boehringer-Ingelheim Pharma GmbH & Co. KG. **H.J. Draheim:** A. Employment/Salary (full or part-time);; Boehringer-Ingelheim Pharma GmbH & Co. KG.

Poster

440. Sodium Channel Dysfunction in Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 440.02

Topic: B.03. Ion Channels

Support: 5U01DK116311

Title: Action potential conduction in the mouse and rat vagus nerve is dependent on multiple tetrodotoxin sensitive voltage-gated sodium channels (Nav1s)

Authors: *S. NAIR¹, N. PAVELKOVA⁴, S. H. HADLEY², C. MURPHY⁵, T. TAYLOR-CLARK³;

¹Univ. of South Florida, Univ. of South Florida, Tampa, FL; ²Univ. of South Florida, Univ. of South Florida, Plattsburgh, NY; ³4202 E Fowler Ave, Univ. of South Florida, Tampa, FL; ⁴MPP, USF, Tampa, FL; ⁵Wake Forest Univ., Winston-Salem, NC

Abstract: Action potential conduction in excitable cells depends on voltage-gated sodium channels (Nav1), of which there are nine subtypes. Selective inhibition of action potential conduction in specific neuronal populations is one approach to alleviating morbidities associated with inappropriate neuronal activity (e.g. pain). The vagus nerve contributes to multiple sensory and autonomic functions regulating the cardiopulmonary and gastrointestinal systems. However, little is known of vagal Nav1 channels, which hinders Nav1 blocker use. Previous studies suggest that tetrodotoxin (TTX)-sensitive Nav1.1, 1.2, 1.3, 1.6 and 1.7 and TTX-insensitive Nav1.8 and Nav1.9 are expressed in these nerves. Here, we studied electrically-evoked compound action potentials (CAPs) in the mouse (C57BL6) and rat (Sprague-Dawley) vagus nerve *ex vivo*. We measured the amplitude of the A- and C-waves in the CAP under control conditions and following the administration of selective Nav1 inhibitors such as TTX, PF-05089771 (PF), ICA-121341 (ICA), LSN-3049227 (LSN) and ProTX-II. Although these inhibitors have been well characterized in their block of human Nav1, gaps exist in our knowledge of their selectivity for mouse and rat Nav1. PF blocks Nav1.7 but this is likely limited for rat; ProTX-II blocks rat Nav1.7. ICA blocks Nav1.1, Nav1.3 and probably Nav1.6; LSN blocks Nav1.2, Nav1.6 and Nav1.7. The A- and C- waves of the CAPs were completely abolished by TTX in both mice and rat vagus nerve, confirming that TTX-sensitive Nav1s are necessary for action potential conduction. In the mouse vagus nerve, inhibition of A-wave by PF and LSN but not ICA, suggests that A-wave conduction is mediated by Nav1.7 and to a minor extent Nav1.2. The mouse C-wave was abolished by LSN and by a combination of PF and ICA, indicating that it is mediated by Nav1.7 and Nav1.6. In the rat CAP studies, we confirmed that PF has limited affinity for Nav1. Instead, ProTX-II induced a robust C-wave block and a mild A-wave block. ICA showed similar inhibition of C-wave and a mild block of A-wave; we presume this effect of ICA is via Nav1.7. LSN abolished the A- and C-wave of the rat CAP. Such data indicate that rat A- and C-waves are mediated by Nav1.7, Nav1.2 and Nav1.6. Overall, our data indicates that multiple Nav1 subtypes (TTX-sensitive) contribute to vagal CAPs. Our findings also indicate that there is inter-species variability in the contribution of each subtype to vagal CAP conduction.

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Poster

440. Sodium Channel Dysfunction in Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 440.03

Topic: B.03. Ion Channels

Title: Discovery of positive modulators for treating SCN2A-related disorders such as autism spectrum disorder

Authors: *P. KARILA¹, E. P. LEBOIS², Å. JÄGERVALL¹, P. SPRATT⁴, K. BENDER⁴, D. LAL⁵, M. WEÏWER², J. CAMPBELL², H.-R. WANG², S. CHOI², A. GHOSHAL², D. BAEZ², M. FITZGERALD², M. FLEISHMAN², Z. FU², S. IQBAL², K. PEREZ DE ARCE², J. SACHER², Q. XU², G. FENG², Y.-L. ZHANG³, E. SCOLNICK², J. PAN², J. PIHL¹, F. WAGNER², J. COTTRELL²;

¹Collectricon AB, Collectricon AB, Mölndal, Sweden; ²Stanley Ctr. Therapeut., ³Ctr. for the Develop. of Therapeut., Broad Inst., Cambridge, MA; ⁴Dept. of Neurol., UCSF Weill Inst. for Neurosciences, San Francisco, CA; ⁵Cleveland Clin., Cleveland, OH

Abstract: *SCN2A* genetic variants are causal for a number of neurodevelopmental disorders, including autism spectrum disorder (ASD), intellectual disability (ID), and infantile seizure disorders. *SCN2A* encodes the voltage-gated sodium channel Nav1.2, with loss-of-function variants causing ASD and ID, and gain-of-function variants causing infantile seizure disorders. Given the genetic linkage to disease, we set out to discover small molecule positive modulators of Nav1.2 for the treatment of ASD. We first established a novel primary high-throughput screening (HTS) assay to allow the detection of voltage changes in Nav1.2-expressing HEK cells in response to electrical stimulation. This physiologically relevant assay platform enabled the completion of an HTS of approximately 80,000 compounds, where we identified 378 Nav1.2 activators and 1837 inhibitors. Follow up concentration-response assay yielded a number of potent and efficacious activators, and select compounds were followed up in a synaptic function model where a synaptically transmitted response to electrical stimulation was quantified in primary murine cortical cultures to confirm the compounds' effect. Subsequent electrophysiological characterization of one hit, BRD4032, revealed that it activates Nav1.2 by changing both the voltage sensing mechanism and the inactivation kinetics of the channel, and that BRD4032 elicited marked widening of the action potential waveform in *Scn2a*^{+/-} mouse brain slices. Finally, *in vivo* EEG studies showed significantly altered alpha, beta, and delta frequency bands in *Scn2a*^{+/-} mice, indicating that these alterations may be useful translational biomarkers. Taken together, we have successfully implemented a robust screening strategy to identify Nav1.2 modulators and are poised to follow up on the selectivity, mechanism of action, and *in vivo* effects of these compounds.

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Poster

440. Sodium Channel Dysfunction in Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 440.04

Topic: B.03. Ion Channels

Support: Fritz Thyssen fellowship

Title: Pathogenic gating pore current conducted by autism-related mutations in the $\text{Na}_v1.2$ sodium channel

Authors: *A. ELTOKHI¹, T. M. GAMAL EL-DIN¹, C. W. TSCHUMI², M. QUINLAN², J. LI¹, L. S. ZWEIFEL², W. A. CATTERALL¹;

¹Dept. of Pharmacol., ²Dept. of Psychiatry & Behavioral Sci., Univ. of Washington, Seattle, WA

Abstract: The rapidly advancing identification of genomic variants associated with autism spectrum disorder (ASD) profoundly impacts research approaches. Hundreds of risk genes cause susceptibility along a continuum of rare and common alleles, confirming the genetic nature of this disorder. Recently, we discovered that ASD gating charge mutations in the KCNQ/K_v7 potassium channel cause gating pore currents and impaired firing of dopamine neurons when expressed in brain slices (Gamal El-Din et al., *PNAS* 2021). The voltage-gated $\text{Na}_v1.2$ channel encoded by *SCN2A* ranked high among the ion channel genes having mutations in individuals with ASD. $\text{Na}_v1.2$ has a central pore formed by pore-forming modules from four domains (D-I to D-IV). Each domain contains 6 transmembrane segments. S1-S4 form the voltage sensor domain, and S5 and S6 surround a central ion pore. Mutations that alter arginine gating charges (R) in the voltage sensor of $\text{Na}_v1.2$ are among those most frequently associated with ASD. We hypothesized that the gating charge mutations in $\text{Na}_v1.2$ would induce gating pore current by causing an ionic leak through the mutant voltage sensor. We developed a novel method using the BacMam vector that captures a maximum expression of functional $\text{Na}_v1.2$ channels at the cell surface of human embryonic kidney (HEK) cells in order to detect small gating pore currents. Our results showed that ASD mutations in the R2 gating charge in D-II (R853Q) of $\text{Na}_v1.2$ caused gating pore current in the resting state of $\sim 0.5\%$ of the central pore current, which was blocked when the voltage sensors activate. The gating pore current was ~ 4 -fold selective for K^+ over Na^+ , and its amplitude was increased substantially by reducing extracellular Ca^{2+} . Gating charge mutants in R1 (R1626Q) and R2 (R1629H) in D-IV of $\text{Na}_v1.2$ conducted smaller gating pore currents at the resting state and both mutations induced small positive shifts in the voltage dependence of inactivation. To test the physiological significance of these gating pore currents, experiments in progress will express $\text{Na}_v1.2$ ASD mutants in spontaneously active dopamine neurons of the ventral tegmental area of mice using CRISPR/Cas methods and slice electrophysiology, which revealed major impairment of electrical excitability by expression of K_v7 channels conducting gating pore current (Gamal El-Din et al, *PNAS*, 2021). We hypothesize that R1 and R2 mutations of $\text{Na}_v1.2$, similar to K_v7 channels, will increase resting membrane conductance by inducing an inward leak and a shunt conductance at the resting membrane potential. Understanding this common pathophysiology at the circuit level will give new insights into the underlying mechanisms of ASD.

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Poster

440. Sodium Channel Dysfunction in Disease

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Program #/Poster #: 440.05

Topic: B.03. Ion Channels

Support: UGC-BCR-97

Title: Computational Analysis of isoform specific blockers for Na_v1.2 from different peptides of honeybee venom

Authors: *S. A.P., G. B A;
SASTRA Univ., Thanjavur, India

Abstract: Sodium Channel (Na_v) is a complex multimeric trans-membrane protein, with a core alpha subunit and auxiliary beta-subunits. Of the 9 different isoforms, Na_v1.2 and Na_v1.5 are responsible for neuronal and cardiac action potential respectively. There very few drugs that modulate specific Na_v isoforms. Many potential therapeutic effects of bee venom therapy have been demonstrated, but few research has been carried out with Na_v. The aim is to computationally predict how honey bee venom proteins target Na_v1.2 and Na_v1.5 isoforms. Mellitin (2MLT), Tertiapin (1TER), and Phospholipase A2 enzyme (1POC) of *Apis mellifera* were retrieved from PDB. The structure of Na_v 1.2 and Na_v 1.5 was obtained from PDB (6J8E & 6LQA) and docked with the three bee venom peptides using ZDOCK, with the findings analysed using PISA. Pymol was used to examine the docked protein results. The docking results shows that, 27 residue peptide melittin binds to the transmembrane helices of domain II and IV of α subunit of both Na_v1.2 and Na_v1.5. These were predominantly hydrophobic interactions. In phospholipase A2, hydrophobic interactions were observed in domain I, II and IV transmembrane helices and cytoplasmic region of both the isoforms. The 21-residue peptide Tertiapin predominantly bind to the pore forming and extracellular regions of Na_v1.5 across all the four domains of the α -subunit. In Na_v 1.2 interactions were seen in domain I, II and IV pore forming regions. Few interactions (945 GLU, 948 TRP, 949 ASP) were seen in cytoplasmic region which connects domain I and II, this interaction is not observed in Na_v 1.5 which had a mix of both hydrophobic and hydrophilic interactions. From the analysis, tertiapin could be a ligand of interest which blocks Na_v 1.2 differently form Na_v1.5. This study opens up a lot of possibilities for treatment of neuronal channelopathies. We will also investigate the importance of intracellular and extracellular loop amino acid interactions with toxin components using electrophysiological tests. Thus, different animal toxins will significantly improve our understanding of the biophysical and pharmacological characteristics of channels, especially in terms of distinguishing unique channel activity.

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Poster

440. Sodium Channel Dysfunction in Disease

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Program #/Poster #: 440.06

Topic: B.03. Ion Channels

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Title: Regulation of hyperexcitable neuron carrying epilepsy-associated sodium channel Nav1.2-L1342P variant by hiPSC-derived microglia

Authors: *Z. QUE¹, M. OLIVERO-ACOSTA¹, K. WETTSCHURACK¹, J. ZHANG¹, X. CHEN¹, J. WU¹, Y. ZHAO¹, B. DEMING¹, N. CUI¹, I. CHEN², T. XIAO², B. SKARNES³, Y. YANG¹;

¹MCMP, ²Purdue Univ., West Lafayette, IN; ³The Jackson Lab., Farmington, CT

Abstract: Rapid progress in the discovery of genetic variants in *SCN2A* from patients with epilepsy and autism highlights the importance of understanding the pathophysiology of *SCN2A* variants. Nav1.2, encoded by gene *SCN2A*, is predominantly expressed in the excitatory neurons across the brain and is responsible for action potential firing and propagation. *De novo* variant L1342P of Nav1.2 was discovered in multiple patients with encephalopathy and intractable seizures. To study the functional consequence of this mutation, we generated genome-edited iPSCs carrying the Nav1.2-L1342P variant and differentiate them into cortical neurons. We found the cortical neurons carrying Nav1.2-L1342P variants display both increased intrinsic and network excitability. Recent studies have suggested a central role of microglia in the regulation of neurodevelopment and neuronal excitability. While the study of microglia in the chemical (KA, PTZ)-induced experimental seizure model provided insights regarding the functions of microglia, it is not known, however, how human microglia would respond to human neurons carrying disease-causing genetic variants in a naturally occurring pathological environment. To investigate the role of microglia in the context of neurons carrying the disease-causing mutation L1342P, we established both 2D and 3D co-culture systems by adding microglia into the neuronal culture. Interestingly, we found increasing microglial calcium activities with a preference in process territory in the co-culture with L1342P neurons. Since microcephaly was reported as one of the important developmental impairments in patients carrying Nav1.2-L1342P, we are investigating how microglia would respond to the abnormal local environment

and remodel the neuronal network during neural development. Our data allows us to explore the role of microglia in regulating Nav1.2 mutation-associated disease phenotypes, deepening our understanding of the interplay between neurons and immune cells under the clinically relevant pathophysiological condition.

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Poster

440. Sodium Channel Dysfunction in Disease

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National Institutes of Health National Institute of Neurological Disorders and Stroke (NINDS) Grants R01 NS117585

Title: Modeling epilepsy-related SCN2A gain-of-function mutation L1342P with CRISPR-edited human-induced pluripotent stem cell-derived cortical spheroids.

Authors: ***M. OLIVERO ACOSTA**¹, **Z. QUE**², **T. NGUYEN**³, **C. OTTERBACHER**¹, **M. WANG**¹, **N. CUI**¹, **X. CHEN**², **J. ZHANG**², **W. C. SKARNES**⁴, **K. WETTSCHURACK**¹, **Y. YANG**²;

²MCMP, ¹Purdue Univ., West Lafayette, IN; ³Medicinal Chem. and Mol. Pharmacol., Purdue Univ., Lafayette, IN; ⁴The Jackson Lab., Farmington, CT

Abstract: The SCN2A gene encodes for sodium channel Nav1.2, a protein that mediates action potentials in neurons. SCN2A pathogenic mutations have been associated with epilepsy. An example is the gain-of-function (GoF) L1342P mutation, identified in several patients with microcephaly and drug-resistant seizures. Human-induced pluripotent stem cells (hiPSCs) can be used to generate neuronal cultures that model neurological diseases. In our recent work, we have demonstrated that hiPSC-derived 2D-neuronal monolayers carrying the CRISPR/Cas9-edited L1342P-mutant channel display a marked hyperexcitability phenotype (Que, Olivero-Acosta et al., 2021). However, the impact of the GoF-L1342P mutation on neurodevelopment remains unknown. Cortical spheroids are in-vitro 3D cellular aggregates that resemble features of the human cortex, in which cortical neurons arrange themselves in patterns similar to the postnatal

brain (Sloan et al., 2018). These spheroids possess glutamatergic neurons and astrocytes (Yoon et al., 2019), and when mature, they display robust electrical activity. Human brain spheroid models have been previously used to recapitulate features of neurodevelopmental disorders. In addition, they are used as platforms to screen for therapeutical interventions. In the present study, we present the use of CRISPR/Cas9-edited hiPSCs to generate cortical spheroids carrying the epilepsy-related SCN2A GoF mutation L1342P to study its effect on neuron development and further characterize its disease phenotypes. We will also test the use of Antisense oligonucleotides (ASOs), which are synthetic nucleotide molecules that target messenger RNA (mRNA) and are used to inhibit the expression of disease-causing proteins. The disease phenotypes will be assessed by immunocytochemistry, organoid morphology assessments, microelectrode array (MEA), and patch-clamp recordings. Our results will provide insight into how Nav1.2 gain-of-function mutations may affect neuronal excitability and development, as well as present a platform suitable for testing therapeutic interventions to advance personalized precision medicine.

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Poster

440. Sodium Channel Dysfunction in Disease

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Program #/Poster #: 440.08

Topic: B.03. Ion Channels

Support: Showalter Research Trust
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Title: Reversible social deficits by the genetic restoration of Nav1.2 in adult mice

Authors: *J. ZHANG, X. CHEN, M. EATON, B. A. DEMING, J. WU, Z. QUE, Y. ZHAO, M. I. OLIVERO ACOSTA, K. WETTSCHURACK, Y. LIN, N. CUI, Y. YANG;
Medicinal Chem. and Mol. Pharmacol., Purdue Univ., West Lafayette, IN

Abstract: Genetic variants in the voltage-gated sodium ion channel Nav1.2 (encoded by gene *SCN2A*) are strongly linked with autism spectrum disorder (ASD), epilepsy, as well as other neurodevelopmental disorders according to recent whole-exome sequencing studies in humans. Nav1.2 channel is a major voltage-gated sodium channel in the central nervous system (CNS)

supporting action potential (AP) firing. Nav1.2 is predominantly expressed in the principal neurons of the cortico-striatal circuit, including pyramidal neurons in the medial prefrontal cortex (mPFC) and medium spiny neurons (MSNs) in the striatum. The current paradigm suggests that Nav1.2 gain-of-function variants enhance neuronal excitability resulting in epilepsy, whereas Nav1.2 loss-of-function or protein-truncating variants (collectively referred to as Nav1.2 deficiency) impair neuronal excitability contributing to autism. This paradigm, however, does not explain why 20%~30% of patients with Nav1.2 deficiency still develop seizures. Recently, using different mouse models, both Dr. Bender's and our (Dr. Yang's) labs reported a counterintuitive finding that severe Nav1.2 deficiency results in increased intrinsic neuronal excitability in brain slices, challenging the current dogma. However, how the *ex vivo* increased intrinsic neuronal excitability contributes to the *in vivo* neuronal activity is elusive. Also, how severe Nav1.2 deficiency affects social behavior, a hallmark behavior that is impaired in ASD, remains unknown. Here, we find that Nav1.2-deficient mice, generated by the gene-trap strategy, show major social impairments. Interestingly, the social behaviors can be bi-directionally modulated by the genetic manipulations of *Scn2a* expression. By analyzing signals of c-fos, an immediate-early gene, in cleared brain samples from mice undergoing social stimulation, we find that Nav1.2-deficient mice show altered neuronal activities in multiple brain regions. Using *in vivo* calcium imaging of freely moving mice as a surrogate to monitor neuronal activity, we perform additional studies to further understand how Nav1.2 deficiency perturbs the neuronal functions and brain circuits resulting in social deficits. Our study is expected to reveal the cellular and circuit basis underlying Nav1.2 deficiency-related behavior abnormalities, which will help to advance targeted intervention for Nav1.2-related disorders.

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Poster

440. Sodium Channel Dysfunction in Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 440.09

Title: WITHDRAWN

Poster

440. Sodium Channel Dysfunction in Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 440.10

Topic: B.03. Ion Channels

Support: NIH Grant R01MH12435101 (FL)
U18DA052504 (FL)
Technology Commercialization Program (FL)

Title: Ifgf14 peptide derivative differentially regulates $\text{Na}_v1.2$ and $\text{Na}_v1.6$ function

Authors: *P. ARMAN¹, Z. HAGHIGHIJOO¹, Y. XUE¹, P. WANG¹, H. CHEN¹, J. ZHOU¹, D. ANTUNES³, F. LAEZZA²;

¹Univ. of Texas Med. Br., Galveston, TX; ²Dept. of Pharmacol. & Toxicology, Univ. of Texas Med. Br., Friendswood, TX; ³Dept. of Biol. & Biochem., Univ. of Houston, Houston, TX

Abstract: Voltage-gated Na^+ channels (Na_v) are the molecular determinants of action potential initiation and propagation because of their role in mediating ionic flow (Na^+). Out of the nine voltage-gated Na^+ channels ($\text{Na}_v1.1$ - $\text{Na}_v1.9$), $\text{Na}_v1.2$ and $\text{Na}_v1.6$ are of particular interest because of their expression and distribution throughout the central nervous system (CNS), especially in pyramidal neurons. Although the α -subunit can sufficiently confer transient Na^+ currents (I_{Na}), in vivo these channels exist alongside β -subunits and in addition are modulated by auxiliary proteins like intracellular fibroblast growth factor 14 (FGF14) through protein:protein interactions (PPI). Previous studies have identified ZL0177, a peptidomimetic derived from a short peptide sequence thought to mediate the FGF14: $\text{Na}_v1.6$ PPI, as a functional modulator of $\text{Na}_v1.6$. In this report, ZL0177 was chosen for selectivity evaluation against $\text{Na}_v1.2$ and $\text{Na}_v1.6$. We observed statistically significant changes in peak I_{Na} density as well as shifts in both $V_{1/2}$ of activation and steady-state inactivation that were isoform specific. To that end, ZL0177 effectively decreased $\text{Na}_v1.6$ and $\text{Na}_v1.2$ mediated peak I_{Na} density at $10 \mu\text{M}$. However, preliminary studies indicate that, at $10 \mu\text{M}$, ZL0177 caused statistically significant shifts in $V_{1/2}$ of activation that were isoform specific when compared to their corresponding controls. In addition, ZL0177 ($10 \mu\text{M}$) caused a selective shift in $V_{1/2}$ of steady-state inactivation for $\text{Na}_v1.2$, with no effect in $\text{Na}_v1.6$. In silico docking predicted hydrogen bond interactions of ZL0177 with residues Ile1896 and Asp1856 in the $\text{Na}_v1.2$ and Ser1838 and Ile1886 in $\text{Na}_v1.6$ as well as π - π interactions with Phe1859 in the $\text{Na}_v1.2$ and His1843 in $\text{Na}_v1.6$. In conclusion, ZL0177 produced concomitant reduction in $\text{Na}_v1.2$ and $\text{Na}_v1.6$ peak I_{Na} , but exhibited selectivity towards voltage-sensitivity of activation and steady-state inactivation. These ZL0177 isoform-specific effects might be driven by the differential interactions of the compound with $\text{Na}_v1.2$ and $\text{Na}_v1.6$ residues. This study provides useful information for the development of novel isoform-specific probes and future neurotherapeutics against Na_v channels.

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Poster

440. Sodium Channel Dysfunction in Disease

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Program #/Poster #: 440.11

Topic: B.03. Ion Channels

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John Sealy Memorial Endowment Funds (F.L.)
NIH Grant R01 DA038446 (J.Z.)
NIH Grant P30 DA028821 (J.Z.)
CPRIT RP150578 (C.S.)

Title: Expanding the Druggable Genome: Pharmacologically Targeting Protein:Protein Interaction Interfaces for Neuropsychopharmacological Probe Development

Authors: *N. M. DVORAK¹, P. A. WADSWORTH¹, D. S. ENGELKE², A. K. SINGH¹, N. NGUYEN³, C. M. TAPIA¹, P. WANG¹, H. CHEN¹, R. T. POWELL³, C. STEPHAN³, F. H. M. DO MONTE², J. ZHOU¹, F. LAEZZA¹;

¹Dept. of Pharmacol. & Toxicology, Univ. of Texas Med. Br., Galveston, TX; ²Dept. of Neurobio. & Anat., Univ. of Texas Hlth. Sci. Ctr., Houston, TX; ³Inst. of Biosci. & Technol., Texas A&M Hlth. Sci. Ctr., Houston, TX

Abstract: Perturbations of neuronal excitability cause neuropsychiatric disorders. Current therapeutics target receptors, enzymes, and transporters to ameliorate the aberrant neuronal activity; however, these approaches are associated with intolerability and delayed therapeutic onset. Thus, there is a need to identify new surfaces to target to develop improved neuropsychopharmacological agents. Whereas protein:protein interaction (PPI) interfaces have been targeted in the oncology field to develop antineoplastic agents with lessened side effects, these surfaces have not yet been explored in central nervous system (CNS) drug discovery. Among the CNS interactome, the PPI between the voltage-gated Na⁺ (Na_v) channel isoform 1.6 (Na_v1.6) and its auxiliary protein fibroblast growth factor 14 (FGF14) represents an attractive target on account of its regulation of the output of medium spiny neurons (MSN) of the nucleus accumbens (NAc). To test this, we screened ~45,000 compounds against the complex using a cell-based assay. This screening, in tandem with a Lipinski's analysis, potency studies, and selectivity studies identified 4 non-toxic compounds with favorable drug-like properties that had potent and selective effects on the FGF14:Na_v1.6 complex. Surface plasmon resonance revealed that the 4 compounds had binding to FGF14 or Na_v1.6. Then, a combination of patch-clamp electrophysiology and molecular docking was used, which revealed that the 4 ligands had conserved effects on Na_v1.6 channel inactivation, effects on MSN firing, and predicted interactions with residues at the FGF14:Na_v1.6 PPI interface with established roles in regulating Na_v1.6 channel inactivation. Due to its superior potency, 1028 was selected as a representative ligand from this class for mechanism of action studies. Consistent with our molecular docking studies and models of Na_v channel inactivation, 1028 was shown to bind to FGF14, modulate FGF14:Na_v1.6 complex assembly, and manipulate Na_v1.6 channel inactivation through a mechanism dependent upon an intact interaction between FGF14^{R117} and the Na_v1.6^{D1846:R1866} salt bridge. Consistent with this 1028 was shown to potentiate MSN firing through a mechanism dependent upon FGF14^{R117} *ex vivo*. *In vivo*, 1028 was shown to potentiate firing rates of accumbal neurons and help sustain motivation in satiated states. Overall, we show that small molecule modulation of the FGF14:Na_v1.6 complex increases Na_v channel availability through

manipulating the interaction between FGF14^{R117} and Nav1.6^{D1846;R1866}, which increases MSN firing and leads to maintenance of motivation in satiated states.

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Poster

440. Sodium Channel Dysfunction in Disease

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Program #/Poster #: 440.12

Topic: B.03. Ion Channels

Support: Merit Review Award B9253-C
Merit Review Award BX004899
NIH MSTP T32GM007205

Title: The role of polybasic motifs in the L1 of Nav1.7 in channel trafficking to the distal axonal membrane of sensory neurons

Authors: *S. TYAGI^{1,2,3,4}, G. HIGERD-RUSLI^{1,2,3,4}, D. GEBERT^{1,3,4}, M. ALSALOUM^{1,2,3,4}, S. LIU^{1,3,4}, F. DIB-HAJJ^{1,3,4}, S. G. WAXMAN^{1,3,4}, S. D. DIB-HAJJ^{1,3,4};
¹Neurol., ²Med. Scientist Training Program, Yale Sch. of Med., New Haven, CT; ³Ctr. for Restoration of Nervous Syst. Function, ⁴Ctr. for Neurosci. and Regeneration Res., VA Connecticut Healthcare Syst., West Haven, CT

Abstract: Non-addictive treatment of chronic pain represents a major unmet clinical need. Peripheral voltage-gated sodium channels are an attractive target for pain therapy because they are critical for initiation and propagation of action potential in primary afferents that detect and transmit noxious stimuli. Nav1.7 is the best validated peripheral sodium channel involved in human pain, and previous work has shown that it is transported in vesicles in sensory axons which also carry Rab6a, a small GTPase known to be involved in vesicular packaging and transport. Understanding the mechanism of the association between Rab6a and Nav1.7 could lead to therapeutic modalities which take advantage of the interactions between these two proteins to decrease trafficking of Nav1.7 to the distal axonal membrane. Previous studies have shown that polybasic motifs (PBM) regulate the interaction of a viral cellular protein and Rab11. In this study, we explored if two Polybasic Motifs (PBM) in the L1 of human Nav1.7 (476RRNRRKKK; 510RRK) were responsible for association with Rab6a and if site-directed mutagenesis of either of these motifs could 1) reduce co-trafficking of Nav1.7 and Rab6a, and 2) reduce expression of Nav1.7 at the distal axonal surface. We generated Nav1.7 constructs that contained Alanine-substitutions at each of the charged residues in the two L1-PBM (Nav1.7 476AANAAAAA; Nav1.7 510AAA). Each of these constructs were modified to fuse an additional transmembrane segment affixed with a HaloTag enzyme at the extracellular surface,

which enabled the use of Optical Pulse-chase Axonal Long-distance (OPAL) imaging to investigate co-trafficking of these channels with Rab6a in live sensory axons. Mutations of the PBMs did not alter gating properties of the mutant channels or the expression level as determined by voltage-clamp recordings. Our studies use the OPAL imaging method to assess the effect of mutating the PBMs on trafficking of Nav1.7 channels in sensory neurons and the association of the channel with Rab6A proteins. These studies may shed light on mechanisms that regulate Nav1.7 trafficking in sensory neurons.

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Poster

440. Sodium Channel Dysfunction in Disease

Location: SDCC Halls B-H

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Program #/Poster #: 440.13

Topic: B.03. Ion Channels

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PhRMA Foundation (P.A.W)

Title: Disruption of the FGF13:Nav1.7 complex as a novel anti-pain therapeutic strategy

Authors: ***A. K. SINGH**¹, N. M. DVORAK¹, J. DI RE¹, M. BERNABUCCI¹, O. FOLORUNSO¹, C. M. TAPIA¹, P. WANG¹, H. CHEN¹, K. M. HANKERD², J. WANG², F. K. KADAKIA⁴, P. A. WADSWORTH¹, P. M. GRACE⁵, S. DAVIDSON⁴, K. RAJARATHNAM³, J. ZHOU¹, J.-H. LA², J. CHUNG², D. GREEN², F. LAEZZA¹;

¹Dept. of Pharmacol. & Toxicology, ²Dept. of Neuroscience, Cell Biology, & Anat., ³Dept. of Biochem. & Mol. Biol., Univ. of Texas Med. Br., Galveston, TX; ⁴Dept. of Anesthesiol., Univ. of Cincinnati, Cincinnati, OH; ⁵Dept. of Symptom Res., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: The voltage-gated Na⁺ (Nav) channel Nav1.7 is a molecular determinant of action potential firing of dorsal root ganglia (DRG) sensory neurons. Despite the canonical role of the pore-forming α subunit in conferring this function, protein:protein interactions (PPI) between the α subunit and its auxiliary proteins are necessary for the full physiological function of the Nav1.7 channel. Among such auxiliary proteins, fibroblast growth factor 13 (FGF13) is of particular prominence, and its PPI with the C-terminal domain of the Nav1.7 channel regulates conversion of noxious stimuli into persistent DRG firing and consequently pain sensation. Crucially, in

response to painful stimuli, there is an increase in FGF13 expression and a corresponding increase in transient Na^+ current (I_{Na}) and excitability of DRG neurons, which collectively contribute to inflammatory pain. As these electrophysiological and behavioral responses are attenuated by genetic deletion of FGF13 and resultant reductions in FGF13:Nav1.7 complex assembly, targeting the PPI could represent a novel therapeutic strategy for pain management. To test this hypothesis, we first employ a peptidomimetic derived from the PLEV motif of the $\beta 12$ sheet of FGF13 (PW164) and show that the ligand inhibits FGF13:Nav1.7 complex assembly. Functionally, PW164 prevents FGF13-mediated potentiation of Nav1.7 currents, reduces channel availability in heterologous cells and human DRG neurons, and suppresses firing in donor-derived DRG neurons. In preclinical pain models associated with hyperactivity of Nav1.7 channels, intradermal injection of PW164 prevents capsaicin-induced mechanical hypersensitivity at the level of single afferent fibers and nociceptive behavior without affecting normal mechanosensitivity and, furthermore, reduces postoperative mechanical hypersensitivity. Secondly, we employ a peptidomimetic derived from the FLPK motif of the $\beta 12$ sheet of FGF13 (ZL192). Whereas PW164 reverses the potentiated Nav1.7-mediated I_{Na} induced by noxious stimuli, ZL192 augments Nav1.7-mediated I_{Na} , suggesting that FGF13 is able to bidirectionally control electrophysiological responses to painful stimuli. Overall, these studies demonstrate that pharmacological manipulation of the FGF13:Nav1.7 complex confers selective anti-hyperalgesic effects by acting exclusively on hyperactive Nav1.7 channels associated with nociception without compromising normal sensory function.

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Poster

440. Sodium Channel Dysfunction in Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 440.14

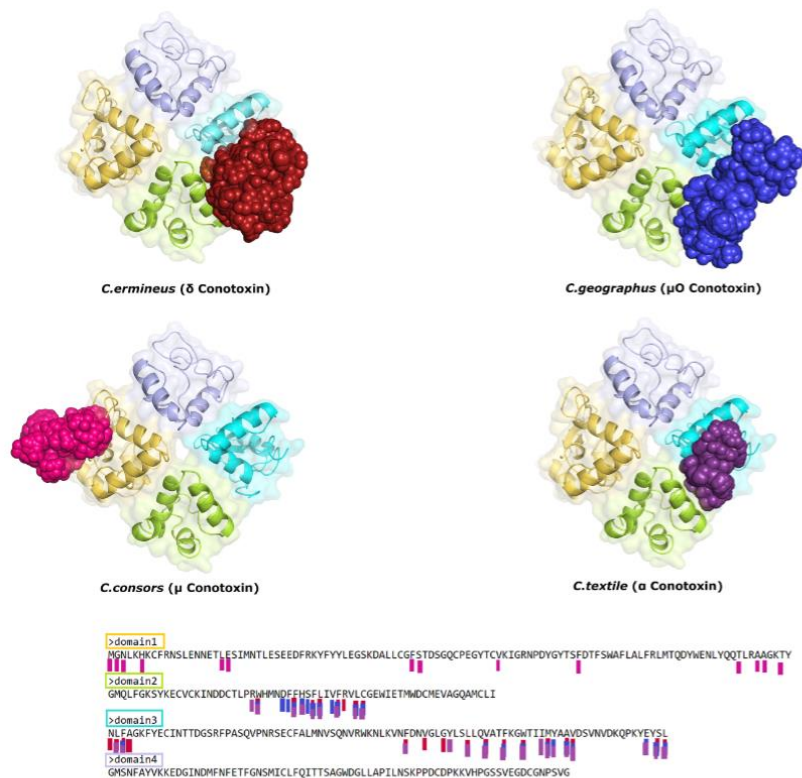
Topic: B.03. Ion Channels

Support: UGC-BCR-97

Title: Computational Insights into Conotoxin Variants - A drug candidate for pain Sodium channel

Authors: *C. KADIRIMANGALAM, S. NATESAN, G. SRINIVASAN, S. A.P, K. GUPTA, G. B A;
Sastra Deemed to be Univ., SASTRA Univ., Thanjavur, India

Abstract: The development of novel drugs to treat a variety of neurological disorders is oriented on voltage-gated sodium channels (Na_v). $\text{Na}_v1.7$ being one of the nine sodium channel isoform mainly involved in neuropathy pain. With recently solved $\text{Na}_v1.7$ structure, a promising drug candidate for pain-related diseases targeting $\text{Na}_v1.7$ will be available. Conotoxins are mainly small disulfide-rich peptides from the venom of cone snails with diverse composition, and biological functions. Conotoxins could be a selective ligand for certain subtypes of Na_v . This work aims to computationally analyse potential binding sites in $\text{Na}_v1.7$ for four distinct conotoxins that might be the beginning point for potential pain killers. Four different peptides of cone snail venom from *C. consors* μ -Conotoxin (2YEN), *C. geographus* μ O-Conotoxin (2N8H), *C.textile* α - conotoxin (6OTA) and *C.ermineus* δ -conotoxin (1G1Z). The structure of the $\text{Na}_v1.7$ (6J8H) was retrieved from PDB. ZDOCK was used to dock $\text{Na}_v1.7$ with the four cone snail venom peptides, and the analysis on interacting residues was performed using PISA. The docking results demonstrate that 3 distinct conopeptides (*C. geographus*, *C.ermineus*, *C.textile*) bind primarily to the pore forming regions of domain II (D II) and domain III (D III), whereas *C. consors* interacts with pore forming regions of domain I (D I) in $\text{Na}_v1.7$. Figure 1 shows the binding pockets of all conopeptides with $\text{Na}_v1.7$. In D III, hydrophobic interactions (F1343, F1405, W1408) are prominent in δ , α and μ O- conotoxins and hydrophilic interactions (W908, D912, H915, R922) have also been identified in *C.ermineus* for D II. In contrast, *C. consors* μ -Conotoxin exhibits distinct hydrophobic interactions (F317, F344, V331) in D I, whereas no other interactions were seen in D II and D III. Thus *C. consors* μ -Conotoxin has unique binding site which makes it a candidate for unique $\text{Na}_v1.7$ blocker. Hence, the utilization of toxins will considerably increase our understanding of the biophysical and pharmacological characteristics of channels, particularly in distinguishing specific channel activity.



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Poster

440. Sodium Channel Dysfunction in Disease

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 440.15

Topic: B.03. Ion Channels

Support: NIH R35HL155671

Title: Roles of Nav1.9 in Inflammatory Mediator-Induced Activation of Mouse Airway Vagal C-fibers.

Authors: J. S. KIM, H. SUN, S. MEEKER, *B. J. UNDEM;
Med., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: We used male C57/BL6 Nav1.9 knockout (KO) mice to evaluate the role of Nav1.9 in the chemical activation of vagal C-fiber terminals in the mouse lungs. Single-cell rt-PCR of lung-labeled neurons revealed that 90% of TRPV1-expressing vagal sensory neurons innervating the lungs express Nav1.9 mRNA. We isolated the mouse lungs with the vagus nerves and vagal ganglia intact. Action potentials were recorded using extracellular electrodes positioned near the relevant cell bodies in the vagal ganglion. The stimuli were applied to the receptive field via perfusion through the mouse trachea. The PAR1 agonist, TFLLR(10 μ M), consistently activates nodose C-fiber terminals in mouse lungs in wild-type (WT) but not in PAR1^{-/-} mice. A 1ml perfusion of TFLLR(10 μ M) evoked 270 \pm 62 action potentials in WT nodose C-fibers (n=23), but only 85 \pm 38 action potentials in Nav1.9^{-/-} mice (P< 0.01, n=29). PAR1 is a GPCR stimulus, so we next evaluated α , β -methylene ATP that stimulate nodose C-fibers in the mouse lung via activation of the ionotropic P2X2/3 receptor. 1ml perfusion of α , β -methylene ATP activated nodose terminals averaged 93 \pm 18 action potentials (n=33), whereas the same treatment elicited only 13 \pm 4 action potentials in the Nav1.9^{-/-} mice (P=0.0001, n= 35). The C-fibers of both WT and Nav1.9^{-/-} were equally activated by rapid punctate mechanical stimulation of the receptive field with von Frey fibers. At the patched clamped cell soma, there was no difference in activation or passive electrophysiological characteristics of nodose neurons isolated from WT versus KO animals. There was no significant difference in the rheobases 125 \pm 32 and 102 \pm 23 pA, respectively, as well as AP threshold -36 \pm 1 and -38 \pm 1 mV, respectively. However, bath-applied α , β -methylene ATP was more effective in evoking action potentials in neurons isolated from WT (58 % responding) vs. KO (8 % responding). The data reveal that Nav1.9 plays an important role in the activation of nodose C-fiber terminals evoked by inflammatory mediators that act via GPCRs or ionotropic receptors but is less critical in the activation by rapid punctate mechanical stimulation or rapid current injection. We hypothesize that Nav1.9 is an intriguing target for treating chronic coughing, excessive reflex bronchospasm, and secretions associated with inflammatory airway disease without interfering with the protective reflexes evoked by mechanical activation that may occur during aspiration.

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Poster

440. Sodium Channel Dysfunction in Disease

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 440.16

Title: WITHDRAWN

Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.01

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: 1ZIAN003135-10

Title: Unraveling the substantia nigra pars lateralis: electrophysiological intrinsic properties of non-canonical dopaminergic neurons.

Authors: *L. SANSALONE;

Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Dopaminergic neurons (DA) located in the substantia nigra pars lateralis (SNL) increase their firing in response to salient stimuli and have been shown to be critical for learning during aversive or threatening behaviors (Menegas et. al. **2018**). While the properties of DA neurons in the neighboring substantia nigra pars compact (SNC) are well characterized, there are few studies that examine the action potential firing patterns of SNL DA neurons. Therefore, we set out to define the intrinsic action potential firing properties of dopaminergic neurons within the SNL from 2-6 months old mice. To identify SNL neurons, we used a combination of either retrograde labeling from the tail of the striatum or Calb1-Cre and VGlut2-Cre mice bred with DAT-Flp Ai65. We found that the rate of natural pacemaking in SNL DA neurons was slightly lower compared to firing in the classic DA neurons located in the ventromedial SNC (*SNL*: 2.1 ± 1.0 Hz, $n = 29$; *SNC*: 2.8 ± 0.7 Hz, $n = 13$). While SNC DA neurons showed highly regular pacemaker firing consistent with past literature, action potential firing in SNL neurons was highly irregular (*SNC*: 4.0 ± 1.7 %, $n = 13$; *SNL*: 28.8 ± 15.4 %, $n = 29$). In addition, we found that SNL DA neurons exhibited higher input resistances, maximal firing rates (100 pA - *SNL*: 36.08 ± 14.38 Hz, $n = 11$; *SNC*: 10.84 ± 3.48 Hz, $n = 10$) and smaller voltage sags relative to SNC DA neurons (*steady voltage*: -70 mV, VGlut2+ *SNL*: 6.6 mV \pm 3.6 mV, $n = 15$; *SNC*: 24.4 ± 5.0 mV, $n = 11$), indicative of weaker I_H recruitment. Comparison of Calb1+ and VGlut2+ DA neurons in SNL showed no significant differences in spontaneous firing rates and spiking characteristics, suggesting that these cells belong to the same subtype. Therefore, we find that SNL DA neurons are clearly distinct in their firing properties compared to SNC DA neurons, consistent with their different functional role. Testing synaptic inputs, we found that the frequency of “miniature” EPSCs (mEPSC) was substantially higher in SNL DA neurons relative to SNC DA neurons suggesting strong excitatory input onto SNL DA neurons (*mEPSC freq*, *SNL*: 14.3 ± 9.8 Hz, $n = 8$; *SNC*: 1 Hz \pm 0.5 Hz). Interestingly, the frequency of inhibitory inputs to SNL was low (mIPSC freq, SNL DA neurons *SNL*: 0.6 ± 0.2 Hz, $n = 4$) which was comparable to rates in SNC. Together, both suggest that SNL neurons have a higher ratio of excitatory to inhibitory input. Given the role of SNL DA neurons in salience and learning responses to threat, the high-frequency firing that we observe, coupled with the stronger excitatory input that they receive, may be a critical feature underlying SNL DA neuron’s ability to respond rapidly to salient stimuli.

Disclosures: L. Sansalone: None.

Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.02

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Compagnia di San Paolo, Progetto Trapezio
Italian Miur, 2015FNWP34

Title: Altered firing of midbrain dopaminergic neurons by exogenous α -synuclein oligomers

Authors: *V. CARABELLI^{1,3}, G. TOMAGRA^{1,3}, F. CESANO^{2,3,4}, C. FRANCHINO¹, E. CARBONE^{1,3}, A. MARCANTONI^{1,3};

¹Drug Sci. Dept., ²Chem. Dept., Univ. of Torino, Torino, Italy; ³NIS Interdepartmental Ctr., Torino, Italy; ⁴INSTM-UdR, Torino, Italy

Abstract: The aim of this work was to characterize the firing patterns of cultured midbrain dopamine (DA) neurons, dissociated from substantia nigra TH-GFP mice embryos, and to investigate the effects of exogenous α -synuclein oligomers on circuit maturation. To this purpose, midbrain neurons were cultured on microelectrode arrays (MEAs) and their activity was monitored since 7 until 21 days in vitro (DIV). Within this temporal range, the network exhibited different patterns of spontaneous firing activity, which was mainly sporadic at 7-9 DIV and became dominated by bursts after 14 DIV. In order to understand the role of DA neurons in sustaining the spontaneous firing, we applied selective blockers to silence the GABA_A, AMPA and NMDA-mediated transmission. Under these conditions, we could still detect a residual spontaneous firing activity that exhibited either high-rate or low-rate pace-making features or even non-pace-making activity. After characterizing the firing of DA neurons, we investigated the effect of α -synuclein oligomers at different stages of network development. By adding α -synuclein oligomers to the culture medium we found that α -synuclein progressively reduced the spontaneous firing in a time- and concentration-dependent manner ($K_d = 1.03 \mu\text{M}$), impaired burst generation and reduced network synchronism. These findings uncover the effects of exogenous α -synuclein at different stages of network development and provide new evidence for the early detection of neuronal function impairment.

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Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.03

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Effects of oxytocin treatment on mesolimbic dopamine functioning in male and female B6 and D2 mice

Authors: *M. K. ESTES, J. P. MANUS, R. PACE, P. NALAN, M. N. COOK, D. B. LESTER; Psychology Dept., Univ. of Memphis, Memphis, TN

Abstract: Systemic oxytocin administration has been shown to decrease behavioral responses to drugs of abuse. Previously, our lab demonstrated that subchronic oxytocin administration reduces baseline dopamine release and attenuates dopaminergic responses to nomifensine in the nucleus accumbens (NAc) of adult male B6 mice. Sex differences in the effects of oxytocin administration on dopamine transmission had not been examined in either B6 or D2 mice. The purpose of the current experiment was to compare the effects of systemic oxytocin administration on mesolimbic dopamine transmission in male and female B6 and D2 mice. Mice received an intraperitoneal injection of either oxytocin (1 mg/kg) or saline every 48 hours for 16 days (8 total injections). Forty-eight hours after the last injection, *in vivo* fixed potential amperometry was used to assess NAc dopamine release and synaptic half-life before and after an injection of nomifensine, a dopamine transporter (DAT) inhibitor. Oxytocin's effects on dopamine functioning were both sex- and strain-dependent. In B6 mice, oxytocin administration did not alter baseline dopamine release or synaptic half-life, but oxytocin-treated male mice displayed decreased dopaminergic response to DAT inhibition via nomifensine. In D2 mice, oxytocin administration did not alter baseline dopamine release but did decrease the synaptic half-life of dopamine, which is an indication of faster reuptake and more efficient DAT. Oxytocin did not alter the dopaminergic effect of DAT inhibition via nomifensine in D2 mice. Interestingly, both of the oxytocin-induced differences observed in B6 and D2 mice are related to DAT functioning. Following a nomifensine injection, only oxytocin-treated female D2 mice showed an increased percent change in dopamine half-life. These findings demonstrate sex and strain differences on dopamine functioning following oxytocin treatment and highlight the need for more in-depth research on the effects of long-term administration of oxytocin.

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Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.04

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Age-dependent modulation of layer V pyramidal neurons' excitability by dopamine D1 receptor in mouse primary motor cortex

Authors: *V. PLATEAU, F. GEORGES, J. BAUFRETON, M. LE BON-JÉGO; Inst. des Maladies Neurodégénératives, CNRS UMR 5293, Bordeaux, France

Abstract: Like the basal ganglia but to a lesser extent, the primary motor cortex (M1) is innervated by dopaminergic terminals, in both superficial and deep layers. Furthermore, it has been demonstrated that dopamine (DA) receptors are expressed in M1. To date, there is strong body of evidence that DA is instrumental for M1 but the level of understanding of DA action in M1 is rather macroscopic. Indeed, the specific location and function of DA receptors remain poorly understood. Very few is known concerning the distribution and the effect of DA receptor on specific subtypes of neurons in M1 during the growth and at adult state. Thus, the aim of this study was to investigate the impact of D1 receptor activation and blockade on pyramidal neurons (PNs) intrinsic properties at different ages in the layer V of M1. For this purpose, young (P16-P25 old) and adult (6-12 weeks old) male and female mice expressing the green fluorescent protein (GFP) in D1 receptor-expressing cells were used (D1-GFP mice). First, we quantified and made a cartography of the different types of PNs expressing D1 receptor within M1 in young and adult mice using immunohistochemistry for molecular markers of cortical- (Satb2) and subcortical- (Ctip2) projection PNs. Then, we investigated the modulation of D1 receptor on M1 layer V PNs' excitability and intrinsic properties in young and adult mice. To this aim, we performed whole cell patch clamp recordings of M1 layer V PNs, coupled with pharmacology to either activate (with 2.5 μ M SKF 81297) or block (with 1 μ M SCH 23390) D1 receptor. To ensure the results obtained were not due to a network effect, all recordings were made with the fast synaptic transmission blockers DNQX (50 μ M), AP V (20 μ M) and GABAzine (10 μ M). Our results suggest an age-dependent modulation of M1 layer V PNs' excitability. Indeed, an increase of the excitability of M1 layer V PNs was induced by D1 receptor activation both in adult (n = 17) and young animals (n = 9), with a stronger effect in young mice. However, the effect of D1 receptor blockade shifted from a decrease of the excitability of M1 layer V PNs in young mice (n = 9) to an increase in adult mice (n = 12). Taken together, these results clarify the impact of D1 receptor activation and blockade on M1 layer V PNs. Several physiological processes might depend on these effects, such as motor execution, planning and learning.

Disclosures: V. Plateau: None. F. Georges: None. J. Baufreton: None. M. Le Bon-Jégo: None.

Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.05

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: DA051450

Title: Intrinsic excitability of direct pathway spiny projection neurons in the dorsolateral striatum is increased in mice trained to self-administer methamphetamine.

Authors: *S. CHOI, S. M. GRAVES;
Dept. of Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: Methamphetamine (meth) is a potent psychostimulant and its illicit use in the US has increased in recent years (NSDUH 2020). The dorsolateral striatum (DLS) receives dopaminergic input from the substantia nigra pars compacta and has been implicated in drug-seeking behaviors. Lesioning, non-specific dopamine receptor antagonism, and D1 dopamine receptor antagonism in the DLS all decrease drug-seeking behaviors (Vandershuren *et al.*, *J Neurosci* 25:8665, 2005; Murray *et al.*, *Neuropsychopharm* 37:2456, 2012; Murray *et al.*, *Biol Psych* 76:15, 2013; Gabriele & See *Brain Res* 1417:27, 2011; Li *et al.*, *J Neurosci* 35(21):8232, 2015); however, underlying neuronal dysfunction that may contribute to maladaptive drug-seeking is unclear. The DLS is mainly comprised of direct pathway spiny projection neurons (dSPNs) that express D1 dopamine receptors and indirect pathway spiny projection neurons (iSPNs) that express D2 dopamine receptors (Gerfen and Surmeier, *Annu Rev Neurosci* 34:441, 2011). To determine the consequence of meth self-administration on DLS neuron function, male mice expressing eGFP and tdTomato under the control of *Drd2* and *Drd1a* receptor regulatory elements, respectively, were implanted with chronic indwelling jugular vein catheters and trained to self-administer meth (0.1 mg/kg/infusion) for 10 consecutive days on a fixed ratio 1 schedule of reinforcement using active and inactive nose poke manipulanda; infusions were paired with a cue light and self-administration sessions were 2 hr per day during the light cycle. Each mouse that self-administered meth was paired with a saline-yoked control subject. Mice were group housed and provided food and water *ad libitum* throughout the duration of the study; on the last day of meth self-administration mice received on average approximately 20 infusions resulting in approximately 2 mg/kg meth. After 1-4 days of abstinence in the home cage subjects were euthanized and *ex vivo* brain slices prepared entailing the DLS; iSPN and dSPN intrinsic excitability was assessed by depolarizing current injection while neurons were patched in whole-cell configuration. Meth self-administration resulted in an increase in DLS dSPN intrinsic excitability; depolarizing current injections generated more action potentials in DLS dSPNs from mice trained to self-administer meth than saline-yoked control mice. In contrast, meth self-administration had no effect on DLS iSPN intrinsic excitability when measured at 1-4 days of abstinence. These data suggest that meth self-administration produces a dSPN dominant DLS that may contribute to meth-seeking behavior.

Disclosures: S. Choi: None. S.M. Graves: None.

Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.06

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: 1R01AA025652-01
1R01AA025652-01
T32AA014127

Title: Effects of acute and prenatal alcohol exposure on posterior parietal cortex.

Authors: *V. LICHERI, S. L. OLGUIN, B. Y. JACQUEZ, C. VALENZUELA, J. L. BRIGMAN;

Dept. of Neurosciences, Univ. of New Mexico, Albuquerque, NM

Abstract: Alcohol consumption during pregnancy can lead to behavioral and cognitive deficits that persist throughout the lifespan. Collectively known as Fetal Alcohol Spectrum Disorders (FASDs) and they include impairments in learning and working memory, executive and social behavior. Previously, we have demonstrated that moderate prenatal alcohol exposure (PAE) impairs cognitive control on a touchscreen continuous performance task and alters frontal and parietal cortical power during performance. In addition, previous studies have shown PAE positively modulates the amplitude of spontaneous post-synaptic current (sIPSCs) in pyramidal neurons and alters the number of GABAergic interneurons expressed in orbitofrontal cortex (OFC). In the present study we tested whether our PAE model was sufficient to alter neurotransmission in posterior parietal cortex (PPC), one of major cortical association areas involved in multiple cognitive processes. At the beginning, we decided to characterize the action of acute alcohol exposure on intrinsic excitability in pyramidal and interneurons expressed in this brain region. In order to measure the intrinsic properties we performed whole-cell current clamp recordings in slices obtained from adult male and female C57BL/6J mice (PND 90-120). Specifically, we injected 13 current pulses equally spaced from -300 to +300 pA. The resting membrane potential, input resistance, rheobase, action potential (AP) threshold and AP frequency were assessed during baseline (ACSF perfusion), following 10 min of bath-application of alcohol (50 mM). The data collected show that acute alcohol exposure reduces the excitability in interneurons, while we did not observe significant differences in pyramidal neurons. To further examine whether alcohol affected the GABAergic transmission in PPC in PAE offspring, whole-cell patch clamp recordings were performed in slices coming from saccharine controls (SAC) and PAE male and female mice sacrificed at PND 90-120 days. sIPSCs were isolated pharmacologically using 50 μ M AP5 and 10 μ M CNQX to block NMDA and AMPA receptor-mediated currents. We observed that our PAE model also modulated the GABAergic transmission in adult offspring. Taken together, these findings could explain a mechanism of cognitive impairments observed in PAE offspring and also during alcohol abuse providing an important tool for developing new effective therapies for executive dysfunctions observed in FASDs.

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Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.07

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Indiana Alcohol Research Center (46-832-50 P64)

Title: Converging Effects of Chronic Pain and Binge Alcohol Consumption on Anterior Insular Cortex Neurons Projecting to the Dorsolateral Striatum

Authors: *Y. YIN, D. HAGGERTY, S. ZHOU, B. ATWOOD, P. SHEETS;
Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Patients with chronic pain are twice as likely to meet the criteria of alcohol use disorder (AUD). There is evidence that both chronic pain and AUD alter similar brain pathways, but this area is understudied. Studies show that anterior insular cortex (AIC) is a brain region involved in both chronic pain and alcohol addiction. However, most of the prior studies focused on local lesions or excitation/inhibition in the AIC, which lack circuit specificity. Dorsolateral striatum (DLS) is a major efferent target of the AIC and is known to govern habitual-directed behaviors that are altered by drugs of abuse including alcohol. The goal of this work is to elucidate the converging effect of binge alcohol consumption and chronic pain on AIC-DLS circuit. Here, we performed intracranial injections of retrograde beads into the DLS of mice to label AIC neurons that project to the DLS (AIC-DLS neurons). We subsequently performed the spared nerve injury (SNI) surgery in injected mice to induce chronic pain behavior. Both control (sham) and SNI mice then underwent the drinking-in-the-dark (DID) paradigm for 3 weeks to model binge-like alcohol drinking. Surprisingly, our results show that SNI mice consume significantly less alcohol during the 3-week DID session. After completing DID, we performed whole-cell patch-clamp in acute brain slices to measure intrinsic and synaptic properties of AIC-DLS neurons. Our recording analyses revealed that AIC-DLS neurons from SNI-alcohol mice display an increased frequency of action potentials in response to depolarizing current steps compared to SNI-water and sham-alcohol mice. This indicates that the combination of SNI and alcohol consumption increases the intrinsic excitability of AIC-DLS neurons. In addition, analyses revealed a significant increase in the frequency, but not amplitude, of miniature excitatory postsynaptic currents (mEPSCs) recorded in AIC-DLS neurons from SNI-alcohol mice compared to SNI-water and sham-alcohol animals. These data indicate that increased excitability of AIC-DLS neurons observed in SNI-alcohol may be due to increased presynaptic activity at glutamatergic synapses. We are currently performing conditioned place preference (CPP) test and saccharin DID to further understand whether there is a difference in the reward value of alcohol between SNI and sham mice. Overall, our data suggest that chronic pain and alcohol drinking have an interaction effect on both intrinsic excitability and synaptic transmission of AIC-DLS neurons in mice, which may be critical in altering motivational behaviors associated with alcohol drinking in chronic pain states.

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Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

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Program #/Poster #: 441.08

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant DC004450
NIH Grant R35NS116798
Promotion of Science KAKENHI Grant 22K06838

Title: Sodium leak channel NALCN underlies noradrenergic modulation of cartwheel interneurons of the cochlear nucleus

Authors: T. NGODUP¹, T. IRIE³, *L. TRUSSELL²;
¹Oregon Hearing Res. Ctr., ²Oregon Hlth. and Sci. Univ., Portland, OR; ³Div. of Pharmacol., Natl. Inst. of Hlth. Sci., Kanagawa, Japan

Abstract: In the dorsal cochlear nucleus, feedforward inhibition from glycinergic cartwheel cells potently shapes the output of fusiform principal neurons. Previous studies (Kuo & Trussell, 2011) found that in cartwheel cells, noradrenaline (NA) inhibited spontaneous spiking, and the postsynaptic effects of that spiking, yet paradoxically enhanced evoked inhibitory currents in postsynaptic cells. These actions could be attributed to the effects of spontaneous firing on presynaptic depression. However, the identity of the effector channel underlying the inhibition of spontaneous firing by NA remained unknown. Here, we report that the Na⁺ leak channel, NALCN, is the primary target of $\alpha 2$ NA receptor action in cartwheel cells. We developed a mouse line in which NALCN is deleted from glycinergic neurons. NALCN KO mice showed reduced spontaneous firing compared to WT mice. Puff application of NA to cartwheel cells resulting in outward currents (due to block of inward current) in WT mice but not in NALCN conditional knockout mice. GABA-B receptor activation by baclofen puff also resulted in an outward current. Such baclofen currents are usually attributed to GIRK K⁺ channels. Yet, in WT cartwheel cells, only 40% of outward baclofen current was blocked by the GIRK blocker Ba²⁺, suggesting an additional mediator of baclofen action. This mediator is likely NALCN, as 80% of outward baclofen current was blocked by Ba²⁺ in NALCN knockout mice. NA application hyperpolarized, and significantly increased firing threshold, in cells from wild-type mice but not from NALCN knockout mice. Finally, we isolated NALCN-mediated currents by blocking Ca²⁺, K⁺, and voltage-gated Na⁺ channels. NA or baclofen inhibited the isolated NALCN currents in WT mice but not in NALCN KO mice. Moreover, the two modulators appeared to act on the same population of NALCN, as application of baclofen completely occluded responses to subsequent application of NA. Therefore, NALCN is a downstream effector of inhibitory G protein-coupled receptors in cartwheel cells.

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Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.09

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIMH R01MH113007

Title: OTR activation directly excites Type I and Type III neurons of dorsolateral bed nucleus of the stria terminalis (BNST_{dl})

Authors: *W. FRANCESCONI¹, F. BERTON¹, V. OLIVERA-PASILIO¹, S. H. OLSON¹, V. GRINEVICH², J. DABROWSKA¹;

¹Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL; ²Dept. of Neuropeptide Res. in Psychiatry, German Cancer Res. Ctr., Heidelberg, Germany

Abstract: The dorsolateral bed nucleus of the stria terminalis (BNST_{DL}) has emerged as a key brain region translating an exposure of unpredictable threats into sustained fear. BNST_{DL} contains three types of neurons functionally classified as Type I, II, and III in rats and monkeys. The BNST_{DL} is innervated by oxytocin (OT) and vasopressin (AVP) fibers and has high expression of OTR and V1aR. We have previously shown that in male rats, OT modulates BNST_{DL} activity in a cell type-specific manner, including a direct excitatory effect on Type I BNST_{DL} neurons. However, the role of V1aR in the modulation of BNST_{DL} activity is unknown. Here, using *in vitro* patch-clamp electrophysiology, we demonstrate that AVP excites both Type I and Type III BNST_{DL} neurons. In Type III neurons, AVP induced a leftward shift of input/output relationship ($F(1,5) = 3.77, P=0.0046, n=6$), but this AVP effect was abolished in a presence of V1aR/OTR antagonist ((d(CH2)5¹, Tyr(Me)², Arg⁸)-Vasopressin, 1 μ M, $F(1,3)=0.5036, P=0.5291, n=4$). In contrast, in a presence of SR49059 (5 μ M), a selective and potent V1aR antagonist, AVP still induced a leftward shift of input/output relationship in Type III neurons ($F(1,6.54, 23.15) = 13.54, P=0.0003, n=10$), suggesting that V1aR do not mediate excitatory effect of AVP. Next, we used a selective and potent OTR antagonist, OTA, d(CH2)5(1), D-Tyr(2), Thr(4), Orn(8), des-Gly-NH2(9)]-Vasotocin, 0.4 μ M). Notably, AVP did not affect the steady state firing frequency of Type III neurons ($F(1,2)=7.516, P=0.1113, n=7$) in the presence of OTA. To establish the possible direct effect of OTR activation we studied the effect of [Thr4, Gly7]-oxytocin (TGOT, 0.4 μ M), a potent and selective OTR agonist and we show a leftward shift of input/output relationship, demonstrating a significant increment of steady state frequency ($F(1,24.5, 14.94) = 30.75, P<0.0001, n=8$) in Type III neurons. We also measured the latency of the 1st spike at the same current pulse before and after TGOT application. This revealed a significant shortening in the latency of the 1st spike after TGOT ($P=0.0007, t=4.646, df=11, n=12$, paired *t*-test), an effect potentially mediated by D-type potassium current (*ID*) via Kv1.2 potassium channels present on Type III neurons. Finally, we recorded from brain slices containing the BNST of OTR-Cre rats (Cre-recombinase under OTR promoter) injected into the BNST_{DL} with AAV-DIO-mCherry. From 27 recorded fluorescent neurons from male rats, 67% were characterized as Type III and 33% were characterized as Type I neurons, results consistent with our pharmacological findings. Our results demonstrate that OTR activation directly excites Type I and Type III neurons of the BNST_{DL}.

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Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.10

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Intrinsic neuronal plasticity occurs around puberty in kisspeptin neurons of the arcuate nucleus of female mice

Authors: *Y. ZHANG, W. COLLEDGE, S. JONES;
Univ. of Cambridge, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Neuronal plasticity is well established in developing neuronal circuits where persistent changes in both structural and functional properties underlie maturing circuit functions. Puberty is a transition period from infertility to fertility and is governed by specific neuronal circuits in the hypothalamus, including the kisspeptin neurons of the arcuate (ARC) nucleus and the anteroventral periventricular (AVPV) nucleus that control the hypothalamic-pituitary-gonadal (HPG) axis. We hypothesised that arcuate kisspeptin (*KissI*^{ARC}) neurons from female mice alter their physiological properties from an immature to a mature phenotype as they transition through puberty. We have investigated the properties of these neurons before puberty (22 to 34 days old, with no vaginal opening) and after puberty (6-9 weeks old, with a stable estrous cycle) to determine whether plasticity of intrinsic neuronal properties occurs during this important period of maturation. We prepared brain slices from female mice expressing tdTomato in *KissI*^{ARC} neurons for whole cell current clamp recordings. We evoked action potentials in response to depolarising current injections under physiological conditions. *KissI*^{ARC} neurons from female mice after puberty (n = 31) showed a current input-spike output relationship that initially increased to a maximum and then decreased, indicative of depolarisation block. The current input-spike output relationship was not affected by the stage of the estrous cycle. *KissI*^{ARC} neurons from female mice before puberty (n = 43) also showed a current input-spike output relationship that initially increased to a maximum and then decreased; however, there was a significantly lower maximum number of action potentials and more depolarisation block than seen after puberty. There was no difference in the maximum frequency of firing or in the pattern of firing (measured as the coefficient of variation in the inter-spike intervals). These data suggest that specific forms of plasticity of intrinsic action potential firing properties of *KissI*^{ARC} neurons occurs either just before or during puberty in female mice. The mechanisms of this are currently being investigated.

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Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.11

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Selective SSTR4 agonists mediate membrane hyperpolarization and reduced intrinsic firing in CA1 pyramidal neurons from humanized SSTR4 rats and human

Authors: J. ARTINIAN¹, J. HUBBARD¹, F. GACKIÈRE¹, D. L. BUHL², K. SCHLEICHER², N. ENGLISH², M. ZIENTEK², O. TOURY¹, B. BUISSON¹, R. E. PETROSKI³, N. J. BROADBENT²;

¹Neuroservice, Le Puy-Sainte-Réparate, France; ²Takeda Develop. Ctr. Americas, San Diego, CA; ³Neuroservice USA, San Diego, CA

Abstract: Human studies suggest that aberrant overactivation of the hippocampal network exacerbates neurodegeneration in Alzheimer's Disease (AD). Somatostatin receptor subtype 4 (SSTR₄) is highly expressed in the hippocampus and may play an anti-convulsant role by downregulating CA1 pyramidal cell intrinsic excitability through coupling to Kv7 channels (responsible for the M-current). In addition, SSTR₄ has been shown to promote amyloid-beta (A β) phagocytosis and clearance, and thus SSTR₄ agonists have been proposed for the treatment of AD. Using whole-cell current-clamp recordings in acute hippocampal slices, we examined the effects of novel selective SSTR₄ agonists (TAKEDA proprietary compounds A and B, cpA, cpB) and an antagonist (compound C, cpC) on membrane and firing properties of CA1 pyramidal cells from 6-15-week-old male Sprague-Dawley rats of wildtype (WT) and SSTR₄ humanized (hSSTR₄) transgenic rats. hSSTR₄ rats were generated by knockout of the rat Sstr4 gene then knock in of the human Sstr4 gene. Recordings were also performed in human hippocampal acute slices from surgical resections. After a 5 min baseline of vehicle perfusion, test compounds were added to the bath for 10-15 min. The Kv7 activator retigabine (30 μ M) was used as a positive control at the end of some recordings. Time-locked vehicle arms were used to monitor stability in control conditions. In response to subthreshold square pulses of current, 10 μ M cpA significantly decreased resting membrane potential (RMP) and input resistance compared to vehicle treated cells in hSSTR₄ but not in WT rats. In contrast, the SSTR₄ agonist J-2156 decreased RMP in both WT and hSSTR₄ rats at 100 nM, with no effect at 10 nM. In response to suprathreshold current injections in WT rats, 100 nM J-2156 and 100 nM cpB failed to alter intrinsic firing from RMP. However, 100 nM cpB significantly decreased membrane potential (V_m) and firing rate and increased the rheobase when cells were depolarized by moderate constant current injection (\sim -50 mV), suggesting that the effect may rely on the functional coupling between SSTR₄ and Kv7 channels. In hSSTR₄ rats, consecutive application of cpA and cpB significantly altered firing and V_m in depolarized cells. Concomitant application of the SSTR₄ antagonist cpC prevented the effect of the agonist cpA, indicating a specific role of SSTR₄. Finally, preliminary results suggest similar effects of SSTR₄ agonists in human pyramidal neurons. In summary, activation of SSTR₄ results in hyperpolarization and reduction of the firing rate of CA1 pyramidal neurons from both hSSTR₄ rats and humans, representing promising future therapeutic strategies.

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E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Takeda Development Center Americas. **N. English:** A. Employment/Salary (full or part-time);; Takeda Development Center Americas. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Takeda Development Center Americas. **M. Zientek:** A. Employment/Salary (full or part-time);; Takeda Development Center Americas. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Takeda Development Center Americas. **O. Toury:** None. **B. Buisson:** None. **R.E. Petroski:** None. **N.J. Broadbent:** A. Employment/Salary (full or part-time);; Takeda Development Center Americas. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Takeda Develop. Ctr. Americas.

Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.12

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: ANR/NSF Grant 1608236
Weill Institute Research Support for Female Learners Impacted by COVID-19 Funding

Title: Characterization of conditional bistability, the neuronal substrate of parametric working memory, and its impairment in normal aging and Alzheimer's disease in mice

Authors: ***M. A. H. LEROUX**¹, D. MEDERNACH², B. A. C. DELORD², J. PAZ¹;
¹Gladstone Inst. of Neurolog. Dis., The J. David Gladstone Institutes, UCSF, San Francisco, CA;
²The Inst. of Intelligent Systems and Robotics, Sorbonne Univ., Paris, France

Abstract: Working memory is the ability to maintain and manipulate transient information for a few seconds to minutes. Parametric working memory (PWM), which allows us to retain quantitative information such as numbers, the frequency of a sound, or the size of an object, is the least understood form of working memory. We use PWM unconsciously, for example, to distinguish the sound of a fire engine's siren in a noisy street long enough to make the decision to pull over. Thus, people with PWM impairments can experience life-threatening situations and eventually lose their ability to live independently. Loss of working memory, including PWM, which can occur late in normal aging, is one of the earliest symptoms of Alzheimer's disease (AD).

Despite its crucial role, the mechanisms underlying PWM are still poorly understood. *In vivo* experiments in humans, monkeys and rodents indicate that PWM involves graded persistent activity (GPA) in the prefrontal cortex (PFC). Models of recurrent neural networks such as those found in the PFC suggest that GPA requires conditional bistability (CB). CB is a form of cellular

memory that depends on sustained sub-threshold network inputs to memorize a transient input. In a biophysical model, we previously demonstrated that layer V PFC pyramidal neurons may underlie CB. However, whether CB exists in the rodent PFC, and how it functions, remained unknown.

To address these gaps, we used patch-clamp electrophysiology in acute PFC slices from young adult rats and mice. We found that: (1) a subset of PFC layer V pyramidal neurons exhibits CB; (2) CB is fine-tuned by acetylcholine (ACh) (97% CB neurons with ACh vs. 14% without ACh, $p=0.0002$); (3) carbachol-induced CB relies on changes in fast, medium and slow afterpolarization potentials mediated through calcium-activated non-selective cationic channels; and (4) CB is not modulated by other common neuromodulators such as dopamine, serotonin and noradrenaline. We evaluated the evolution of CB in normal aging and in a mouse model of Alzheimer's disease (5xFAD). CB declined significantly faster in 5XFAD mice (6 months, $p=0.0129$) than in control mice (12 months, $p=0.0277$).

Understanding the mechanisms underlying CB, a novel intrinsic neuronal property of PFC neurons, will be crucial to assess its role in the emergence of PWM. Ultimately, our findings may highlight new therapeutic targets and critical time windows to improve PWM in physiological and pathological aging.

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Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.13

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant NS086933
NIMH Training Grant MH016880

Title: Akt1 KO causes changes in neuronal excitability and plasticity in the prefrontal cortex

Authors: *J. L. HANSON^{1,2}, J. K. KUSHNER^{3,4}, M. N. SVALINA^{3,4}, C. BORSKI^{1,2}, S. M. BACA^{5,3}, M. M. HUNTSMAN³, C. HOEFFER, Jr.^{2,1};

¹Inst. for Behavioral Genet., ²Integrated Physiol., Univ. of Colorado, Boulder, CO; ³Pharmaceut. Sci., ⁴Neurosci. Grad. Program, Univ. of Colorado at Anschutz Med. Campus, Aurora, CO;

⁵Pharmaceut. Sci., Univ. of Virginia Neurosci. Program, Charlottesville, VA

Abstract: The isoform AKT1 of the AKT family of central kinases has been implicated in both psychiatric disease expression and responses to treatment. In addition, genetic variation in AKT1 may interact with cannabis use to effect sensitivity to psychotic disorders, suggesting a mechanism linking cannabinoid signaling and AKT1. Our research group has recently described sex-specific effects of AKT1 modification on anxiety-like behaviors and cued fear memory

extinction. The fear extinction genotype differences were eliminated with AAV-mediated rescue of expression of AKT1 in the medial prefrontal cortex (mPFC). To investigate the mechanisms underlying sex and genotype-dependent changes in behavior, we performed whole-cell current-clamp recordings from principal neurons and interneurons in the mPFC of both male and female *Akt1* KO animals and wildtype littermate controls. Here, we present the active and passive membrane properties and neuromorphological properties of these neurons. We expect to find some differences in intrinsic properties of both principal neurons and interneurons as both cell types normally express AKT1. In addition, we assessed a mechanistic link between the endocannabinoid system and AKT1 by testing for a cannabinoid-dependent form of plasticity - slow self-inhibition (SSI) in both fast spiking (FS) and low-threshold spiking (LTS) interneurons. We found that LTS and not FS interneurons have SSI, similar to previous research done in the somatosensory cortex. This indicates that this cannabinoid-dependent plasticity is present in LTS interneurons in the mPFC. During recording neurons were filled with biocytin and 3D reconstructions were used to measure dendritic branching and length. Confirmation of interneuron sub-types, including somatostatin, parvalbumin, and cholecystokinin-expressing neurons was done immunohistochemically. In addition to these results, we will show preliminary results from conditional *Akt1* KO animals in which *Akt1* was removed selectively from interneurons. Future experiments will include pharmacological manipulations targeting candidate pathways for cannabinoid involvement. We hope these studies will facilitate novel therapeutic avenues involving cell-specific AKT signaling mechanisms to treat neuropsychiatric disorders.

Disclosures: J.L. Hanson: None. J.K. Kushner: None. M.N. Svalina: None. C. Borski: None. S.M. Baca: None. M.M. Huntsman: None. C. Hoeffler: None.

Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 441.14

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Consortium Research Fellows Program

Title: Infrared's long-term inhibition and excitation effects on 3d human brain models

Authors: *S. TRITLEY¹, C. CRUZ¹, L. MALDONADO², G. TOLSTYKH², A. QUTUB¹;
¹Univ. of Texas at San Antonio, San Antonio, TX; ²Air Force Res. Lab., San Antonio, TX

Abstract: Infrared's long-term inhibition and excitation effects on 3D human brain models

Authors: S. C. Tritley^{1,2}, C. A. Cruz¹, L. A. Maldonado², G. P. Tolstykh², A. A. Qutub¹.
Biomedical Engineering, the University of Texas at San Antonio, San Antonio, TX. 2. Air Force Research Laboratory, Joint Base San Antonio, Fort Sam Houston, TX.

Disclosures S.C. Tritley: none; C.A. Cruz: none; L. A. Maldonado: none; G. P. Tolstykh: none; A.A. Qutub: none

Abstract Infrared (IR) exposure can functionally impact the electrochemical properties across a neuronal membrane through fast thermal gradients. Contingent on the delivered energy and duration on the target cells, IR exposure, can inhibit or potentiate neuronal communication. However, much is still unknown regarding the long-term effects of IR exposure on neurons and glia. We have identified from our previous research in mice NG108 cells that IR laser pulses of longer lengths (3-3.5ms, corresponding to 3.8-4.5mJ) inhibited the generation of evoked action potentials (AP) when applied within a range of 0-5ms before AP initiation. Relatively moderate IR pulses (≤ 2.5 ms ~ 3.1 mJ) of IR stimulation reduced the action potential amplitudes and shortened the duration instead of complete inhibition. Despite this, we noticed neuronal membrane blebbing and dysfunction with the multiple and longer dose exposures. To identify long-term effects in a human brain model, we cultured neural progenitor cells in a 3D Matrigel and differentiated them to day 14. We then use a combination of immunocytochemical markers in the 3D cell cultures of neurons and glia at multiple IR stimulus durations (1.5ms-3.5ms) and at different time points after exposure (2 hours-24 hours). In addition to standard DAPI and class III beta-tubulin (Tuj1) staining, we used oxazole yellow (YO-PRO-1), which can identify apoptotic cells, and glial fibrillary acidic protein (GFAP) to observe the effects of IR on astrocytes. We found that stimulating the cultured neurons past 3.0ms had inhibitory effects due to cellular membrane degradation with an influx of YO-PRO-1. The results have helped identify a difference between an efficacious dose of IR stimulation versus a detrimental dose of IR.

Disclosures: S. Tritley: None. C. Cruz: None. L. Maldonado: None. G. Tolstykh: None. A. Qutub: None.

Poster

441. Modulation of Intrinsic Properties

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Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: VA Merit Review

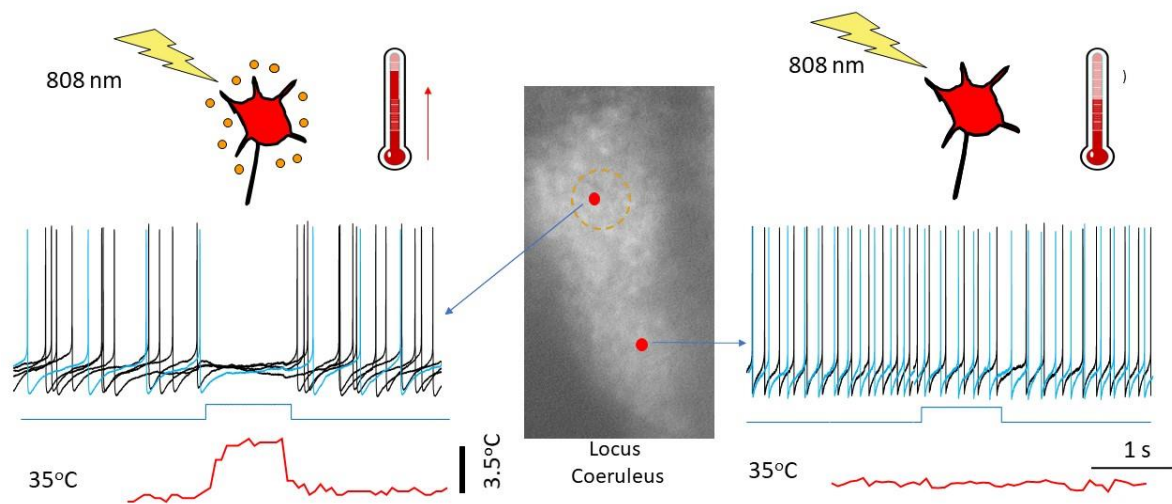
Title: Harnessing the brain's mechanism for thermal resilience as a tool for optical control of neuronal excitability

Authors: *C.-M. TANG^{1,2}, K. YANG²;

¹UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE, BALTIMORE, MD; ²Baltimore VA MC, Baltimore, MD

Abstract: Temperature elicits opposing effects on the many diverse mechanisms that control neuronal excitability. These counterbalancing mechanisms enable resilience of brain function to thermal perturbations. A better understanding of these mechanisms provides insights to how the mammal brain has adapted to unforgiving environmental conditions. The importance of thermal resilience is supported by our finding that its

molecular mechanism is distributed over a range of potassium conductances, not just in a single ion conductance. This neuroprotective mechanism may potentially be harnessed to modulate brain function for clinical and fundamental neuroscience. Harnessing endogenous inhibitory mechanism rather than expressing foreign proteins to produce non-physiologic conditions has the distinct advantage of circumventing the poor tolerance associated with current optogenetic inhibition. NIR light pulses are directed towards brain regions that have been targeted with low concentration of frequency-tuned gold nanorods. Robust inhibition can be achieved with small temperature steps with high temporal-spatial precision. Importantly, repetitive inhibition can be maintained for the full duration of a normal brain slice experiment (>2 hours). Interestingly, but not surprisingly, inhibitory and excitatory neurons exhibit differing thermal responses.



Disclosures: C. Tang: None. K. yang: None.

Poster

441. Modulation of Intrinsic Properties

Location: SDCC Halls B-H

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Program #/Poster #: 441.16

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: DARPA Grant 52554.1.1990740

Title: Verification of neuromodulation with magnetoelectric nanotransducers using motor evoked potentials in primary motor cortex

Authors: ***J. A. SHULGACH**¹, M. D. MURPHY², E. ZHANG⁴, M. ABDEL-MOTTALEB⁵, S. CHEN⁶, S. KHIZROEV⁴, P. LIANG⁶, D. M. GRIFFIN², D. J. WEBER³;

¹Mechanical Engin., ²Neurosci. Inst., ³Mechanical Engin. and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA; ⁴Electrical and Computer Engin., ⁵Biomed. Engin., Univ. of Miami, Coral Gables, FL; ⁶Cell. Nanomed, Irvine, CA

Abstract: Electroactive nanomaterials have emerged within the last 10 years as a unique solution to address the limits of traditional wireless neuromodulation techniques. Because of their small size, piezoelectric properties, and ability to wirelessly transduce external electric, light, or magnetic fields, their use in neurostimulation applications shows great promise. Previous studies have shown that magnetoelectric nanotransducers (MeNTs) implanted in deep brain regions in rodents can be used as neurostimulators powered wirelessly via externally applied magnetic fields. However, no studies have attempted cortical microstimulation with MeNTs in large mammals. In this study, we demonstrate microstimulation of corticomotor neurons in anesthetized nonhuman primates (n = 3) via MeNTs injected into the primary motor cortex (M1). The MeNTs consisted of a cobalt ferrite-barium titanate (CoFe₂O₄-BaTiO₃) core-shell structure 30 nm in diameter suspended in deionized water and were injected in 5-10 μ L amounts via a 50 micron diameter capillary tube integrated into the body of a penetrating microelectrode array (V-probe, Plexon, Inc.). The microelectrode was inserted into the forelimb region of M1 to a depth of 1.5 mm and used to deliver brief trains of intracortical microstimulation (ICMS) pulses for mapping motor evoked potentials (MEPs) in the contralateral forelimb, measured via bipolar and high density electromyographic (HD-EMG) electrode arrays. We compared the amplitude of the MEPs in the pre and post injection conditions from ICMS and found a median percentage increase of 21% after MeNTs were injected. We then placed an electromagnetic (Emag) coil 0.5 cm above the injected sites and delivered brief (~10 ms) trains of 10-500 mA pulses of electrical current at pulse frequencies of 1-300 Hz. MEPs were measured at 15-21 ms latency from the onset of stimulation. Muscle responses were quantified as the ratio of the root-mean-square (RMS) of EMG in the post-versus pre- stimulus intervals. MeNT stimulation evoked responses in the EMG recordings that were 12x smaller than with ICMS at the same sites. Stimulation with Emag also recruited fewer or different muscles compared with ICMS at the same sites. Our results demonstrate that MeNTs can activate neurons with relatively high spatio-temporal resolution. Ongoing efforts are aimed at improving MeNT synthesis and implantation methods and optimizing the Emag coil design to achieve high-efficiency, multisite targeting of MeNT-based ICMS. Ultimately, this work may yield a new class of wireless neurostimulators that could be implanted with minimal surgery and enable precise targeting of multiple structures in the brain.

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Poster

442. Microglial Mechanisms of Development and Physiology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 442.01

Topic: B.09. Glial Mechanisms

Title: Neuronal SIRP α as a temporal regulator of microglial phagocytosis in the visual system during development.

Authors: *P. I. ANDRADE^{1,2}, D. JIANG^{1,2}, C. A. BURGER^{1,2}, N. E. ALBRECHT^{1,2}, V. AKHANOV^{1,2}, R. D. MACKIN^{1,2}, J. H. LIANG^{1,2}, D. P. SCHAFFER³, M. A. SAMUEL^{1,2}; ¹Dept. of Neurosci., ²Huffington Ctr. on Aging, Baylor Col. of Med., Houston, TX; ³Dept. of Neurobio., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: The temporal regulation of microglial phagocytic activity is essential for microglia's ability to establish proper circuits in the developing brain. However, neuron-derived molecular cues that tightly regulate the timing and extent of phagocytic activity remain largely undefined. Our lab identified signal regulatory protein alpha (SIRP α) and its ligand CD47 as surprising permissive cues that temporally regulate microglial phagocytosis. Here, we utilize the visual system (retina and dorsal lateral geniculate nucleus; dLGN) to study the distinct cellular sources of SIRP α in regulating microglial phagocytosis. Our data in the retina suggest that neurons are the dominant cellular source of SIRP α , and neuronal but not microglial SIRP α enables microglial phagocytosis during peak refinement periods. Using cell-type-specific deletion mouse models, we show that neuronal SIRP α is a crucial molecular cue that temporally regulates microglial-mediated refinement by controlling the timing and extent of microglial phagocytosis during development. Prolonging neuronal SIRP α late in development restores microglia's phagocytic activity, while the deletion of neuronal SIRP α results in decreased microglial activity and synapse preservation. Additionally, by genetically manipulating neuron- or microglia-specific CD47 levels, with and without paired SIRP α co-manipulation, we posit that interactions between neuronal SIRP α and neuronal CD47 indirectly regulate microglial phagocytic activity. We are now extending these experiments to the dLGN, which appears to employ neuronal SIRP α signaling in a similar manner. Together, our results suggest that neuronal SIRP α serves as a signaling cue that temporally regulates microglial phagocytosis and refinement via neuronal CD47 binding during early development.

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Poster

442. Microglial Mechanisms of Development and Physiology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 442.02

Topic: B.09. Glial Mechanisms

Support: Conacyt PhD scholarship 713285

Title: Effects of manganese exposure in mice cerebellar microglia primary cultures and macrophages

Authors: *M. MARTÍNEZ HERNÁNDEZ¹, L. HERNÁNDEZ-KELLY¹, M. TORRES RAMOS², L. PÉREZ MARTÍNEZ³, A. ORTEGA SOTO¹;

¹Dept. de Toxicología, Ctr. de Investigación y de Estudios Avanzados del IPN, Ciudad de México, Mexico; ²Unidad Periférica de Neurociencia, Inst. Nacional de Neurología y Neurocirugía Manuel Velasco Suárez, Ciudad de México, Mexico; ³Inst. de Biotecnología, Univ. Nacional Autónoma de México, Cuernavaca, Mexico

Abstract: Microglia cells are the primary resident immunocytes in the cerebellum, continuously function as immune system supervisors to sustain cerebellar homeostasis. Recent studies have demonstrated that cerebellar microglia have a uniquely hyper-vigilant immune phenotype compared to microglia from other brain regions. These findings suggest that microglia contribute to cerebellar vulnerability in ataxia and probably in the toxic effects of xenobiotics. Cerebellar development may be particularly sensitive to such insults, as cerebellar maturation occurs over a relatively long developmental time. Human cerebellar development extends from the early embryonic period until the postnatal years. Elimination of immature, functional redundant synapses during postnatal development is essential for the formation of the functional cerebellar network. While the unique character of cerebellar microglia has only recently been identified, cerebellar astrocytes and in particular radial Bergmann glia are well known for their unique interaction with Purkinje cells (PC) and their plausible regulation of glutamatergic synapses. Additionally, the cerebellum is a target for metal neurotoxicity, like manganese (Mn). It has been shown that Bergman glia cells are affected by acute exposure to Mn, decreasing glucose uptake in an ERK1/2 dependent manner. The establishment of the molecular and cellular process triggered by xenobiotics is fundamental for the development of public health policies. Therefore, the development of suitable *in vitro* models is important, herein we describe a protocol to isolate and characterize cerebellar microglia from post-natal mice. Moreover, using cerebellar microglia and the raw-blue macrophage cell line we were able to characterize the effect of Mn exposure on cell viability and phagocytosis. In conclusion, an easy-to-follow protocol for the culture of primary cerebellar microglia was developed. Mn exposure does not alter the cell viability of cerebellar microglia, but it modifies macrophage phagocytic capacity.

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Poster

442. Microglial Mechanisms of Development and Physiology

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Program #/Poster #: 442.03

Topic: B.09. Glial Mechanisms

Support: T35 OD010956
T32 GM 136559
#2K12CA138464-11
UC Davis SVM Endowment Funds

Title: Characterizing cellular topography and molecular signatures of immune cell populations in the canine brain

Authors: D. A. JIMENEZ, R. G. TOEDEBUSCH, *C. M. TOEDEBUSCH;
Univ. of California, Davis, CA

Abstract: Naturally occurring canine glioma, with shared clinical, histopathological, and molecular features, has a 2-3 fold higher incidence rate than human glioma. Canines are an immunologically outbred host with similar environments to humans. Therefore, companion dogs provide a distinct advantage over genetically and environmentally homogeneous laboratory mice for translational immunotherapeutic development. However, there is a paucity of information on the immune landscape in normal canine brain. Therefore, this proof-of-principle pilot study *tests the hypothesis that healthy canine brain is enriched with regionally distinct, immunocompetent microglia*. Our study aims to define the distinct cellular populations of immune cells in healthy canine brain by single cell RNA sequencing (scRNA-seq). We utilized cryopreserved mononuclear cells from four brain regions of an aged, histopathologically normal canine brain. After thawing, cells were stained and sorted via fluorescent-associated cell sorting to isolate putative microglia (CD11b⁺/CD45^{low/moderate}), macrophages (CD11b⁺/CD45^{high}), and lymphocytes (CD3^{high}/CD11b^{neg}). Cells were combined and processed via the 10X Genomics platform. Sequencing results were aligned to the canine reference genome, ROS_Cfam_1.0. Across brain regions, 2001.2±246.5 cells per sample were sequenced, with 159,652.8±16876.6 reads per cell, ultimately identifying 1354.7±29.0 genes per cell. Approximately 90% of genes were mapped to the reference genome. Gene signatures clustered into 6.6 ±0.75 individual populations, with most clusters exhibiting up-regulation of genes associated with T and B lymphocytes (e.g., *CD3*, *CD79A*). Within each brain region, a single cluster of putative microglia were identified by strong expression of *TMEM119*, *AIF1*, and *P2RY12*. Top up-regulated genes within this cluster included *C1QB*, *C1QC*, *CCL23*, and *APOE*, suggesting activated, pro-inflammatory microglia. These data confirm that scRNA-seq is feasible on cryopreserved canine brain cells and demonstrates that there are distinct clusters of immune cells within the canine brain. However, contrary to our hypothesis, this preliminary analysis indicates that the cellular majority are lymphocytes, not microglia. As myeloid cells are the predominant immune population in human and rodent brains, this raises the possibility that cryopreservation resulted in loss of myeloid cells, yielding an enriched, predominant lymphocyte population.

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Poster

442. Microglial Mechanisms of Development and Physiology

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Program #/Poster #: 442.04

Topic: B.09. Glial Mechanisms

Support: NIH NINDS SBIR Grant 1R43NS102088
NIH NINDS SBIR Grant 2R44NS102088

Title: Modeling Neuroinflammation with iPSC-derived microglia cultured on Synthetic Hydrogels

Authors: W. RICHARDS¹, P. FAVREAU¹, J. KULAS¹, K.-D. CHOI², A. MASSMAN², *S. VISURI¹, **R. GORDON**¹, C. LEBAKKEN¹;

¹Stem Pharm, Inc, Madison, WI; ²BrainXell, Madison, WI

Abstract: Neuroinflammation is a complex response to brain insult involving activation of the innate immune response, release of inflammatory mediators, and the generation of reactive species resulting in downstream effects including vascular compromise, oxidative stress, and neurotoxicity. Neuroinflammation is a key component in many disease etiologies including neurodegenerative disease, stroke, trauma, seizures, neuropsychiatric disorders, and brain tumors. Microglia are the primary innate immune cells in the brain and are responsible for sensing and responding to stimuli. iPSC-derived microglia have become a valuable tool to further understand microglia biology and their role in neuroinflammation and as a tool for assessing compound effects on human microglia function. We have developed synthetic hydrogels which support microglia mono- and neural co-cultures that can be supplied in a pre-plated, sterilized, and ready-to-use format. The hydrogels are formed using multi-armed polyethylene glycol monomers that incorporate adhesion peptides and crosslinkers to form a 5-8 μm coating on tissue-culture polystyrene or cyclic olefin co-polymers. The hydrogels provide a substrate with an elastic modulus of 80-100 kPa as measured by atomic force microscopy. We have utilized these substrates to interrogate the biological response of iPSC-derived microglia to inflammatory stimuli including lipopolysaccharide (LPS) and interferon gamma, and amyloid beta oligomers, and anti-inflammatory cytokines including treatment with IL-4 and -3, and TGF β and IL-10. Microglia adhesion to the substrates is robust and sustained, and the cells are morphologically dynamic. We have compared the results to microglia plated on poly-D-lysine (PDL) and have observed enhanced transcriptional responses to stimuli on the synthetic substrates compared to standard 2-dimensional culture on PDL. Additional responses have been measured by immunoassays, analyzing cell culture supernatants; imaging assays assessing morphological metrics, p65 nuclear translocation and phagocytosis assays. We observe time dependent responses to stimuli. There is a statistically significant p65 nuclear translocation response to 100 ng/mL LPS as early as 30 min with the response peaking at 6 h post treatment and at control levels at 24 h. Inflammatory cytokine and morphological responses peak at 24 h post stimulation. The substrates enable robust read-outs to interrogate human microglia biology in a more physiologically relevant context.

Disclosures: **W. Richards:** A. Employment/Salary (full or part-time):: Stem Pharm, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stem Pharm, Inc. **P. Favreau:** A.

Employment/Salary (full or part-time);; Stem Pharm, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);; Stem Pharm, Inc. **J. Kulas:** A. Employment/Salary (full or part-time);; Stem Pharm, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);; Stem Pharm, Inc. **K. Choi:** A. Employment/Salary (full or part-time);; BrainXell. **A. Massman:** A. Employment/Salary (full or part-time);; BrainXell. **S. Visuri:** A. Employment/Salary (full or part-time);; Stem Pharm, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);; Stem Pharm, Inc. **R. Gordon:** A. Employment/Salary (full or part-time);; Stem Pharm, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);; Stem Pharm, Inc. **C. Lebakken:** A. Employment/Salary (full or part-time);; Stem Pharm, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);; Stem Pharm, Inc..

Poster

442. Microglial Mechanisms of Development and Physiology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 442.05

Topic: B.09. Glial Mechanisms

Support: NRF-2019M3C7A1031534
NRF-2019R1I1A2A01063642
NST Grant CCL21201-100

Title: Polymethyl methacrylate nanoplastics enhance the migration of microglia via the induction of chemokine secretion

Authors: ***J. BAEK**, W. LEE, J. JEONG, D. LEE;
Korea Res. Inst. of Biosci. & Biotechnology(KRIBB), research building, Korea Res. Inst. of Biosci. & Biotech., Daejeon, Korea, Republic of

Abstract: Given the continuously increasing plastic pollution worldwide, human exposure to micro/nanoplastics is an inevitable fate. Despite the existence of various plastics in the ecosystem, most studies are focused on polystyrene (PS) nanoplastics. Upon uptake, nanoplastics can cross the BBB and reach the brain, but the information regarding the potential toxicity of various nanoplastics is limited. In this study, we investigated the biological effect of nanoplastics to microglia using three types of nanoplastics generated from PS, polypropylene (PP), and polymethyl methacrylate (PMMA). Using fluorescence-labeled nanoplastics, we established that PMMA showed notably morphological changes and increased expression of OX-6⁺ reactive microglia compared to other nanoplastics. In addition, we found that PMMA-treated microglia significantly increases the expression of chemokines, especially CXCL1, which induces immune cell migration. These data provide evidence that functional changes and activation of microglia

by exposure of PMMA nanoplastics in pathological conditions may accelerate neurological disorders.

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Poster

442. Microglial Mechanisms of Development and Physiology

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Program #/Poster #: 442.06

Topic: B.09. Glial Mechanisms

Support: AFAR Grant for Junior Faculty A19090
NIH R01 AG075909-01
David Geffen School of Medicine at UCLA Start-up Funds

Title: Lysosome status as a key driver of microglial phenotype and responses to aging

Authors: F. ETIENNE¹, K. HOPE¹, S. SIDHU¹, A. SCHALER¹, C. WEBB¹, A. CALCAGNI², A. BALLABIO³, L. DE BIASE¹;

¹David Geffen Sch. of Med. At UCLA, Los Angeles, CA; ²Baylor Col. of Med., Houston, TX;

³Telethon Inst. of Genet. and Med., Naples, Italy

Abstract: During aging, microglia produce inflammatory factors, show reduced tissue surveillance, altered interactions with synapses, and prolonged responses to CNS insults, positioning these cells to have profound impact on the function of nearby neurons. We recently showed that microglial proliferation and inflammatory factor production during aging varies significantly across basal ganglia nuclei, giving rise to “pockets” of inflammation in ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) beginning in middle age. These early aging responses of VTA/SNc microglia tightly aligned with changes in microglial lysosome status. In these follow-up studies, we sought to reveal the underlying causes of VTA/SNc microglial lysosome overload during aging via detailed analysis of autofluorescent lipofuscin aggregates. Through a combination of imaging and FACS-based analyses, we found that lipofuscin accumulates primarily in CNS neurons and microglia but not other CNS glia, including astrocytes. The lipofuscin aggregates in VTA microglia were larger and filled more of the lysosomal network than those in NAc microglia. As VTA microglia proliferated during aging, lipofuscin aggregates partitioned to one daughter cell, leaving an unburdened daughter cell that may be better equipped to support tissue homeostasis. We are using RNAseq of microglia with high and low lipofuscin burdens from multiple brain regions to further elucidate the relationship between instantaneous aggregate burden and overall cell inflammatory and aging profiles. In addition, we developed a FACS-based protocol to purify cell-free lipofuscin aggregates from microglia and neurons for proteomics, ImageStream analysis, and mechanistic in vitro studies. Finally, we are using genetic strategies to induce lysosome overload with lipofuscin, independent of other changes that occur during aging. These approaches will allow us

to test causal relationships between lysosome status and key microglial functions. Collectively our studies promise to reveal mechanisms that drive regional differences in microglial aging and to highlight therapeutic targets for manipulation of microglial aging responses.

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Poster

442. Microglial Mechanisms of Development and Physiology

Location: SDCC Halls B-H

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Program #/Poster #: 442.07

Topic: B.09. Glial Mechanisms

Support: CIHR
Heart and Stroke Foundation
NSERC
CFI

Title: Longitudinal imaging in the adult mouse brain reveals a subpopulation of mobile microglia that are regulated by interferon gamma signalling

Authors: R. BOGHOZIAN¹, M. CHEEMA¹, *C. BROWN²;
²Med. Sci., ¹Univ. of Victoria, Victoria, BC, Canada

Abstract: Microglia are morphologically dynamic immune cells in the brain. Although some, but not all studies suggest these cells are capable of migration, especially in neonates or after injury, there is little direct evidence supporting this in the adult brain. Further, whether this mobility is evident in all microglia or just a subpopulation, and what molecular mechanisms drive this, are not well known. One issue with previous studies was the dependence on myeloid based reporters that do not discriminate between microglia and macrophages. Furthermore because most cells were labeled, unambiguously resolving long-distance movement was not possible. Here, we performed time lapse *in vivo* imaging of sparsely labelled microglia using adult, tamoxifen inducible microglia reporter mice. In the absence of injury, we find a relatively small population of microglia (~6%) that are mobile over a 24h period. Following the induction of a cerebral microbleed (CMB), the fraction of mobile microglia increases significantly (~22%). These cells typically moved towards the CMB and in some cases migrated over 40µm, especially for cells located 50-200µm from the CMB. Next we tested whether interferon (IFN γ) signalling regulates this phenomenon. First, injecting IFN γ into the bloodstream at the time of CMB increased the proportion of mobile microglia (~39%) and their migration distance. Conversely, microglia specific deletion of IFN γ receptors significantly impaired mobility under both normal and bleed conditions. These findings highlight the diversity of microglia responses to injury and shed light into the signaling mechanisms that drive this unique migratory capability.

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Poster

442. Microglial Mechanisms of Development and Physiology

Location: SDCC Halls B-H

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Program #/Poster #: 442.08

Topic: B.09. Glial Mechanisms

Support: NIH grant NS122316

Title: Microglia Regulation of Neuronal Metabolism and mRNA Translation

Authors: *D. ADLER^{1,3}, A. A. HERNANDEZ³, E. KLANN²;

¹New York Univ. Ctr. For Neural Sci., New York, NY; ²New York Univ. Ctr. For Neural Sci., Brooklyn, NY; ³NYU Sch. of Med., New York, NY

Abstract: Maintaining homeostasis in the brain requires the delicate synchronization of signals and structures to ensure that rapid changes in energy demand are met, on a millisecond timescale, by adequate metabolic supply. One cell type that is known to play an important role in regulating brain tissue-homeostasis is microglia, yet little is known about how these resident-immune cells influence neuronal metabolism during physiological activity. We used a combination of AAV delivered metabolic FRET sensors (for lactate, pyruvate, glucose, and ATP) under astrocytic (GFAP) and neuronal (hSyn1) promoters in the motor cortex of treadmill running mice and imaged these cells using two-photon microscopy to determine how neurons generate ATP during motor activity in wild type (WT) mice, and how this changes in microglia-depleted mice (Cx3CR1^{CreERT2/+}; R26^{DTR/+}). We found that upon initiation of treadmill running, astrocytes exhibited an increase in intracellular lactate, which was followed by an increase intracellular lactate in neurons, first in cortical layer 1 (L1) synapses, and later in layer 2/3 (L2/3) somas. Concomitantly, we found that ATP levels were modestly reduced in L1 synapses, but were increased in L2/3 somas. In contrast, after microglia depletion, lactate failed to increase in astrocytes during treadmill running, and pyruvate was consumed, suggesting that the metabolic allocation of lactate is influenced by microglia. In addition, we have developed a new method to detect the anabolic process of *de novo* protein synthesis during motor activity *in vivo* using intravenously delivered L-azidoholoalanine (AHA) for fluorescent noncanonical amino acid tagging (FUNCAT) in rotarod-trained mice. Using this method in WT mice, we detected robust labeling of cells specifically in motor cortex after one hour of rotor rod training compared to control mice injected with AHA and left in their home cage. Finally, we found that during baseline conditions, *de novo* translation is reduced in motor cortical excitatory (CaMKII) and inhibitory (PV) neurons in microglia-depleted mice. These results suggest microglia play a critical role in the regulation both of astrocytic lactate shuttling and the linked anabolic process of *de novo* translation (Descalzi et al., 2021) that are vital for metabolic homeostasis in the healthy brain. This work was supported by NIH grant NS122316 (E.K.)

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Poster

442. Microglial Mechanisms of Development and Physiology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 442.09

Topic: B.09. Glial Mechanisms

Title: Nerve growth factor influences microglial activity *in vivo* via trka receptors

Authors: *G. BORGONOVO¹, A. TIBERI^{2,1}, S. MARINELLI³, P. PACIFICO^{1,2}, M. CALVELLO¹, W.-B. GAN⁴, S. CAPSONI^{5,1}, A. CATTANEO^{1,3};

¹Bio@SNS Lab. of Biology, Scuola Normale Superiore, Pisa, Italy; ²Inst. of Neuroscience, Natl. Res. Council, Pisa, Italy; ³European Brain Res. Institute, Rome, Italy; ⁴Inst. of Neurolog. and Psychiatric Disorders, Shenzhen Bay Laboratory, Shenzhen, China; ⁵Univ. of Ferrara, Ferrara, Italy

Abstract: The neurotrophin Nerve Growth Factor (NGF), in addition to supporting neuronal survival, differentiation, and plasticity, has pleiotropic actions on non-neuronal cells, especially in the immune system. Indeed, peripheral immune cells such as mast cells and macrophages secrete and respond to NGF, by expressing TrkA receptors. Following early reports showing that NGF promotes *in vitro* migration and proliferation of the resident brain immune cells, microglia, more recently our lab discovered potent immunomodulatory properties of NGF via TrkA on microglia *in vitro*: NGF steers them toward a neuroprotective and anti-inflammatory phenotype, by modulating their motility and phagocytosis. In addition, we showed that TrkA is expressed by microglia in mouse brain acute slices and is upregulated in microglia of Alzheimer's disease model 5xFAD. Here, we provide *in vivo* evidence of a role for NGF signaling in brain microglia. (1) We evaluated the effect of a direct NGF application to the motor cortex of CX3CR1^{GFP/+} mice via *in vivo* two-photon microscopy. We observed an increase in microglial process motility, both in baseline conditions and following laser lesion. Moreover, application of an anti-TrkA antibody reduces microglial motility in CX3CR1^{GFP/+}, suggesting an involvement of TrkA in the modulation of microglial motility by endogenous NGF. The functional output of NGF-TrkA signaling in microglia is dependent on TREM2, since the effects of NGF are abolished in TREM2KO mice. The activity of NGF on microglia is relevant also in pathological conditions: indeed, NGF can rescue a deficient process *in vivo* motility in 5xFAD mice. (2) To better and directly assess the functional role of the microglial NGF-TrkA signaling, we generated a novel transgenic mouse line (CX3CR1^{CreERT/+}::TrkA^{fl/fl}) in which TrkA can be specifically deleted in microglia upon tamoxifen administration. We report that knocking out the microglial NGF-TrkA signaling leads to a remarkable reduction in cortical and hippocampal microglial density, while tridimensional morphology is unaffected with respect to controls. Moreover, TrkA-deprived microglia show higher phagocytosis of spines, leading to a striking reduction of cortical spine density. Lastly, at a behavioral level, microglial NGF-TrkA signaling deletion affects motor learning. Altogether, these data demonstrate that CNS resident microglia express functional TrkA receptors *in vivo* and that TrkA signaling influences pivotal microglia activities.

Modulating the NGF-TrkA axis on microglia *in vivo* might be harnessed as a broad neuroprotective strategy for CNS neuronal populations that are not direct NGF targets.

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Poster

442. Microglial Mechanisms of Development and Physiology

Location: SDCC Halls B-H

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Program #/Poster #: 442.10

Topic: B.09. Glial Mechanisms

Support: NIMH K99/R00 Pathway to Independence Grant
Center for Neuroinflammation and Cardiometabolic Diseases (CNCD) Seed Grant

Title: Effects of constitutive Cx3cr1-cre expression on microglial density and morphology in the developing mouse brain

Authors: *B. DESAI, M. GARVIN, F. MROUE-RUIZ, S. CORREA, F. KAMAU, J. SHEHU, J. BOLTON;
Neurosci. Inst., Georgia State Univ., ATLANTA, GA

Abstract: Cx3cr1-Cre transgenic mice express Cre recombinase under the direction of the Cx3cr1 promoter, and this Cre/lox system is an extensively used tool for genetic manipulations in microglia. Recently, Sahasrabudde et.al, 2022 described the adverse effects of inducible Cx3cr1-Cre expression on microglia, which included a lower cell density, an activated phenotype, an upregulation of their phagocytic function, and DNA damage in the developing brain, suggesting a Cx3cr1-Cre toxicity specific to inducing expression in the early postnatal period. These unintended and non-specific effects of the Cx3cr1-driven Cre expression in microglia also rendered the animals more anxious in adulthood, suggesting an urgent need to validate this genetic tool further. However, the possible detrimental effects of constitutive expression of Cx3cr1-Cre (BAC-Cre) on microglia remain unknown. We hypothesize that due to its continuous expression and accumulation, from embryonic stages through adulthood, constitutive Cx3cr1-Cre expression will lead to an altered microglial density, morphology and phagocytic function as well. Here, we performed confocal imaging of immunolabelled microglia in the paraventricular nucleus (PVN) of the hypothalamus, the central nucleus of the amygdala (CeA), the parietal cortex (PC) and hippocampal subregions (CA1, CA3 and Dentate Gyrus) of Cx3cr1-Cre+ and Cx3cr1-Cre- littermates (postnatal days 7-9). Our preliminary data in the PVN reveal a slightly decreased volume of P2RY12+ microglia in Cx3cr1-Cre+ mice, which we are currently following-up with Sholl analysis to further delineate possible morphological changes. However, no apparent differences in the number of P2RY12+ or Iba1+ microglia have been found with constitutive Cre expression, and no change in the number or volume of CD68+ lysosomes. Analysis of microglial phagocytic cups, proliferation, and potential DNA damage in

the developing PVN is ongoing, along with other brain regions and behavioral studies in adult mice. The results of these experiments will have important implications for the use of the Cx3cr1-driven Cre/lox system to study microglia during development, and they also highlight the need to perform the correct control experiments when using these genetic tools.

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Poster

442. Microglial Mechanisms of Development and Physiology

Location: SDCC Halls B-H

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Program #/Poster #: 442.11

Topic: B.09. Glial Mechanisms

Support: NIMH grant 1R21MH126409-01
Stanley Center

Title: Developing microglia-targeting AAV

Authors: ***S. JEREB**¹, D. CUFFE¹, K. Y. CHAN¹, Q. HUANG¹, A. T. CHEN¹, M. B. JOHNSON¹, B. A. STEVENS², B. E. DEVERMAN¹;

¹Broad Inst., Cambridge, MA; ²Harvard Med. Sch. Neurobio., Boston Children's Hosp., Boston, MA

Abstract: Microglia, the resident macrophages of the brain, play essential roles in brain development, homeostasis, and neurodegeneration. Genome-wide association and exome-sequencing studies have shown that multiple Alzheimer's disease risk variants are located in or near genes that are specifically expressed in microglia, suggesting that these cells likely play a causal role in the disease. In addition, complement and microglia have been shown to play a role in synaptic pruning during postnatal brain development. The process might go awry during development of schizophrenia and bipolar disorder, as genetic studies have linked the complement system to the two diseases. However, studying the various functions of microglia in healthy and diseased brain is hindered by the lack of viral tools for rapidly manipulating their gene expression and delivery of genetically encoded reporters, sensors and effectors. Here, we aimed to develop viral vectors to target microglia, with a focus on capsid engineering of adeno-associated viruses (AAVs). Existing AAVs do not transduce microglia *in vivo* and show poor transduction efficiency *in vitro*. We show that AAV9 particles fail to accumulate inside microglia upon intracranial injection in mice. Therefore, we performed *in vivo* and *in vitro* screens of AAV capsid libraries with a randomized 7-mer amino acid insertion in the AAV9 capsid protein to find capsids that show enhanced transduction of microglia. While our *in vivo* screens failed to yield promising candidate capsids, our *in vitro* screen revealed variants that were strongly enriched in microglia and bone marrow macrophages. We picked the top capsid variants that efficiently bound to or transduced microglia and bone marrow macrophages in the

in vitro screen and generated a second-round library, which we tested *in vivo* and *in vitro*. Surprisingly, the most highly enriched capsid variants recovered from cultured microglia were highly similar to each other and were recovered from microglia only. We tested the ability of the most enriched capsid variants from this cluster to transduce microglia *in vivo*. Indeed, the top capsid variants from the *in vitro* screen were able to transduce mouse microglia *in vivo*. Finally, we introduced additional mutations to the top capsid variants and performed an *in vivo* screen for variants with improved transduction efficiency compared to the variants recovered from the *in vitro* screen.

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Poster

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Topic: B.09. Glial Mechanisms

Support: RO1GM134104 (NIGMS)
USUHS intramural grant funds

Title: Effects of the actin cytoskeleton on microglial-complement interaction

Authors: *S. PAULSON, J. ROTTY;
Uniformed Services Univ., Bethesda, MD

Abstract: Microglia are the resident immune cells of the central nervous system. They respond to phagocytic markers to help maintain homeostasis within the brain. Some of the most relevant phagocytic markers are members of the complement cascade, a pro-inflammatory protein cascade responsible for helping label and clear foreign bodies and debris. Our initial studies use components of the complement cascade, such as C3, as a model substrate to understand how microglia sense and respond to substrate bound cues. These are being modeled through assays using a murine microglial cell line, BV2, and primary microglia. We utilized complement protein driven phagocytosis to measure substrate bound protein sensing and response. We then examined whether key regulators of the actin cytoskeleton, primarily the branched actin polymerizing Arp2/3 complex, contribute to the microglial response to complement. In the future, we plan to better flesh out the connection between the C3 receptor and the actin cytoskeleton in microglia. These studies will aid in the search to clarify how microglia sense complement.

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Poster

442. Microglial Mechanisms of Development and Physiology

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Topic: B.09. Glial Mechanisms

Support: NINDS Grant NS106138
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NINDS Grant NS097775
American Heart Association 20POST3516000

Title: Live imaging of microglial behavior across the lifespan

Authors: *T. TIEU¹, A.-J. N. CRUZ¹, A. Y. SHIH^{1,2,3}, V. COELHO-SANTOS^{1,2};

¹Ctr. for Developmental Biol. and Regenerative Med., Seattle Children's Res. Inst., Seattle, WA;

²Dept. of Pediatrics, ³Dept. of Bioengineering, Univ. of Washington, Seattle, WA

Abstract: Microglia are resident immune cells of the brain with key roles in development, neural plasticity, and defense during brain injury. Their abnormal function has been associated with neurological diseases across the lifespan. While *in vivo* microglial dynamics have been thoroughly characterized in the adult mouse brain, little is known about their normal dynamics during early postnatal development and conversely during brain aging. Microglial responses to vascular injury at these early and late life stages is also unknown. To gain more insight, we used *in vivo* two-photon imaging to explore cortical microglial dynamics in neonatal (postnatal day 9), adult (3-5 months), and aged (21-23 months) microglial-labeled mice (CX3CR1-GFP+/-) under normal physiological conditions and after laser-induced capillary injury. Under basal conditions, microglia in both neonates and aged mice have less ramified processes compared to the adult mice. Microglia preferred contacting the vasculature with their processes rather than their somata, which is more common among microglia in the adult brain. Microglia in the neonate are higher in density, with more mobile somata and processes that extend/retract rapidly at baseline. While aged animals had similar microglia density as adults, their processes were significantly less dynamic. During vascular injury in the adult brain, microglia cells extended their processes in a concerted fashion to surround and contain sites of injury. The aging brain is still capable of reacting to microvascular injury, though the amount of process extension declined. In neonates, the response was uncoordinated with some cells extending rapidly toward the injury, while other cells presenting delayed responses. Curiously, only adults reacted to sham lesions that were made in the parenchyma, and not the capillary wall. Three days post-injury, microglial dynamics had largely recovered to baseline levels in the adult and aged brain, while neonates exhibited sustained microglial aggregation at the injury site and more broadly in surrounding brain regions. Our findings support the idea that basal and injury-evoked responses of microglial cells differ based on life stage. In the neonatal period, microglia are highly dynamic but mount uncoordinated and enduring responses to vascular insult. In the aged brain, microglia exhibit reduced capacity to respond to focal insults. These findings reflect microglial immaturity during development, and a decline of microglial function with aging.

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Poster

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Topic: B.09. Glial Mechanisms

Support: NIH-NINDS (R35) NS127232
Nomis foundation awards

Title: *Srgap2* expression in microglia regulates the development of synaptic connectivity in the neocortex.

Authors: *C. DIAZ SALAZAR, F. POLLEUX;
Columbia Univ., Columbia Univ., New York City, NY

Abstract: Precise patterns of connectivity underlie the functional properties of neural circuits, and ultimately behavioral performance. During cortical development, neurons form immature synapses that can be later on maintained or eliminated in an activity-dependent manner. Microglia, the brain-resident phagocytes, play a key role in shaping synaptic connectivity during development and synaptic plasticity in mature circuits. In the past decade, our lab has demonstrated that the postsynaptic protein SRGAP2 limits synapse density and promotes their maturation cell-autonomously in cortical pyramidal neurons (PNs). Interestingly, in the developing and adult mouse and human cortex, SRGAP2 is expressed at high level in microglia. We tested the potential cell-autonomous function of SRGAP2 in microglia by generating microglia-specific conditional deletion of *Srgap2* (*TMEM119^{Cre-ERT2};Srgap^{F/F}*) with tamoxifen induction at P8, corresponding to the peak of synaptogenesis. Our results revealed that SRGAP2 deletion in microglia leads to a dose-dependent hyper-ramification, suggesting that SRGAP2 limits the complexity of microglial morphology at steady state. We then tested whether microglia-specific effect of SRGAP2 deletion has a non-cell autonomous effect on synaptic development in layer 2/3 PNs. Our results revealed increased synaptic density in basal and apical dendrites of these neurons. Interestingly, *Srgap2*-deficient microglia from juvenile mice exhibited reduced phagocytic activity (as revealed by CD68 staining), suggesting that SRGAP2 expression in microglial cells exerts a cell non-autonomous function on neuronal synapse elimination by modulating synaptic pruning. We are currently exploring if SRGAP2 exerts these effects through its Rac1-GAP activity and/or its ability to induce membrane deformation through its F-BAR domain. We are also testing if the human-specific paralog SRGAP2C which inhibits SRGAP2A function exerts a function in microglial cells where it is co-expressed at the same levels than the ancestral SRGAP2A. Finally, we are also testing if SRGAP2A regulated structural forms of synaptic plasticity in adult circuits. Taken together, we propose a novel role for SRGAP2A in the regulation of synaptic development through its function in microglial cells.

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Poster

442. Microglial Mechanisms of Development and Physiology

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Topic: B.09. Glial Mechanisms

Support: NARSAD Young Investigator Award 26155 (2018-2020, PI: De Biase)
David Geffen School of Medicine Seed Grants (2021, PIs: DeNardo, De Biase, Geschwind, Golshani)

Title: Microglial support of synaptic development in the nucleus accumbens

Authors: *M. GONGWER¹, F. ETIENNE¹, E. MOCA¹, J. RILEY¹, A. ENOS¹, M. HARATIAN¹, C. PRIDANS², L. DENARDO¹, L. DE BIASE¹;
¹Physiol., UCLA, Los Angeles, CA; ²Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: The nucleus accumbens plays a critical role in numerous motivated behaviors, both aversive and rewarding, where it serves as a functional link between the limbic system and motor output regions. Dysfunction in the nucleus accumbens has been linked with multiple psychiatric and neurological disorders, including addiction, mood disorders, and neurodegenerative diseases. While this brain region is widely studied and well characterized in the adult brain, much less is known about the cellular and synaptic development of this region. Our lab recently demonstrated a robust overproduction of nucleus accumbens microglia during the second and third postnatal weeks, after which these cells undergo programmed cell death. Given the emergence of microglia as crucial players in synaptic development, we postulate that this overproduction likely plays an important role in synapse maturation in this region. To probe this hypothesis, we performed whole-cell electrophysiology recordings in acute brain slices from microglia-depleted mice and wild-type littermate controls across early postnatal development. To achieve microglia depletion, we obtained a knockout mouse line for a tissue-specific CSF1R enhancer, *fms*-intronic regulatory element (FIRE). Knockout of this enhancer selectively eliminates microglia and macrophage populations in the brain, peritoneum, heart, and kidney while leaving other macrophage and osteoclast populations intact, significantly reducing off-target effects such as growth retardation in comparison to global CSF1R knockout models. We quantify miniature excitatory and inhibitory postsynaptic currents, AMPA/NMDA ratio, and release probability as probed by paired-pulse ratio in developing medium spiny neurons from this mouse line. Our data suggest that genetic microglial depletion delays functional synapse maturation as measured by some, but not all, of these physiological properties, suggesting a key role for microglia in shaping maturation of nucleus accumbens circuits.

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Poster

442. Microglial Mechanisms of Development and Physiology

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

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Topic: B.09. Glial Mechanisms

Support: ERC consolidator grant

Title: In vivo two-photon STED imaging of microglia-synapse communication

Authors: *N. GOCKEL, S. POLL, M. FUHRMANN;
Neuroimmunology and Imaging Group, German Ctr. of Neurodegenerative Dis. (DZNE), Bonn, Germany

Abstract: Microglia-synapse interactions have been examined in a variety of contexts, *in vitro* and *in vivo*, which revealed an important role for microglia in synaptic pruning and loss. However, the question remains whether microglia are also involved in the process of synapse formation, by serving as a link between pre- and post-synapse. We hypothesize that microglia sense neurotransmitter release at highly active pre-synaptic sites and actively modulate the formation of new spines at the dendritic site. To examine this relationship, we target microglia-synapse communication in the mouse hippocampus *in vivo* under normal physiological conditions related to learning and memory. Our methodological approach goes beyond the current state-of-the-art and for the first time yields microglia-synapse interactions in super-resolution time-lapse imaging of awake mice. We established two-photon (2P) stimulated emission depletion (STED) microscopy via a cranial window *in vivo*, to increase imaging depth and at the same time restrict excitation to a smaller focal point. Our results show improved resolution of 2P STED imaging of microglia-synapse interactions as well as a relationship between dendritic spine formation and previous contact frequency by microglia. These findings will pave the way to visualize nanoscale anatomical structures during microglia-synapse communication *in vivo* and therefore allow longitudinal and correlative studies in combination with behavioral experiments.

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Poster

442. Microglial Mechanisms of Development and Physiology

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Program #/Poster #: 442.17

Topic: B.09. Glial Mechanisms

Title: Microglia innate immune signaling regulates neuronal activity through MEF2C transcription network

Authors: *Y. HUANG, S. AMIN, J. C. UDEOCHU, L. FAN, M. ZHAO, L. GAN;
Helen and Robert Appel Alzheimer's Dis. Res. Inst., Weill Cornell Med., New York, NY

Abstract: Cognitive function requires regulated and balanced excitatory and inhibitory signaling in the brain. The disruption of the balance is commonly observed in Alzheimer's disease (AD). Previous research shows microglia contributes largely to neuroinflammation and neurodegeneration. One question we aim to understand is if microglia innate immune response contributes to the imbalance between excitatory and inhibitory signaling in AD. In the P301S tauopathy model, we find evidence of activation of the innate DNA sensing cGAS pathway specifically in microglia. We then generated P301S; Cgas^{-/-} mouse line. Through single-nuclei RNA sequencing, we identify that compared to P301S mice, P301S; Cgas^{-/-} mice has downregulated interferon signature (Stat1, Rnf213, Ddx60). With immunohistochemistry (IHC) we show significant downregulation of phospho-Stat1 expression in microglia. Pathway analysis of neuronal response from P301S; Cgas^{-/-} animal shows changes in neuronal activity, including upregulation of anti-seizure gene, alterations of K⁺ and Ca²⁺ channel, and neurotransmitter receptor activity. What is common between excitatory and inhibitory neuron is the upregulation of Mef2c, a transcription factor crucial for synaptic transmission, Parvalbumin interneuron development, and neuronal excitability. With IHC, we show significant upregulation of Mef2c immunoreactivity in NeuN⁺ neurons in hippocampus CA1 region of P301S; Cgas^{-/-} mice compared to P301S mice. We also identified a unique enrichment of Mef2c targets in the differentially expressed genes in both excitatory neuron and inhibitory neuron. Further examining 2 previously published human AD single nuclei RNA sequencing dataset (Mathys et al., 2019, Sayed-Kodama et al., 2021), we find significant negative correlation between microglial interferon gene IRF3, RNF213 and neuronal MEF2C expression. Our current data suggests the potential role of cGAS pathway in regulating neuronal excitability. cGAS pathway regulates microglia interferon response. Microglial interferon signals to neurons and modulates the expression of MEF2C and its transcription target genes, leading to changes in neuronal activity.

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Poster

442. Microglial Mechanisms of Development and Physiology

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Topic: B.09. Glial Mechanisms

Support: A.P. is a research fellow of F.R.S-FNRS
FMRH

Title: Phagocytosis and synaptic pruning are enhanced in mechanically activated glial cells

Authors: A. PROCÈS¹, Y. ALPIZAR⁴, B. BRÔNE^{4,5}, F. SAUDOU⁵, S. GABRIELE², *L. RIS^{3,4};

¹Neurosci Dpt, ²Mechanobiology & Biomaterials group, ³Univ. of Mons, Mons, Belgium;

⁴Neurophysiol. laboratory, Univ. of Hasselt, Hasselt, Belgium; ⁵Intracellular Dynamics and Neurodegeneration Team, Grenoble Inst. Neurosci., Grenoble, France

Abstract: In brain tissues, mechanical stresses can lead to complex neuroinflammation events. Both microglia and astrocytes play a significant role in mediating the progression of a mechanical damage. Microglia are the primary immune cells of the central nervous system and respond to pathogens and injury by changing their morphology and migrating to the site of injury to destroy pathogens. Classic ways of microglia activation can be triggered by antigen presentation like chemokines, damaged-associated molecular patterns (DAMPs) or pathogen particles. During brain injuries, astrocytes and microglial cells can undergo mechanical deformations, leading to a possible alternative mechanism of activation. However, while the impact of chemical signaling on astrocytes and microglia function has been studied in much detail, the current understanding of mechanical signaling is very limited. To address this challenge, we studied in vitro the role of mechanically injured microglia and compared this to a classical activation way with lipopolysaccharides treatment. BV2 and primary microglial cells were cultivated on elastic membranes and mechanically activated by a rapid single stretch (< 1 sec) in order to mimic traumatic brain injury (TBI). All experiments were conducted 24 hours post-injury. Our findings indicate that 20% stretch of microglial cells does induce an activation through the increase of IBA1 protein level as well as an increase of actin fluorescence signal. This activation state is found to be simultaneous with the stiffening of BV2 cells as we measure it using a ferule-top nanoindenter. In addition, results of migration show a change in cell behavior suggesting that immune glial cells are mechanosensitive and can adopt an activated state in response to a single mechanical stretch. Nonetheless, we showed a large increase of phagocytosis activity in mechanically activated cells. To understand the consequences generated by this modulation of phagocytic activity, we introduced the microglial cells in microfluidic chambers allowing the isolation of synaptic connections of a cortical neuronal network. We observed an increase in synaptic markers (PSD95 and synaptophysin) inside mechanically activated microglia. Altogether, these results suggest that mechanical activation of microglial cells during traumatic brain injury could be one of the factors leading to neuroinflammation and synaptic pruning in the hours and days following the lesion. Further analysis of the molecular pathways involved in this activation process could lead to new therapeutic strategy to prevent long-term disabilities after trauma.

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Poster

442. Microglial Mechanisms of Development and Physiology

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Program #/Poster #: 442.19

Topic: B.09. Glial Mechanisms

Title: It's not about the destination, it's about the journey: microglial navigation through complex brain tissue

Authors: *A. K. J. BOYCE^{1,2}, A. OCHOA DE AMEZAGA², R. J. THOMPSON¹, V. U. NÄGERL²;

¹Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada; ²Univ. of Bordeaux - CNRS, Bordeaux, France

Abstract: Microglia play diverse roles across physiological and pathological conditions as the principle immune cells of the brain. In physiological conditions, thin and ramified microglial processes perform homeostatic surveillance functions, monitoring and pruning synapses to regulate synaptic plasticity. Under pathophysiological conditions, microglial processes rapidly and autonomously converge on focal injuries independent of cell body movement or retract towards the cell body generating an amoeboid activated morphology during chronic inflammation or neurodegeneration. Investigation of cell-cell interactions between microglia and their targets has relied upon positively labelling microglia and target cells with fluorescent proteins to identify interactions. Thus, although we are beginning to understand the start and end points of microglial navigation, we know very little about the journey itself. Brain tissue is a densely packed meshwork of distinct cell types with unique morphologies. So how do dynamic microglial processes navigate through brain tissue during physiological and pathophysiological conditions? Do cellular bystanders between microglial processes and their targets move aside, as we do in response to a passing emergency vehicle, or do processes navigate otherwise immobile cellular bystanders, either weaving or pushing their way through the tissue? *In vitro*, microglia are typically highly reactive in morphology and function; to ameliorate this, we developed an optimized protocol for maintaining healthy microglia in their surveillance state in organotypic Müller hippocampal slice cultures. Paired with these cultures, we used our novel fluorescence imaging technique, confocal shadow imaging (COSHI), for labeling extracellular fluid with a fluorescent dye to coincidentally generate a sharp inverted contrast image of all cellular structures, providing subcellular resolution of the entire context of an organotypic slice in an unbiased manner. Using COSHI combined with fluorescence lifetime-based separation, we demonstrate the behaviour of EGFP-labeled microglia (CX3CR1-EGFP) amidst the cellular environment of healthy living organotypic brain slices, as well as in response to a local lesion and global excitotoxicity. This study provides a first look into microglial navigation through the environmental context of complex brain tissue in physiological and pathophysiological conditions.

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Poster

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Support: GW4 BioMed DTP PhD funding
ARUK South West small grant

Title: Gamma-frequency oscillations induce changes to the morphology and activation of microglia

Authors: *M. ELLEY, J. WITTON, J. BROWN;
Univ. of Exeter, Exeter, United Kingdom

Abstract: Microglia are the immune cells of the central nervous system, which are responsible for mediating neuroinflammation in both health and disease. Their activity has been implicated in several neurological diseases such as dementia, and it is currently debated as to whether the inflammatory response observed in neurodegenerative disease is causative or compensatory. A recent series of studies has demonstrated that inducing neuronal network oscillations at the gamma frequency of 40 Hz is capable of reducing the neuropathology and cognitive deficits associated with dementia, which appears to occur as a result of microglia activation. To investigate the molecular mechanisms underlying this novel neuron-microglia interaction, we have developed an *ex vivo* assay, using hippocampal brain slices prepared from two mouse models (C57BL/6J and CX3CR1-GFP^{+/+}, which express GFP exclusively in microglia). This assay involves pharmacologically inducing gamma oscillations in the CA3 region of the hippocampus, fixing the brain tissue and then assessing microglia morphology following staining and mounting using two-photon microscopy. We have demonstrated that microglia morphology is altered in response to gamma oscillations, as microglia that have been exposed to these network oscillations have a significantly more amoeboid morphology than those in control conditions, where no oscillations were induced. These data suggest that sustained gamma oscillations result in the activation of microglia. Therefore, we have developed a new reduced model of gamma oscillation-microglia signalling that can be used to gain mechanistic understanding of this novel neuroimmune interaction.

Disclosures: M. Elley: None. J. Witton: None. J. Brown: None.

Poster

442. Microglial Mechanisms of Development and Physiology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 442.21

Topic: B.09. Glial Mechanisms

Support: NIH Grant F32MH124298
URMC SPIN Grant

Title: Frontal cortical microglia respond to dopaminergic signals from the adolescent mesofrontal circuit

Authors: *R. STOWELL, K. H. WANG;
Univ. of Rochester Med. Ctr., Univ. of Rochester, Rochester, NY

Abstract: Psychiatric disorders such as schizophrenia are neurodevelopmental disorders with diverse disruptions to neuronal circuitry. In animal models, adolescent changes in the mesofrontal dopaminergic circuit may contribute to the cognitive deficits associated with neurodevelopmental disorders. Previous work suggests that the adolescent circuit is not just vulnerable, but malleable, providing a window for intervention. Indeed, phasic activation of the adolescent dopaminergic mesofrontal circuit through wheel running or direct optogenetic activation drives dopaminergic bouton outgrowth in the frontal cortex. However, the mechanisms behind this form of adolescent plasticity remain unclear. Microglia, the resident immune cell of the central nervous system, serve diverse roles in circuit refinement throughout development. Importantly, recent evidence has shown that microglia respond *in vivo* to specific neurotransmitter signals. Here, with a combination of transgenic, optogenetic, and viral labeling transfection, we investigated if microglia respond to dopaminergic signals *in vivo* and if their response to these signals impacted mesofrontal plasticity. With *in vivo* two-photon microscopy, we tracked fluorescently labeled microglia and dopaminergic axons in the M2 frontal cortical region pre- and post- stimulation of dopaminergic ventral tegmental area (VTA) neurons. After 2-hours of wheel running, microglia exhibit increased arborization and parenchyma occupation as compared to their pre-run parenchyma occupation. Importantly, this effect was unique to adolescent animals. We then used the refined temporal profile of optogenetics to assay whether this microglial response was a direct consequence of dopamine release. We found with optogenetic stimulation, microglia exhibit a biphasic response to VTA stimulation with an initial reduction in motility and surveillance of the parenchyma during dopamine release and a subsequent extension of processes into the parenchyma after cessation of stimulation. Post-stimulation, microglia make more frequent, but more transient, contacts with axonal boutons. Our pharmacological experiments show reduced surveillance and motility in the presence of D2-type receptor agonists, suggesting that the initial microglial response to VTA stimulation may be mediated by D2 receptors. Future work will address if manipulating dopaminergic receptor signaling systems in microglia will impede mesofrontal plasticity. With an improved understanding of the mechanisms which regulate dopaminergic system development and plasticity, we hope to provide therapeutic targets for neurodevelopmental disorders.

Disclosures: R. Stowell: None. K.H. Wang: None.

Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.01

Topic: B.11. Neuro-Oncology

Support: CRUK
CIHR
University of Toronto

Title: Investigating the role of dopaminergic activity in brain cancer

Authors: *M. WARE^{1,2}, L. LOPES PONTUAL^{1,2}, C. YU^{1,2}, M. KUSHIDA^{1,2}, N. RASTEGAR^{1,2}, S. DOLMA^{1,2}, P. DIRKS^{1,2};

¹Univ. of Toronto, Toronto, ON, Canada; ²Developmental & Stem Cell Biol., The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Glioblastoma (GBM) is an incurable disease of adults and children and the deadliest form of central nervous system (CNS) malignancy. Despite major advances in our understanding of GBM biology in recent years, the prognosis for patients who develop this disease has not improved. If we hope to find new treatments for GBM that are safe and effective, we desperately need to reform our thinking. The brain's chemical milieu is rich with neurotransmitters, and our lab's screen of 680 neuroactive compounds on patient-derived glioblastoma stem cells (GSCs) *in vitro* strongly implicates neurotransmitter pathways as critical regulators of the GSC niche. More specifically, disrupting dopamine signaling by inhibiting its receptor D4 on GSCs causes substantial GSC apoptosis *in vitro* and attenuates GBM growth *in vivo* (Dolma *et al.*, Cancer Cell, 2016). Dopamine (DA) is a catecholamine neurotransmitter that is essential for reward learning and movement and has numerous roles in cognition. Consequentially, dysregulation of DA signaling is associated with a diversity of brain diseases ranging from drug addiction to schizophrenia to Parkinson's. Our research aims to determine how DA signaling affects normal neural stem cell (NSC) and tumorigenic GSC populations, as we hypothesize that GSCs arising/residing in DA projection zones exploit dopaminergic (DAergic) activity for GBM growth. Toward these aims, we have developed *in vivo* model systems and harnessed them to study NSC and GSC niches in the context of either controlled activation or depletion of DAergic neurons. These manipulations of the brain's DAergic neurons are achieved using optogenetic stimulation, genetic depletion, and neurotoxin-mediated ablation in mice. Ultimately, unraveling the dopaminergic influence on GBM may contribute to a redeployment of existing treatments—that modulate DA signaling and are already approved to treat CNS disorders—to patients with this deadly brain cancer.

Disclosures: M. Ware: None. L. Lopes Pontual: None. C. Yu: None. M. Kushida: None. N. Rastegar: None. S. Dolma: None. P. Dirks: None.

Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.02

Topic: B.11. Neuro-Oncology

Support: College of Medicine Research Award (COMGRAD)
Breast Cancer Society of Canada

Title: Prognostic implication of nerve distribution in breast cancer.

Authors: *P. J. DESAI, J. HALL, I. SASSMANNSHAUSEN, A. KRISHNAN;
Anat. Physiol. Pharmacol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Recent studies demonstrated that peripheral nerves modulate breast cancer progression. In particular, parasympathetic and sensory nerve signaling was shown to suppress breast cancer, while sympathetic signaling was shown to promote the same. A possible association between nerve activity and breast cancer metastasis was also suggested. However, a quantitative association between overall tumor-nerve density and breast cancer metastasis has not been explored well. In this study, we examined total nerve density in 36 human breast tumors and established their association with metastatic incidence. The samples were procured from Alberta Cancer Research Biobank following the guidelines approved by the Biomedical ethics committee. Our sample cohort contained 18 breast tumors from patients that developed metastasis and another 18 breast tumors from patients that did not develop metastasis within the first five years of diagnosis. Immunohistochemical analysis was performed to examine the distribution of different classes of nerve fibres, and western blot was performed to assess the expression of corresponding receptors. Our result showed an association between decreased total nerve density in the tumor microenvironment and metastatic incidence. However, class-specific nerve density analysis and expression of their corresponding receptors showed mixed results. Overall, our results suggest that class-specific nerve density analysis may be used to predict breast cancer metastasis. Additional studies involving a large set of samples corresponding to various stages of the disease are warranted to further establish an association between nerve density and stage-specific progression of breast cancer.

Disclosures: P.J. Desai: None. J. Hall: None. I. Sassmannshausen: None. A. Krishnan: None.

Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.03

Topic: B.11. Neuro-Oncology

Title: Lateral proteome transfer between sensory neurons and breast cancer

Authors: *Y. WU, J. BORNIGER;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Emerging evidence suggests that the nervous system plays a critical role in cancer pathogenesis. Reciprocally, cancers can alter forms and functions of the nervous system. Crosstalk between the nervous system and cancer occurs through direct nerve-cancer interactions or via the regulation of other cell types in the tumor microenvironment. The peripheral nervous system is a crucial component of the tumor microenvironment that remains understudied. In our *in vitro* model, co-cultured breast cancer cells show direct contact with sensory neurons, and exhibit significantly increased rates of proliferation and migration especially in the presence of stroma. Using RNA sequencing, we observed that co-cultured breast cancer cells upregulate multiple cell signaling pathways, including cell adhesion, migration and proliferation. Moreover, we demonstrated that nascent proteins and mitochondria are transferred from sensory neurons to cancer cells using bioorthogonal labeling and immunostaining, respectively. These cellular components trafficking may implicate in cellular homeostasis, damaged cell repair, tumor progression, and immunoregulation. Our research focuses on identifying the trafficked proteins, the structure of cellular connections and their functionalities by using mass spectrometry, electron microscopy and microelectrode array. Since cells respond to environmental changes by altering their protein ensemble, our *in vivo* model uses bioorthogonal labeling and click chemistry ligation of nascent proteins to monitor precise proteome changes within specific brain regions in response to internal (e.g., cancer development) and external stimuli. By this means, a methionyl-tRNA synthetase mutant is expressed under a cell-type-specific promoter through the Cre-Lox recombination system, which allows non-canonical amino acids (ncAA) to incorporate into newly synthesized proteins within specific cell populations. The ncAA-tagged proteins can be ligated to dyes for visualization or to affinity probes for enrichment and identification. As a result, the proteome changes can be distinguished from the complex existing protein pool, and the sample complexity is decreased by enriching a subset from specific cell populations. This study is expected to illuminate black boxes in the crosstalk between the nervous system and peripheral cancers, promote the identification of molecular mechanisms involved in their interactions, new cancer biomarkers, and define potential anti-cancer targets for therapeutic interventions.

Disclosures: Y. Wu: None. J. Borniger: None.

Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.04

Topic: B.11. Neuro-Oncology

Support: Ride for Dad
Prostate Cancer Fight Foundation
Saskatchewan Health Research Foundation

Title: Imbalance in hormone-neurotransmitter signaling modulates neuroendocrine differentiation of cancer cells

Authors: *S. SHRESTHA, B. CHOWDHURY, A. KRISHNAN;
Anatomy, Physiology, and Pharmacol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Recent studies showed that sympathetic nerves play a major role in promoting prostate cancer. Selective denervation of sympathetic nerves and targeted depletion of sympathetic signals were shown to suppress prostate tumorigenesis and progression. In addition, a critical role for sympathetic signaling for the induction neuroendocrine differentiation (NED) of prostate cancer cells was also shown. NED is a prerequisite for the development of treatment-resistant neuroendocrine prostate cancer (NEPC). However, the underlying stimulating factor for the sympathetic signaling-dependent induction of NED was not clear. On the other hand, long-term therapies involving new-generation anti-androgen drugs have been identified as a risk factor for the induction of NED and the development of NEPC. Here, we tested if androgen deprivation is a stimulating factor for the sympathetic signaling mediated NED in prostate cancer cells. We used the androgen receptor-positive (AR⁺) cell line LNCaP for this study. The cells were treated with a combination of testosterone and the sympathetic neurotransmitter norepinephrine (NE) for one week. The testosterone was then withdrawn for a 24-72 h time interval, followed by the expression of the molecular markers of NED was examined in these cells. The cells maintained in the testosterone + NE combination treatment at the respective time intervals were used as the corresponding controls. Our mRNA analysis showed that the NED molecular markers, including chromogranin B and synaptophysin, were highly upregulated in the testosterone-deprived groups, especially at the 48 h and 72 h time intervals, compared to the combination group. Overall, our result suggests that an imbalance in the hormone and neurotransmitter signaling triggers NED in cancer cells. This imbalance may be critical for the development of neuroendocrine cancers, including NEPC.

Disclosures: S. Shrestha: None. B. Chowdhury: None. A. Krishnan: None.

Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.05

Topic: B.11. Neuro-Oncology

Support: Cancer Research Society Grant 19188
Canadian Cancer Society Research Institute 701740

Title: Norrin/frizzled4 signalling controls the microenvironment to suppress medulloblastoma

Authors: *N. T. POKRAJAC^{1,3}, N. J. A. TOKAREW², A. GURDITA^{2,3}, V. A. WALLACE^{1,3};
¹Donald K Johnson Eye Institute, Krembil Res. Inst., ²Vision Sci., Univ. Hlth. Network, Toronto, ON, Canada; ³Lab. Med. and Pathobiology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Crosstalk between pre-tumour and stromal cells in the microenvironment plays a pivotal role in tumour initiation and progression. In the cerebellum, canonical Wnt signalling mediated by Norrin/Frizzled4 (Fzd4) activation in meningeal endothelial cells inhibits the development of Sonic hedgehog medulloblastoma (Shh-MB) in mice. Here we investigated the mechanism of Norrin/Fzd4-mediated tumor initiation in granule cell progenitors (GCPs), the cell of origin of Shh-MB, in two mouse models of spontaneous MB, *Ptch*^{+/-} and *NeuroD2-Smo*^{A1+/-}. To characterize Norrin-dependent stromal remodelling we used immunohistochemistry and RNAscope and to quantify preneoplasia we used light sheet fluorescence microscopy (LSFM) to image cleared cerebella. We show that Norrin/Fzd4 signalling is required to maintain the activation of state, marked by Lyve1 and Mrc1 expression, of pvMΦs in the meninges during the critical window for tumorigenesis, P7 to P14. In vivo pvMΦ depletion, by intracranial administration of clodronate liposomes or systemic CSF1R inhibition, phenocopies the effects of Norrin deficiency on preneoplasia and tumor progression in Shh-MB prone mice, indicating that pvMΦs suppress Shh-MB tumorigenesis. Finally, we found that both Norrin/Fzd4 signalling and pvMΦs can attenuate preneoplasia and tumorigenesis by inhibiting GCP proliferation and promoting GCP differentiation and migration during postnatal cerebellar development. Taken together, our results identify an unanticipated cross talk between endothelial cells and pvMΦs, mediated by Norrin/Fzd4 signalling, in the control of preneoplasia in the cerebellum.

Disclosures: N.T. Pokrajac: None. N.J.A. Tokarew: None. A. Gurdita: None. V.A. Wallace: None.

Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.06

Topic: B.11. Neuro-Oncology

Support: Good Samaritan Foundation of Legacy Health

Title: Adenosine kinase isoforms in cellular proliferation

Authors: R. GESESE¹, B. BUI¹, J. REEMMER¹, J. M. COOK¹, R. M. ROSE¹, O. MITIUKHIN², V. S. BROVARETS², J. N. SARKARIA³, *H.-Y. SHEN¹;

¹Legacy Res. Inst., Portland, OR; ²Inst. of Bioorganic Chem. and Petrochemistry of the Natl. Acad. of Sci. of Ukraine, Kyiv, Ukraine; ³Mayo Clin. Comprehensive Cancer Ctr., Rochester, MN

Abstract: Aims: The adenosinergic signaling pathway is revealed to participate in cancer pathology and the adenosine metabolic enzyme, adenosine kinase (ADK) is dysregulated in cancers with disturbance of its two isoforms - ADK long (ADK-L) and short (ADK-S) isoforms. This study aimed to characterize ADK isoform-mediated actions on cellular proliferation and to evaluate ADK inhibitors in the suppression of glioblastoma cell proliferation. **Methods:** We

used a retroviral approach to establish in vitro system model of cultured baby hamster kidney (BHK) cell lines that have selective expression patterns of isoforms in a subcellular refined manner. Two lines of patient-derived glioblastoma multiforme (GBM) cells with distinct expression patterns of ADK isoforms were tested to evaluate the action of ADK inhibitors on cellular proliferation. MTT assay and xCELLigence real-time cell analysis were used to determine cell viability and proliferation, and Western blot assay and immunocytochemistry (ICC) were performed to evaluate cellular ADK expression profiles. ADK inhibitor 5-iodotubercidin (5-ITU) and NSC compounds were used to differentiate isoform-specific actions of ADK in BHK and GBM cell lines. **Results:** (i) Distinct ADK expression profiles were identified in our generated BHK lines; BHK-AK2 line with complete deletion of both isoforms, resulting in significantly lower proliferation vs wild-type BHK (BHK-WT) cells; (ii) BHK-AK/L and BHK-AK/S lines with solely induced human ADK-L or ADK-S led to differently increased proliferation vs BHK-AK2 cells; (iii) 5-ITU suppressed proliferation of BHK cells with a predominant effect on ADK-S isoform, i.e., in BHK-WT and ADK-AK/S; conversely, NSC-B showed an ADK-L referent effect in the suppression of BHK cell growth. (iv) NSC-B effectively suppressed the proliferation of two GBM cell lines. **Conclusion:** Together, we established an *in vitro* cellular platform for the development of ADK inhibitors as a potential therapeutic antitumor application.

Disclosures: **R. Gesese:** None. **B. Bui:** None. **J. Reemmer:** None. **J.M. Cook:** None. **R.M. Rose:** None. **O. Mitiukhin:** None. **V.S. Brovarets:** None. **J.N. Sarkaria:** None. **H. Shen:** None.

Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.07

Topic: B.11. Neuro-Oncology

Support: NIH R21 DA049253-02
NIH R01 NR019531-01

Title: Acute Lymphoblastic Leukemia and Methotrexate Impact the Transcriptional Response in Microglia and Astrocytes in the Prefrontal Cortex

Authors: ***A. B. DAVIS**¹, J. L. BOLLINGER¹, C. CANTELON¹, E. S. WOHLER¹, T. M. REYES²;

¹Pharmacol. and Systems Physiol., Univ. of Cincinnati, Cincinnati, OH; ²Pharmacol. and Systems Physiol., Univ. of Cincinnati, Col. of Med., Cincinnati, OH

Abstract: Acute Lymphoblastic Leukemia (ALL) is one of the most prevalent childhood cancers, representing around 23% of all childhood cancer diagnoses. Survivorship has increased due to the success of chemotherapeutic protocols, involving drugs like methotrexate (MTX).

While MTX has increased survival rates, it has also been linked to long term cognitive deficits which occur in between 40% and 70% of all survivors. Reducing the incidence of long-term side effects like cognitive deficits in survivors is a priority. Our preliminary studies have indicated that neuroinflammatory responses due to MTX treatment may be related to the deficits seen in ALL survivors. Neuroinflammation engages specific immune cell populations namely, microglia, the resident immune cell, and astrocytes, an important glial cell in the brain. Transcriptional changes associated with the specific cell populations in relation to ALL and MTX is not known. Therefore, to investigate the roles of microglia and astrocytes in the neuroinflammatory responses in early life cancer+chemotherapy exposed animals, we have created a mouse model that incorporates both cancer and chemotherapy exposure which leads to cognitive deficits. C57BL/6 x DBA F1 mice were randomly assigned to an injection of cultured L1210 cells, mouse leukemic cell line, or saline on postnatal day 19 (P19). Starting on P21 the mice that received cancer began a 4-cycle chemotherapy regimen using MTX, vincristine, and leucovorin; the control group received saline injections. One day following the final chemotherapy exposure, prefrontal cortex (PFC) was dissected from the brains. Microglia and astrocytes were isolated from the PFC using a Percoll density gradient and fluorescence activated cell sorting (FACS). RNA isolated from the sorted cells were used for RNA-sequencing to identify a broad range of gene expression changes. Transcriptional profiles of both microglia and astrocytes in response to early life cancer+chemotherapy exposure will be presented.

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Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.08

Topic: B.11. Neuro-Oncology

Title: High levels of TrkB.T1 induces deregulation of synaptic genes in human gliomas and in different stem cell lines

Authors: *L. MERINO-GALAN¹, S. ARORA², P. J. PADDISON², E. C. HOLLAND², M. EVANS¹, S. S. PATTWELL¹;

¹Ben Towne Ctr. for Childhood Cancer Res., Seattle Children's Res. Inst., Seattle, WA; ²Div. of Human Biol., Fred Hutchinson Cancer Res. Ctr., Seattle, WA

Abstract: Gliomas are the most common type of brain tumor in both children and adults. Communication between neurons and cancer cells is a fundamental component of brain cancer pathophysiology. Neuronal activity drives the proliferation and growth of glial malignancies through direct electrochemical synaptic communication and paracrine signaling, being brain derived neurotrophic factor (BDNF) one of the activity-dependent secreted drivers. BDNF binds to TrkB receptor, and both are fundamental for proper synaptic functioning and plasticity

throughout life. While prior studies have revealed the involvement of *NTRK2* gene (coding for TrkB) in cancer, the complex post-translational modifications and intricate splicing patterns are often ignored. Recent work from our group has shown that the TrkB.T1 splice variant is upregulated in various human gliomas and that overexpressing TrkB.T1 enhances PDGF β -driven aggressiveness *in vivo*. TrkB.T1 has been described to regulate Ca²⁺ intracellular signaling, which has an essential role in the establishment of functional synaptic connections. Thus, our aim was to study the contribution of high TrkB.T1 expression levels to the synaptic physiology using a synaptic gene ontology analysis. For this purpose, we explored different RNAseq datasets: 1. Highly correlated genes with *NTRK2* in human low-grade glioma (LGG) and glioblastoma (GBM); 2. Deregulated genes in human glioma stem cells (GSC) expressing high levels of TrkB.T1 vs. GSCs expressing low levels of TrkB.T1; 3. Deregulated genes in human neural stem cells (NSC) overexpressing TrkB.T1 vs. full-length TrkB. 60/350 and 43/350 genes have synaptic annotations in GBM and LGG, respectively. Interestingly, 6 presynaptic compartments were enriched in GBM including the synaptic vesicles and synaptic cytosol, but not the postsynapse, as well as 9 fundamental synaptic processes including the synaptic vesicle cycle, endocytosis, exocytosis and synaptic organization. However, none of the synaptic compartments or processes was significantly enriched in LGG, suggesting that the aggressiveness grade of gliomas could be related to synaptic mechanisms. Interestingly, 151/1249 deregulated genes in GSCs were mapped to SynGO, with enrichment of 13 synaptic compartments including the postsynaptic density membrane and specialization, and 23 synaptic processes mainly involved in signaling and organization. These results are consistent with the involvement of TrkB receptors in the establishment of malignant neuron-to-glioma synapses, and open new directions for research to understand mechanisms of TrkB.T1 signaling in oncogenesis and neuron-glioma communication.

Disclosures: L. Merino-Galan: None. S. Arora: None. P.J. Paddison: None. E.C. Holland: None. M. Evans: None. S.S. Pattwell: None.

Poster

443. Neuro-Oncology

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.09

Topic: B.11. Neuro-Oncology

Support: This study is funded by a developmental funds award from Comprehensive Cancer Center, St. Jude Children's Research Hospital.

Title: Studying metabolic effects of PI3K/mTOR inhibitor GDC-0084 in patient derived xenograft model of diffuse intrinsic pontine glioma

Authors: *L. SANCHEZ HERNANDEZ¹, Z. COLEMAN¹, J. OCASIO ADORNO², H. TAN³, E. PAVAO¹, K. CHAKRABORTY¹, P. BAGGA¹;

¹Diagnos. Imaging, ²Developmental Neurobio., ³Proteomics and Metabolomics, St. Jude Children's Res. Hosp., MEMPHIS, TN

Abstract: Diffuse intrinsic pontine gliomas (DIPG) appear in infancy with a survival rate of less than 10% after two years of diagnosis. Radiation therapy is the only available option for the treatment of this disease. Recently, a new brain-penetrant PI3 Kinase/mTOR inhibitor, Paxalisib (GDC-0084), has shown promising response to this disease and has reached clinical trial phase (NCT03696355, NCT05009992). We aim to characterize and assess tumor metabolism in response to GDC-0084 using stable isotopically labeled metabolic tracers. In our study, we used three patient-derived DIPG cell lines, HSJD-DIPG-007, SJ-DIPG-x7 and SJ-DIPG-x37. 1x10⁶ DIPG cells were treated with vehicle (DMSO) or with 0.5 μ M GDC-0084 for 24 hours in a medium containing; 1) U13C-Glucose + L-Glutamine, 2) U13C-Glutamine + D-Glucose, or 3) U13C-Acetate + D-Glucose + L-Glutamine. The cell extracts were collected in cold 80% acetonitrile and further processed to extract metabolites. We performed LC-MS/MS analysis to measure isotopologues of intermediates involved in different metabolic pathways including glycolysis, TCA cycle, amino acid (AA) biosynthesis and fatty acid (FA) biosynthesis. We observed that each cell line shows different behaviors depending on their sources of carbon. A significant reduction in glycolysis was observed in all three cell lines with GDC-0084 treatment. Further, a reduction in TCA cycle intermediates (succinate m+2, fumarate m+2 and oxoglutarate m+2) was observed only in SJ-DIPG-x37. A lower TCA cycle intermediate isotopologues enrichment from U13C-Glutamine in SJ-DIPG-x37 cell line treated with GDC-0084 pointed towards impaired glutamine reductive carboxylation. U13C-acetate allows the measurement of FA synthesis by incorporation of ¹³C-labeled acetyl-CoA into the long chain FAs. Treatment with GDC-0084 reduced oleate and vaccenic acid only in HSJD-DIPG-007 and ST-DIPG-x7 cells, demonstrating a disruption in saturated and unsaturated FAs. Further, measurement of glycolytic and mitochondrial ATP synthesis of these 3 cell lines by XF-Seahorse ATP Real Time Assay showed a significant reduction in the global energy obtained from both glycolytic pathway and oxidative phosphorylation in mitochondria of all the 3 cell lines. In vivo metabolic tracing analysis to carry out the characterization of the flux rates in key metabolic pathways in DIPG PDX models are currently underway. These studies combined with in vitro findings will help us design novel complementary therapies to target DIPGs

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Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.10

Topic: B.11. Neuro-Oncology

Title: 8 hidroxyquinoline decreases viability and induces apoptosis in glioma cells

Authors: ***K. M. LÓPEZ**¹, C. FLORES DE LOS ÁNGELES³, M. LARA-LOZANO⁴, N. PEREZ SILVA⁵, J. SEGOVIA⁶, M. ROJAS QUINTANA⁷, P. VERGARA⁶, J. A. GONZALEZ-BARRIOS⁸, E. BAUTISTA²;

²Biotech. Fac., ¹UPAEP, Puebla, Mexico; ³Univ. Popular Autónoma Del Estado De Puebla, Atlixco, Mexico; ⁴DFBN, Ctr. De Investigación Y De Estudios Avanzados De, Ciudad de México, Mexico; ⁵Univ. Popular Autónoma Del Estado De Puebla, Puebla, Mexico; ⁶Cinvestav-IPN, Mexico 07300 DF, Mexico; ⁷Univ. Popular Autónoma de Puebla, Puebla, Mexico; ⁸Hosp Regional Octubre, ISSSTE, Mexico DF, Mexico

Abstract: Glioblastoma is the most frequent and aggressive primary malignant tumor of the central nervous system. It has been reported that cancer cells have a high demand for iron compared with non-cancer cells; this effect is associated with the induction of cell proliferation. Based on the above, iron chelators are proposed as an alternative adjuvant (Gaur et al., 2018). 8-Hydroxyquinoline (8-HQ) is a molecule with anti-microbial, anti-inflammatory, anti-neurodegenerative, and anti-cancer activity related to its iron and copper chelating capacity. Therefore, in this work, we evaluated the 8-HQ effect on glioblastoma cell viability, iron levels, cyclin D, and activation of caspase 3 in the line of glioma C6. The results show that the different concentrations of 8-HQ (31.5-100µM) decrease the mitochondrial activity of C6 glioma cells at 3, 6, 12, and 24 h after treatment compared to the control group. The concentration of 1000 µM showed the most toxic effect; while concentrations of 62.5 and 125 µM for 24 h of incubation reduced mitochondrial activity by 50%, the loss of viability was confirmed by trypan blue assay. On the other hand, 62.5µM of 8HQ reduces cyclin D1 levels and intracellular iron concentration after 6 and 24 h but not after 12 h of treatment. Also, 0.9 µM of 8HQ decreased by 20% in the intracellular iron and induced caspase three activations after 24 h of treatment. These findings suggest that 8HQ and iron chelators are worth further investigation as a potential pharmacological agents in the therapy of brain tumors.

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Poster

443. Neuro-Oncology

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.11

Topic: B.11. Neuro-Oncology

Support: NIH Grant R01NS105556-02

Title: The Generation of Zinc Finger MYND-Type Containing 8 (ZMYND8) Knockout mIDH1 Glioma Cells using Lentiviral Vectors

Authors: *D. MUKHERJI¹, S. CARNEY^{2,3,4,5}, K. BANERJEE^{3,4}, F. NUÑEZ^{3,4}, J. COSTELLO⁷, P. LOWENSTEIN^{2,3,4,5,6}, M. CASTRO^{2,3,4,5};

¹Undergraduate Program in Neurosci., Univ. of Michigan, Ann Arbor, MI; ²Cancer Biol. Training Program, ³Dept. of Neurosurg., ⁴Dept. of Cell and Developmental Biol., ⁵Rogel Cancer Ctr., ⁶Grad. Program in Immunol., Univ. of Michigan Med. Sch., Ann Arbor, MI; ⁷Dept. of Neurolog. Surgery, Univ. of California San Francisco, San Francisco, CA

Abstract: Mutations in the gene encoding for isocitrate dehydrogenase 1 (IDH1^{R132H}) account for about 80% of low-grade gliomas (LGGs). Patients who carry this mutation have been shown to have better prognosis than those with wildtype IDH1. The IDH1 mutation is a gain of function mutation that converts alpha-ketoglutarate to the oncometabolite 2-hydroxyglutarate (2HG). The production of 2HG leads to inhibition of DNA and histone demethylases, specifically the KDM family and TET2 methylcytosine dioxygenases, resulting in hypermethylation. It has been shown that pharmacological inhibition of mIDH1 using AGI-5198 leads to decreased levels of 2HG and therefore could reveal biological pathways dependent on mIDH1. We obtained a patient derived glioma cell culture (GCC) (SF10602; J. Costello, UCSF) which endogenously expresses mIDH1 and is resistant to radiation. We treated the SF10602 GCC with 5µM AGI-5198 and we found downregulation of the chromatin reader ZMYND8. ZMYND8 has been shown to have a role in DNA repair and is recruited at sites of DNA damage. Based on these findings, we hypothesized that ZMYND8 may play a role in the radio-resistance of mIDH1 glioma cells. In order to investigate our hypothesis, we obtained lentiviral vectors from Wang et. al (2021), the team from UT Southwestern. These lentiviral vectors contained single-guide RNAs (sgRNAs) targeting ZMYND8 and CRISPR Cas9. We transfected HEK293T cells with the lentiviral, envelope, and packaging vectors in order to produce lentiviral particles. After incubation, lentiviral particles were collected from the conditioned media in order to transfect our mIDH1 mouse neurospheres. ZMYND8 KO cells were identified using puromycin selection and the lack of expression was confirmed by Western Blotting. We then exposed the cells to increasing doses of radiation and we observed reduced cellular viability in the KO cells using CellTiter-Glo assay. This work suggests that ZMYND8 plays a role in the radio-resistance of mIDH1 gliomas.

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Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.12

Topic: B.11. Neuro-Oncology

Support: Barncancerfonden
Åke Wibergs Stiftelse

Title: Therapeutically reprogramming microglia to anti-tumoral activation states in diffuse midline glioma, H3K27 mutant

Authors: *L. KEANE, M. POSADA PEREZ, B. JOSEPH;
Inst. of Envrn. Med., Karolinska Inst., Stockholm, Sweden

Abstract: Diffuse midline glioma, H3K27M mutant, formerly known as diffuse midline glioma (DIPG) is one of the most challenging paediatric cancers to date with poor treatment options and dismal survival outcomes. New treatment options are desperately needed. Previously we found that DIPG-induced activation of microglia leads to a tumour-supportive phenotype that is associated with a transient H3K27me3 downregulation suggesting a role for this pathway in regulating this activation state. However, it should be noted, that microglia do not carry the H3K27M mutation when analysed in DIPG patient tissue, otherwise characteristic of the cancer cells. EZH2 is a histone methyltransferase and the catalytic subunit of the polycomb repressor complex 2 (PRC2), responsible for tri-methylation of histone H3 at lysine 27. We found that repression of *Ezh2* in microglia induced an anti-tumour phenotype resulting in decreased cancer cell invasion capability, increased microglial phagocytosis, and increased microglia-mediated tumour cell death. Antisense RNA strategies were used to target *Ezh2* gene expression in BV2 microglia and we have now carried out RNA sequencing on scramble BV2 or si*Ezh2* microglia co-cultured with DIPG tumour cells to further understand how EZH2, PRC2 and H3K27me3 regulates DIPG-induced microglia activation states. Further work has found that activation of the microglial tumour supportive phenotype, in this context, is determined by a complex epigenetic mechanism involving both H3K4me1 and H3K27me3 and their associated methyltransferases. These histone marks are often associated with bivalent regulation of distinct gene transcription programs, and we are currently investigating if this is the case for DIPG-induced activation states using chromatin immunoprecipitation techniques. Overall, our results indicate that microglia, can be reprogrammed epigenetically by EZH2 inhibition to exert anti-tumoral responses and that microglia represent a new therapeutic avenue in DIPG. This future work should provide novel mechanisms governing pro-tumoral microglial activation states and new therapeutic targets that can be combined with EZH2 inhibition to enhance anti-tumoral microglia activation states.

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Poster

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Topic: B.11. Neuro-Oncology

Support: Fulbright U.S. Scholar Grant Program
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Title: Lipidomic analysis of a glioma cell line

Authors: K. K. RODRIGUEZ¹, A. SANGAMI¹, M. R. DOMINGUES², *E. E. SERRANO¹;
¹Biol. Dept., New Mexico State Univ., Las Cruces, NM; ²Dept. of Chem., Campus Universitário de Santiago, Univ. of Aveiro, Aveiro, Portugal

Abstract: Lipids encompass a diverse repertoire of hydrophobic and amphipathic organic compounds that are essential for cell structure and function. In the body, the brain's lipid content is second only to adipose tissue. Lipids support brain function in a variety of roles; they serve as components of cell membranes, energy sources, and mediators of immunity and inflammation. Lipidomics can help uncover the role of lipids in neural signaling and development as well as in pathological conditions of the nervous system. When combined with transcriptomics, lipidomics can enrich knowledge of molecular pathways for brain and sensory system function and potentially identify biomarkers of neurodegeneration. Lipidomics is useful in determining the impact of treatments on experimental systems and also may facilitate standardization and analysis of experimental cell culture systems to enhance reproducibility. This pilot study was designed to determine the lipidome of the F98 glioma cell line (ATCC CRL-2397; *Rattus norvegicus*) that is widely used for *in vitro* and *in vivo* studies of brain tumors. F98 cells were cultured according to the manufacturer's specifications and harvested for untargeted lipidome analysis. Six replicate samples were shipped on dry ice to the UC Davis West Coast Metabolomics Center for lipid profiling. Lipids were extracted according to protocols reported in Matyash V. et al. (J. Lip. Res.49; 2008; 1137-1146) and analyzed with an Agilent 6530B Q-TOF. LC/MS data were processed with MS-DIAL software (Version 4.48). Preliminary analysis identified over 3500 unique lipids. Peak spectra for 523 lipids could be annotated to assign nomenclature and external database identifiers (InChI key; LIPIDMAPS). Phosphatidylcholines, sphingomyelins, and phosphatidylethanolamines comprised almost half of the annotated lipids; triglycerides, ceramides, cholesterol, and diacylglycerols also were well represented in the F98 lipidome. It is significant that annotation was not possible for the 3000+ lipids whose relative abundance was estimated at ~30%. Moreover, the amount of cell culture mass required for lipid extraction using current methods imparts a technical challenge for lipidomics in comparison with transcriptomics where RNA-seq methods have been developed for single cell analysis. Outcomes from this research provide a framework for examining how the F98 lipidome is modulated by experimental treatments and afford the possibility of F98 cell line authentication via lipid profiling. Future research will examine the response of the F98 lipidome to plant bioactive compounds.

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Poster

443. Neuro-Oncology

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Program #/Poster #: 443.14

Topic: B.11. Neuro-Oncology

Title: Synergistic cytotoxic effect of the combination of albendazole with melatonin on human glioma cells

Authors: *M. CERÓN¹, C. RIOS², B. PINEDA OLVERA³, F. PALOMARES⁴, H. JUNG⁵;
¹Univ. Autónoma Metropolitana, Inst. Nacional De Neurología Y Neurocirugía, Mexico, Mexico; ²Neurochemistry, Natl. Inst. Neurology, Neurosurg, Mexico City, Mexico; ³NEUROINMUNOOGIA, INSTITUTO NACIONAL DE NEUROLOGIA Y NEUROCIROGIA, Mexico City, Mexico; ⁴Inst. Nacional de Neurología y Neurocirugía, Ciudad de México, Mexico; ⁵Facultad de química, Univ. Nacional Autónoma de México, Ciudad de México, Mexico

Abstract: Synergistic cytotoxic effect of the combination of albendazole with melatonin on human glioma cells. Glioblastoma (GB) is the most aggressive tumor of the central nervous system (CNS) in adults, with a poor prognosis. Currently, standard treatment involves maximal surgical resection, followed by radiation therapy and concurrent chemotherapy with temozolomide (TMZ); however, the median overall survival is 12-15 months. Previous studies have reported the anticancer activity of albendazole (ALB) and melatonin (MLT), in various types of cancer cells, therefore, main objective of the present study was to evaluate the effect of the combination ALB + MLT on U87 and RG2 cells of the human glioma. Cell viability was evaluated with the MTT technique. Drug interaction was determined by the Chou-Talalay method. The morphological changes were determined by light microscopy. Results showed that in the both cell lines, ALB, ALBSO and MLT displayed cytotoxic activity in a concentration-dependent manner. In RG2 cell lines, the combinations of ALB + MLT, which presented a mortality approximately 80%, showed synergistic and additive cytotoxic effects, while in the U87 line, all the combinations presented synergism. Our results showed that ALB treatment in combination with MLT was better than with the individual drugs and TMZ control and further studies in in vivo glioma models are warranted.

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Poster

443. Neuro-Oncology

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Topic: B.11. Neuro-Oncology

Support: Barncancerfonden travel grant

Title: Cranial irradiation causes three waves of microglial activation in juvenile mouse hippocampus

Authors: *A. LASTRA ROMERO, A. M. OSMAN, C. F. D. RODRIGUES, K. BLOMGREN;
Women's and Children's Hlth. Dept., Karolinska Institutet, Stockholm, Sweden

Abstract: Radiotherapy is an effective tool in the treatment of high-grade brain tumors; However, it often results in long-lasting side effects, such as cognitive deficits, particularly in young cancer survivors. The mechanisms underlying these undesirable consequences are unknown, but one proposed cause is depletion of hippocampal neurogenesis. This is linked to irradiation-induced neuroinflammation that may prevent surviving neural stem/progenitor cells (NSPCs) in the subgranular zone (SGZ) of the hippocampus to generate new neurons and change their fate to become glial cells instead. Therefore, we aimed to determine the spatio-temporal profile of the inflammatory response in the hippocampus after cranial irradiation (IR). Mice were subjected to 10 Gy whole-brain IR on postnatal day 21. Hippocampal tissue was collected 6 hours, 1 day, 1 week, 2 weeks and 6 weeks post-IR, and processed for transcriptomic, proteomic and histologic analyses. We found that IR caused multiple inflammatory responses in hippocampus. The first wave occurred within hours post-IR and was associated with NSPC death. The second wave, 2 weeks after IR, was characterized by reactivation of microglia (MG) and featured molecular signatures linked to interferon-signaling. In the third wave, MG entered a state of senescence. At this stage, we observed a 50% loss of MG in IR mice compared to controls. Together, these observations show that IR to the juvenile brain causes multifaceted MG activation, senescence and degeneration that may contribute to the development of long-lasting cognitive deficits in children treated with radiotherapy.

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Poster

443. Neuro-Oncology

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Program #/Poster #: 443.16

Topic: B.11. Neuro-Oncology

Title: TiO₂-znp nanoparticles functionalized with folic acid as photosensitizers in photodynamic therapy against glioma cells

Authors: *E. ORTIZ-ISLAS¹, G. JARDON GUADARRAMA², C. RODRIGUEZ-PEREZ¹, M. MANRIQUEZ-RAMIREZ³;

¹Natl. Inst. of Neurol. and Neurosurg., Mexico City, Mexico; ²Autonomous Metropolitan University-Xochimilco, Mexico City, Mexico; ³Natl. Polytechnic Inst., Mexico City, Mexico

Abstract: TiO₂-ZnPc nanoparticles functionalized with folic acid as photosensitizers in photodynamic therapy against glioma cells

Authors:*E.E ORTIZ-ISLAS¹, G. JARDON-GUADARRAMA², C.E. RODRÍGUEZ-PÉREZ¹, M.E. MANRÍQUEZ-RAMÍREZ³ ¹Neuropharmacology Molecular and Nanotechnology

Laboratory, National Institute of Neurology and Neurosurgery, México City, México; ²Health Care Department, Autonomous Metropolitan University-Xochimilco, México City, México; ³ISIQIE, National Polytechnic Institute, México City, México.

DisclosuresE.E. Ortiz-Islas: None. G. Jardon-Guadarrama: None. C.E. Rodríguez-Pérez: None. M.E. Manríquez-Ramírez: None

AbstractGlioblastoma (GBM) is the most common primary and most malignant neoplasm of the central nervous system that develops mainly in adults, about 50% of cases occur in people 65 years of age or older. The current standard treatment is trimodal, including maximal safe resection of the tumor, followed by local radiotherapy and concurrent and adjuvant chemotherapy with temozolomide. Tumor progression after surgical resection is also very common, with a fatal outcome expected within one year. Photodynamic therapy (PDT) is a promising and clinically approved, less invasive treatment of several cancers. This therapeutic process follows photon excitation of the photosensitizer (Ph), leading to forming organic radicals and oxygen species (ROS). ROS generated causes irreversible oxidation of one or more critical cellular components leading to cellular damage and cancer death. Natural and synthetic dyes have been tested as Phs in PDT procedures such as zinc phthalocyanines (ZnPc). This work reports the synthesis, characterization, and toxicity assay of TiO₂ nanoparticles functionalized with folic acid (FA) and ZnPc as prominent Ph for PDT for GBM therapy. TiO₂-FA-ZnPc nanoparticles were prepared by the sol-gel method adding the FA and ZnPc during the hydrolysis and condensation of the TiO₂ precursor. An *in vitro* toxicity study on the C6 cellular line was performed using the known MTT assay. Characterization results by Infrared spectroscopy reveal suitable obtaining and functionalization of TiO₂ nanoparticles. The UV-Vis studies report that when TiO₂ was combined with ZnPc, in the UV-Vis spectrum of TiO₂-FA-ZnPc, a new peak was observed at about 640nm within the therapeutic window. The MTT assay revealed that TiO₂-FA-ZnPc in all used concentrations (10-500 µg/ML) did not show a toxic effect on the C6 cell compared with the control cell. This result indicates that it's necessary to apply light to activate the nanoparticles to produce ROS and exert their toxic effect on C6 cells.

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Poster

443. Neuro-Oncology

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Program #/Poster #: 443.17

Topic: B.11. Neuro-Oncology

Support: Coleman Fung Gift

Title: Electrophysiological Signature of Inhibitory Interneuron Loss in Glioma

Authors: *A. SURAPANENI¹, A. A. AABEDI², J. KAUR², D. BRANG³, J. S. KUO⁴, J. D. R. MILLÁN¹, S. L. HERVEY-JUMPER²;

¹Univ. Of Texas At Austin, Austin, TX; ²Univ. of California San Francisco, San Francisco, CA;
³Univ. of Michigan, Ann Arbor, TX; ⁴China Med. Univ., Taichung City, Taiwan

Abstract: Glioma infiltration into brain tissue has been reported to cause cell death of inhibitory interneurons, which alters the balance between excitation (E) and inhibition (I) in tumor-infiltrated regions and correlates with epilepsy. Impaired inhibition in local neural networks can be inferred by a reduction in the power law exponent of the electrophysiological power spectrum. Furthermore, cortical gamma oscillations, thought to be the key neural mechanism for cognitive control, require phasic inhibition of pyramidal neuron networks by interneurons. Previous work has shown that signals from glioma-infiltrated cortex have decreased entropy and impaired modulation in response to the complexity of a language task. It is possible that interneuron death plays a role in the deficient cognitive processing in glioma-infiltrated tissue. We aim to identify an electrophysiological signature consistent with interneuron death in patients with gliomas. We hypothesize that the power spectrum originating from tumor-infiltrated regions will have a decreased power law exponent and gamma power compared to spectra from normal parenchyma during a language task. Patients (n = 13) with dominant hemisphere perisylvian gliomas were intraoperatively implanted with a subdural electrocorticographic grid and performed a picture naming task. Electrodes were registered as overlying regions of tumor infiltration or normal parenchyma using the T2-FLAIR signal. Power in the low-gamma (30-57Hz), mid-gamma (63-79Hz), and high-gamma (80-200Hz, excluding harmonic multiples of 60Hz with 6Hz bandwidth) was estimated using multitaper spectral analysis during speech production in tumor-overlying electrodes and nontumor-overlying electrodes. Additionally, the aperiodic spectral component in the gamma range was identified from averaging auto-spectra generated by irregular resampling of the time-domain data, and the power law exponent was fitted. Linear mixed effects modeling was used to estimate the difference in parameters in tumor-overlying regions compared to normal parenchyma at the group level, after accounting for patient-specific clustering. Significant reductions were observed in the power of low-gamma (p<0.05), mid-gamma (p<0.01), and high-gamma (p<0.05) range and the power law exponent (p<0.05) in tumor-overlying electrodes compared to nontumor-overlying electrodes. Electrodes overlying tumor regions and normal parenchyma had similar spatial coverage at the group level. This shows that alterations in E:I balance in neural circuits and cognitive control characteristic of loss of interneurons can be identified in glioma-infiltrated tissue.

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Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.18

Topic: I.06. Computation, Modeling, and Simulation

Title: Exploratory evaluation of the effective relevance of 2D monolayer vs 3D spheroid culture of highly pure cortical glutamatergic neurons for brain cancer drug screening

Authors: *K. DONNELLY¹, G. R. SOUZA², M. L. HENDRICKSON³, B. RIVOIRE¹;
¹Biotect Services, Fort Collins, CO; ²Greiner Bio-One, Frickenhausen, Germany; ³BrainXell, Madison, WI

Abstract: Brain cancer therapeutics have a high failure rate in early clinical trials. Improving predictability of *in vitro* models for brain cancer is critical for effectively guiding downstream animal and human studies. Two-dimensional (2D) cell culture with primary cells and cell lines has traditionally been used to test candidate drugs in preclinical *in vitro* assays. Recently, three-dimensional (3D) cell culture has become a promising alternative as a more physiologically relevant model. In this study, we explore differences between a monolayer culture and two progressive spheroid models of human cortical glutamatergic neurons (BrainXell) seeded at 20,000 cells per well in 96-well plates. For monolayer culture, cells were seeded in a poly-D-lysine (PDL)-coated flat-bottom plate. For 3D culture via ultralow attachment (ULA), cells were seeded in a BIOFLOAT™ treated round-bottom plate (faCellitate GmbH). For 3D culture via nanoparticle levitation, cells were treated with Nanoshuttle (Greiner BIO-One) and seeded in a ULA round-bottom plate (Greiner) and exposed to a magnet for 1 hour to encourage sphere formation. Monolayer and spheroid maturation was documented by phase contrast microscopy over the course of the cultures. On day 21, monolayer cells (n = 9 wells) and 3D cells (n = 4 spheres) were immunocytochemically (ICC) stained for MAP2 (neuronal marker), VGlut1 (glutamatergic neuron marker), and DAPI (nuclear marker) to confirm cell identity. All cultures showed positive staining for glutamatergic neurons. Neuronal features were consistent with in-life observations of the monolayer culture, but absent in 3D cultures; therefore, ICC must be used to confirm cellular identity of spheroids. Drug inhibition was tested by applying staurosporine in duplicate two-fold serial dilutions starting at 800 nM. After 72 hours, cell toxicity was measured via luminescent ATP and a dose-response curve and inhibitory concentration at 50% of viable cells calculated for each culture. Cytotoxicity was 42% less in ULA spheroids (IC₅₀ = 79 nM) compared to 2D adherent cells (IC₅₀ = 187 nM). In contrast, levitated spheroids (IC₅₀ = 171 nM) responded similarly to 2D cultures with an 8% decrease in cytotoxicity. This finding was surprising because drug inhibition of spheroids, regardless of generation method, was expected to be lower than for monolayer cells due to cell aggregation. These results suggest that different methods of 3D *in vitro* cell culture modeling can help investigators narrow in on important properties of their developing drug candidate before testing in *in vivo* models, leading to better clinical trial outcomes in the brain cancer field at large.

Disclosures: **K. Donnelly:** A. Employment/Salary (full or part-time);; Biotect Services. **G.R. Souza:** A. Employment/Salary (full or part-time);; Greiner Bio-One. **M.L. Hendrickson:** A. Employment/Salary (full or part-time);; BrainXell. **B. Rivoire:** A. Employment/Salary (full or part-time);; Biotect Services.

Poster

443. Neuro-Oncology

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 443.19

Topic: F.03. Stress and the Brain

Title: Role of locus coeruleus noradrenergic neurons in breast cancer progression

Authors: *A. BERISHA, J. C. BORNIGER;

Neuroscience/Cancer Ctr., Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The locus coeruleus (LC) is a norepinephrine-producing nucleus found in the dorsal pons of most vertebrates. The LC is implicated in many neurological diseases, including depression, Alzheimer's and Parkinson's but its potential role in cancer is unexplored. Overall, there are at least three major efferent pathways originating from the LC: 1) ascending pathway projections to the cortex, 2) the cerebellar pathway and 3) descending pathway projections to the spinal cord. Moreover, LC activity has been shown to bidirectionally alter pupil dilation, a marker of arousal often used to indirectly infer LC activity levels across multiple species. LC-mediated pupil dilation may act through disinhibition of parasympathetic pathways with possible contribution of sympathetic pathways controlling the iris dilator muscle. Interestingly, recent research suggests that the sympathetic nervous system (SNS) may modulate multiple physiological processes that promote progression of primary tumors (e.g., breast) and subsequent metastasis. The goal of the current project is to investigate whether aberrant activity of the LC contributes to enhanced tumor progression and subsequent metastases of peripheral tumors, induced by the SNS in animal models of breast cancer. We used a combination of optogenetics and pupillometry to artificially stimulate a subset of neurons in the LC and assess pupil dilation to validate successful viral infection and channelrhodopsin-2 (ChR2) expression. In addition, adult mice (aged >4 months) were subject to orthotopic injections of the mouse mammary cancer cell line E0771 cells into the ninth mammary gland. We found that daily optogenetic stimulation of noradrenergic neurons in the LC resulted in enhanced tumor progression. Additionally, pseudorabies virus (PRV) tracing was used to characterize the anatomical connection between the tumor and the brain and 'map' the neuronal projections that originate in the LC and innervate the peripheral tumor. We found viral fluorescent protein expression in Tyrosine Hydroxylase (TH) expressing neurons in the LC after PRV injection into the primary tumor, indicative of a tumor-brain neural circuit. Furthermore, TRAP2 mice were used as an unbiased approach to "trap" neurons active during different stages of cancer progression. Co-localization of TH and tdTomato was observed in the LC throughout different stages of tumor progression, indicating that the LC is 'active' during different stages of tumor progression. Potential findings could provide important insight into the claim that the brain and cancer in the periphery engage in bi-directional communication.

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Poster

444. Neuroinflammation and Immune Actions: *In Vivo* Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 444.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HU20C0187

Title: Drug Repurposing for Alzheimer's Disease by Modulating Tau Pathology

Authors: *J. HAN¹, J.-H. JEONG¹, K. SUH¹, H. KIM², I. MOOK-JUNG^{1,2};

¹Seoul Natl. Univ. Col. of Med., Seoul Natl. Univ., Seoul, Korea, Republic of; ²Seoul Natl. Univ. Col. of Med., SNU Dementia Res. Ctr., Seoul, Korea, Republic of

Abstract: Alzheimer's Disease (AD) is characterized by accumulation of amyloid beta plaques and neurofibrillary tangles, which ultimately lead to neuronal cell death. Millions of people are suffering from AD, yet clinical therapies targeting AD are still under development. Here we propose FDA-approved Drug A for its potential therapeutic effects on AD. In a mouse model of AD, oral administration of Drug A alleviated tau pathology and prevented neuronal cell death, resulting in improved cognitive functions. We also found that Drug A reduced total and hyperphosphorylated tau by modulating mTOR pathway, which is responsible for lysosomal degradation of abnormal tau proteins. These data reveal that FDA-approved Drug A is effective in ameliorating AD pathology, and suggest Drug A as a prospective therapy for AD.

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Poster

444. Neuroinflammation and Immune Actions: *In Vivo* Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

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KHIDI HI18C0630

Title: QPLEXTM biomarkers distinguish cognitively normal individuals with cerebral amyloid deposition across a broad age range and diverse brain regions

Authors: *D. LEE^{1,3}, J. PARK^{1,3,4,5}, K. JUNG⁶, J. KIM⁶, J. JANG⁶, S. KWON⁶, M. BYUN⁷, D. YI⁸, G. BYEON⁸, G. JUNG⁸, Y. KIM⁹, D. LEE^{8,2,10}, S.-H. HAN^{1,3,4}, I. MOOK-JUNG^{1,3,4},
¹Dept. of Biomed. Sci., ²Dept. of Psychiatry, Col. of Medicine, Seoul Natl. Univ., Seoul, Korea, Republic of; ³Col. of Medicine, Seoul Natl. Univ., SNU Dementia Res. Ctr., Seoul, Korea, Republic of; ⁴Col. of Medicine, Seoul Natl. Univ., Neurosci. Res. Inst., Seoul, Korea, Republic of; ⁵Dept. of Neurodegenerative Dis., Univ. Col. London, London, United Kingdom; ⁶QuantaMatrix, Seoul, Korea, Republic of; ⁷Dept. of Psychiatry, Pusan Natl. Univ. Yangsan Hosp., Busan, Korea, Republic of; ⁸Dept. of Neuropsychiatry, Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; ⁹Seoul Natl. Univ. Boramae Med. Ctr., Seoul Natl. Univ. Boramae Med. Ctr., Seoul, Korea, Republic of; ¹⁰Inst. of Human Behavioral Medicine, Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder with beta-amyloid (A β) and tau known to be key pathological players. Early detection of A β deposition in the brain is crucial because A β accumulation precedes the onset of AD symptoms. Previously, we have reported the applicability of the QPLEXTM Alz plus assay kit, a blood-based biomarker panel, for prescreening of A β accumulation. Here, we specifically applied the kit to a large cohort of cognitively normal (CN) individuals of varying ages to see if the kit could detect early signs of A β accumulation. In a cohort of 221 CN participants with or without brain A β , the biomarkers in the QPLEXTM panel were characterized based on age groups (1st-3rd tertile) and across various brain regions with amyloid deposition. The 3rd tertile group, consisting of individuals over 65 years, was the most suitable age group for the application. In this group, a receiver operating characteristic curve analysis showed that the area under the curve (AUC, discrimination power) was 0.878 with 69.7% sensitivity and 98.4% specificity. Plasma galectin-3 binding protein (LGALS3BP) and periostin (POSTN) levels showed significant correlations with all four brain regions investigated. Posterior cingulate-precuneus (PC-PRC) was closely associated with plasma A β 1-40 level, consistent with previous findings that the region is especially vulnerable at the early onset stage of AD. Furthermore, the combinational panel with plasma A β 1-42 levels maximized the discrimination efficiency and achieved an AUC of 0.921 with 95.7% sensitivity and 67.3% specificity. Our findings suggest that QPLEXTM Alz plus assay is useful for prescreening brain A β levels in CN individuals, especially those over 65 years, and can help prevent the disease progression via early detection of disease initiation.

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Poster

444. Neuroinflammation and Immune Actions: *In Vivo* Models

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 444.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HU20C0187

Title: Long-term excessive NE exposure to brain induces tau aggregation and behavioral disorders

Authors: ***J.-H. JEONG**, D. KIM, S. CHUNG, I. MOOK-JUNG;
Seoul Natl. Univ. Col. of Med., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: The two neuropathological hallmarks of Alzheimer's disease (AD) are the intracellular aggregation of hyperphosphorylated tau and the extracellular deposition of amyloid beta plaques. The locus coeruleus (LC), the major source providing norepinephrine (NE) to brain, is one of the earliest sites of neurofibrillary tau formation in AD. Although the LC-supplied NE plays a key role in a variety of brain functions, including cognition, emotion, locomotion, and the sleep-wake cycle, the contribution of NE to the tau aggregation remains elusive. To clarify the role of NE in the brain associated with tau, we injected intraperitoneally (IP) either N-(2-chloroethyl)-N-ethyl-bromo-benzylamine (DSP4), a selective neurotoxin for noradrenergic neurons, to make NE depletion or Reboxetine (RBX), a drug of the norepinephrine reuptake inhibitor (NRI), to augment the degree of NE in the three-month-old tau transgenic mice expressing mutant human P301L tau for two months. Because we began to inject these drugs into the young tau mice before the known onset of tau pathology, we were able to examine whether the changes of the NE level promote or delay the onset of tauopathy. Interestingly, we observed that the only mice treated with RBX had cognitive deficits in Y-maze and novel object recognition test (NOR) as well as motor dysfunctions in rotarod test. In addition, we discovered that the mice with RBX gained more aggregation of hyperphosphorylated tau in both cortex and hippocampus than the other mice did by performing western blotting and immunohistochemistry. These results suggest that excessive NE in the brain may be related to the hyperphosphorylation of tau either directly or indirectly, which could be explained why the LC is the first region of the tau deposition in AD. Thus, the approach targeting the LC-NE system to regulate the NE level in the brain may be a pivotal therapy to AD.

Disclosures: **J. Jeong:** None. **D. Kim:** None. **S. Chung:** None. **I. Mook-Jung:** None.

Poster

444. Neuroinflammation and Immune Actions: *In Vivo* Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 444.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HI20C0187
HU20C0198

Title: Amyloid- β Activates nlrp3 inflammasomes by affecting microglial immunometabolism through the syk-ampk pathway

Authors: *K. SUH¹, E. JUNG², J. HAN¹, H. KIM³, H.-S. KANG³, W.-S. CHOI³, I. MOOK-JUNG⁴;

¹Seoul Natl. Univ., ²Seoul Natl. Univ., Seoul, Korea, Republic of; ³Chonnam Natl. Univ., Gwangju, Korea, Republic of; ⁴Seoul Natl Univ. Col. Med., Seoul Natl Univ. Col. Med., Seoul, Korea, Republic of

Abstract: Neuroinflammation is considered one of major factors in the pathogenesis of Alzheimer's disease (AD). In particular, inflammasome activation, including NLRP3 inflammasome in microglia, is regarded as fundamental for the pro-inflammatory response of immune cells. However, the precise molecular mechanism through which the NLRP3 inflammasome is associated with AD pathologies remains unclear. Here we show that amyloid- β activates the NLRP3 inflammasome in microglia by activating Syk and inhibiting AMPK. Deactivated AMPK induces metabolic dysregulation, mitochondrial fragmentation and reactive oxygen species formation, leading to the activation of the NLRP3 inflammasome. In addition, flufenamic acid (FA), a member of non-steroid anti-inflammatory drugs, was found to effectively inhibit activation of the microglial NLRP3 inflammasome by regulating Syk and AMPK. Importantly, FA has marked therapeutic effects on major AD pathologies and memory function *in vivo* in microglia-dependent way. All together, these findings demonstrate the molecular mechanism of microglial NLRP3 inflammasome activation by amyloid- β , which act as an important mediator of neuroinflammation. Also, we suggest that repurposing of FA for inhibiting microglial activation of the NLRP3 inflammasome is a potential treatment for AD.

Disclosures: K. Suh: None. E. Jung: None. J. Han: None. H. Kim: None. H. Kang: None. W. Choi: None. I. Mook-Jung: None.

Poster

444. Neuroinflammation and Immune Actions: *In Vivo* Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 444.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HU20C0187

Title: The role of microglial Axl receptors in the formation and engulfment of beta-amyloid plaques in Alzheimer's disease

Authors: *J. KIM¹, J. PARK^{1,2,3,4}, I. MOOK-JUNG^{1,2,3};

¹Dept. of Biochem. and Biomed. Sci., ²Neurosci. Res. Inst., ³SNU Korea Dementia Res. Ctr., Seoul Natl. Univ., Seoul, Korea, Republic of; ⁴Dept. of Neurodegenerative Dis., Univ. Col. London, London, United Kingdom

Abstract: Microglial function of forming beta-amyloid (A β) plaque has been observed in several studies for Alzheimer's disease (AD). However, whether the formation of A β plaque serves as a protection or worsens the progression of AD pathology is still controversial. Axl, one of the

members of the TAM receptor kinase family (Tyro3, Axl, Mertk) expressed in microglia, has been found to be associated with the formation of A β plaques with supports of its ligand, Gas6. We herein identify regulation of A β plaques via microglial Axl-Gas6 complexes using CRISPR-Cas9 base edited Apolipoprotein E (ApoE) isogenic induced pluripotent stem cells (iPSCs). By co-culturing human iPSC-derived microglia (iMG) and iPSC-derived neurons (iNs), we investigate whether the plaque formation can be promoted or inhibited by microglia. Our findings reveal that the role of Axl-Gas6 in the controls of A β plaque-formation and provide an insight for anti-A β therapeutic approaches.

Disclosures: J. Kim: None. J. Park: None. I. Mook-Jung: None.

Poster

444. Neuroinflammation and Immune Actions: *In Vivo* Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 444.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HU20C0187

Title: Microglia engulf beta-amyloid plaques colocalized with dystrophic neurites via TREM2 in the 3D brain assembloids

Authors: *J. HAN¹, J. PARK^{1,2,3,4}, I. MOOK-JUNG^{1,2,3};

¹Dept. of Biochem. and Biomed. Sciences, Col. of Medicine, Seoul Natl. University., Dept. of Biochem. and Biomed. Sciences, Col. of Medicine, Seoul Natl. University, Seoul, Seoul, Korea, Republic of; ²Neurosci. Res. Institute, Med. Res. Center, Col. of Med., Seoul, Korea, Republic of; ³SNU Korea Dementia Res. Center, Col. of Med., Seoul, Korea, Republic of; ⁴Dept. of Neurodegenerative Disease, UCL Queen Square Inst. of Neurology, Univ. Col. London, London, United Kingdom

Abstract: Microglia play an important role in synaptic elimination via engulfing dystrophic neurons by triggering receptors expressed on myeloid cells 2 (TREM2). However, the mechanisms of TREM2-mediated phagocytosis toward beta-amyloid (A β) in the progression of Alzheimer's disease (AD) is still controversial. For this study, we utilize induced pluripotent stem cell (iPSC)-derived microglia, brain organoids, and our advanced 2D/3D/4D co-culture models (2D co-culture models or 3D brain assembloids; with or without live-cell imaging) with loss-of-function of TREM2 variants. We herein identify the mechanism of A β clearances via microglial TREM2. Moreover, since A β plaques are found coated with dystrophic neurons, we investigate whether TREM2-expressing microglia preferentially engulfs beta-amyloid with dystrophic neurons. Consequently, we provide an insight how TREM2 expressing microglia contribute to the phagocytosis of A β plaques in the progression of AD.

Disclosures: J. Han: None. J. Park: None. I. Mook-Jung: None.

Poster

444. Neuroinflammation and Immune Actions: *In Vivo* Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 444.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HU20C0187
NRF Grant 2020R1A6A3A13070845

Title: Sting activation promotes brain inflammation and memory impairment in alzheimers disease model

Authors: *S. CHUNG, J.-H. JEONG, J.-C. PARK, H. CHOI, D. LEE, J. KIM, J. HAN, D. KIM, E. JUNG, I. MOOK-JUNG;
Seoul Natl. Univ. Col. of Med., Dept. of Biomed. Science, Seoul Natl. Univer, Seoul, Korea, Republic of

Abstract: Neuroinflammation accelerates neurodegeneration in Alzheimer's disease (AD), the prominent cause of dementia. Here, we report that cyclic GMP-AMP synthase (cGAS) - Stimulator of interferon genes (STING) pathway is activated in AD and STING inhibition reduces neuroinflammation, A β burden, and tau phosphorylation in Alzheimer's disease model mice. cGAS-STING pathway plays a central role in ectopic DNA recognition and following interferon-related pro-inflammatory responses. Interestingly, both protein levels were elevated in two independent AD model mice, especially enriched in A β plaque-associated microglia. A β activated cGAS-STING downstream signaling molecules and STING inhibition reduced these responses in microglia. In App^{NL-G-F}/MAPT double-KI mice, which manifest A β and tau pathology without artifact of protein overexpression, pharmacological STING inhibition reduced both pathologies and rescued memory impairment. Furthermore, microgliosis and brain inflammation were reduced by STING inhibition. These results indicate that microglial STING activation contributes to neuroinflammation and worsens A β and tau pathogenesis in AD mouse model. Thus, the modulation of microglial phenotype through the STING regulation may provide a therapeutic strategy to cure AD.

Acknowledgments: We sincerely appreciate Dr. Takaomi Saïdo at RIKEN Center for Brain Science for providing the App^{NL-G-F} and MAPT knock-in mice.

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Poster

444. Neuroinflammation and Immune Actions: *In Vivo* Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 444.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ministry of Health & Welfare, Republic of Korea HU20C0187

Title: Cholesterol derivative-A defects microglial function accelerates Alzheimer's disease-like pathology.

Authors: *H. CHOI¹, H. KIM³, J. HAN¹, J. JEONG¹, J. KIM¹, S. PARK², H. SONG², D. LEE², I. MOOK-JUNG^{1,3};

¹Seoul Natl. Univ., ²Dept. of Agr. Biotech., Seoul Natl. Univ., Seoul, Korea, Republic of; ³SNU Dementia Res. Ctr., Seoul, Korea, Republic of

Abstract: Genome-wide analysis suggests a strong association between immune response, lipid metabolism, and Alzheimer's disease(AD) pathology. However, the exact pathogenesis and their relationship are still poorly understood. Cholesterol derivative-A is a type of oxysterol known in the peripheral macrophages of the viral infectious disease model, which uniquely has an immune regulative function. An enzyme producing Cholesterol derivative-A, is mainly expressed in microglia in the brain, and its expression is significantly increased in the AD model. In this study, we wanted to find out how Cholesterol derivative-A regulates microglia and how their quantitative changes affect the progression of AD disease. In vitro primary microglia, Cholesterol derivative-A induces proinflammatory cytokines and defects phagocytosis. Cholesterol derivative-A treatment in a young 5XFAD model, in which A β pathology has not yet been accumulated, reduces microglial phagocytosis and accelerates A β accumulation. Overall, the results suggest that Cholesterol derivative-A could play a significant role in defecting the function of microglia and accelerating AD pathology.

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Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 445.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Modeling Amyloid Time to predict cognitive decline in a heterogeneous sample in multi-site, dual-tracer study.

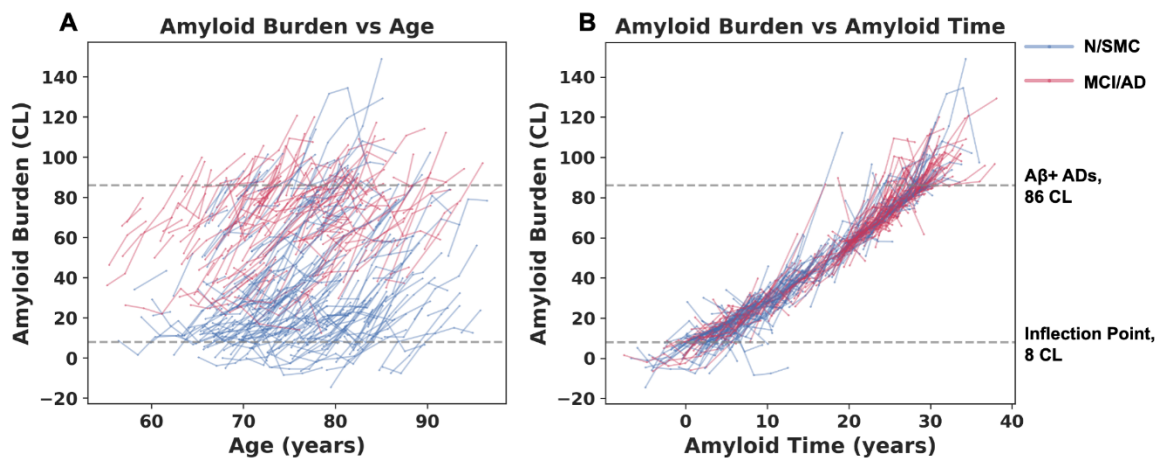
Authors: *J. LEE¹, G. BLAZHENETS², T. M. HARRISON¹, S. L. BAKER³, J. GIORGIO^{1,4}, R. LA JOIE², W. JAGUST¹, S. LANDAU¹;

¹Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA; ²Memory and Aging

Center, Dept. of Neurology, Weill Inst. for Neurosciences, Univ. of California, San Francisco, San Francisco, CA; ³Lawrence Berkeley Natl. Lab., Berkeley, CA; ⁴The Univ. of Newcastle, Newcastle, Australia

Abstract: A nonlinear yet consistent beta-amyloid (A β) trajectory over the course of Alzheimer’s Disease (AD) has been observed, but the role of A β accumulation duration in predicting cognitive decline is unclear. Our goal was to examine the predictive value of a metric derived from A β burden and A β accumulation rate, “A β Time”, in a heterogeneous multi-site longitudinal dataset with two A β PET tracers. Our sample consisted of 692 Alzheimer’s Disease Neuroimaging Initiative (ADNI) participants (57% unimpaired, 43% impaired) who had ≥ 2 18F florbetaben or 18F florbetapir A β PET scans (followup = 3.9 ± 2.3 yrs) used to assess baseline cortical A β and annual A β change in standardized units (centiloids (CL)). Participants were deemed likely to be on the AD pathway based on baseline A β + status or, in the unimpaired, A β -status with positive A β slope. We modeled A β annual change as a function of A β burden using cubic splines and ridge regression, setting lower and upper CL limits to restrict the model to the A β growth phase. The lower limit was an inflection point (8 CL), the earliest reliable detection of A β accumulation based on a series of one-sample t-tests (A β slope > 0 ; $t = 2.9$, $p = .002$); upper limit was the mean A β burden in A β + AD-dementia individuals (86 CL). Using the model that describes A β change as a function of A β over the course of disease, we then estimated A β Time (years of A β accumulation past the inflection point). Unlike evaluating A β trajectories with respect to age (Fig. 1A), A β time aligns individual A β trajectories to the inflection point (Fig. 1B). Finally, we compared A β Time and baseline A β as predictors of cognitive decline in linear regression models for 163 unimpaired individuals with > 1 PACC (Preclinical Alzheimer Cognitive Composite) assessment (followup = 4.6 ± 2.4 yrs), covarying for age, sex and ApoE4. A β Time performed similarly to A β burden in predicting PACC slopes (AICc weight = 69%), perhaps due to the approximately linear accumulation rate within the modeled interval. Nonetheless, A β time can be utilized to align individual trajectories in order to delineate major events on the AD pathway.

Figure 1:
Longitudinal β -amyloid (A β) trajectories for individuals on the Alzheimer’s disease (AD) Pathway



A) Individual trajectories plotted against age at scan B) Individual trajectories realigned to Amyloid Time (estimated duration of A β accumulation since inflection point). Each solid line represents an individual participant. Red lines represent cognitively unimpaired (N or SMC); blue lines represent cognitively impaired (MCI or AD). Horizontal dashed lines indicate 8 CL (inflection point) and 86 CL (mean of A β + ADs). CL = Centiloids; N = cognitively normal; MCI = mild cognitive impairment; SMC = subjective memory complaints; AD = Alzheimer’s disease dementia syndrome

Disclosures: **J. Lee:** None. **G. Blazhenets:** A. Employment/Salary (full or part-time); Walter Benjamin Fellowship, German Research Foundation (DFG). **T.M. Harrison:** None. **S.L. Baker:** F. Consulting Fees (e.g., advisory boards); Genentech. **J. Giorgio:** None. **R. La Joie:** None. **W. Jagust:** None. **S. Landau:** None.

Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 445.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG034570
AG062542

Title: Alzheimer's pathology alters network interactions during processing of repeated stimuli in cognitively intact older adults

Authors: ***J. GIORGIO**^{1,2}, J. N. ADAMS³, A. MAASS⁴, W. J. JAGUST¹, M. J. BREAKSPEAR²;

¹Helen Wills Neurosci. Inst., Univ. of California, Berkeley, CA; ²Univ. of Newcastle, Newcastle, Australia; ³UC Irvine, Irvine, CA; ⁴German Ctr. for Neurodegenerative Dis. (DZNE), Magdeburg, Germany

Abstract: Background: In cognitively intact older adults (OA) the presence of AD pathology (β -amyloid/A β , pathological tau) disrupts normal cortical processes that subserves the ability to recognise repeated stimuli. To uncover this, we used generative modelling of functional and molecular imaging (fMRI/PET) to probe how AD pathologies directly affect these processes. **Methods:** 66 subjects (45 cognitively normal OA, 21 Younger Adults) performed an fMRI task involving novel and repeated scenes and objects. 42 OA had measures of A β using PIB-PET and cross-sectional entorhinal cortex tau (EC-tau) measured using flortaucipir (FTP)-PET. We decomposed the fMRI data into functional networks and then used Dynamic Causal Modelling (DCM) to infer cortical interactions supporting responses to repeated stimuli. We used a hierarchical approach to uncover how individual differences in these interactions are related to AD pathologies. Finally, in a subset (n=32) with multiple measures of EC-tau we ran leave one out validation to use these network interactions to predict rate of longitudinal tau accumulation. **Results:** Five networks with BOLD activity significantly related to the task design (**figure 1**) were selected for DCM analysis. We modelled these time-courses as a fully connected system (except between LOC and PPA) allowing stimulus repetition to modulate any connection. We observed very strong evidence that AD pathologies shift the functional interactions between MTL and DMN from inhibitory to excitatory when stimuli are repeated (**figure 2**). These changes are driven by local pathologies with A β disinhibiting the input of the DMN on the MTL and EC-tau disinhibiting the influence of the MTL on the DMN. Finally, we show excitation of the MTL by the DMN when stimuli are repeated is predictive of rate of tau accumulation

($r(30)=0.45, p=0.005$). **Conclusions:** Here, we find that AD pathologies disrupt local cortical processing of repeated stimuli with $A\beta$ increasing the gain of the DMN, which in turn over stimulates the MTL in an excitatory feedback loop, a potential mechanism for EC-tau accumulation.

Figure 1.

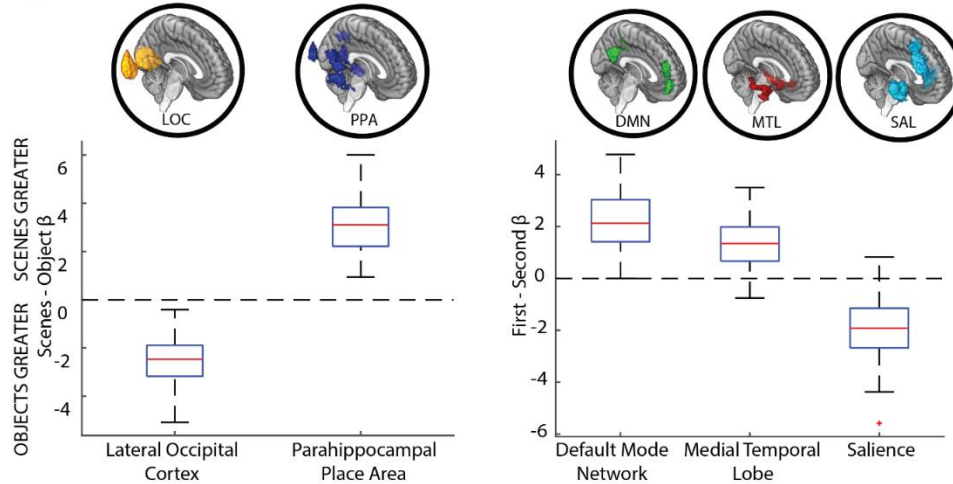
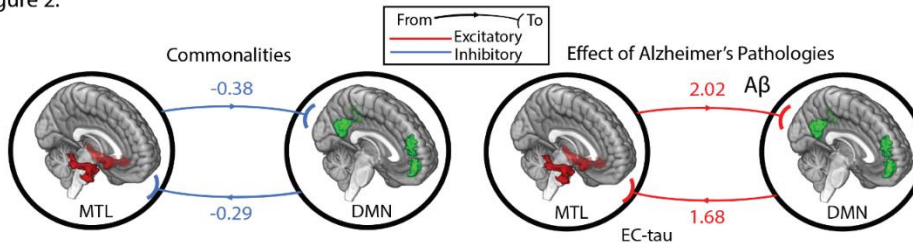


Figure 2.



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Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 445.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 2T32NS095939-06

Title: Tertiary sulci in medial parietal cortex are particularly vulnerable to atrophy in aging and Alzheimer's disease

Authors: *S. A. MABOUDIAN¹, E. H. WILLBRAND^{1,2}, W. J. JAGUST^{1,3}, K. S. WEINER^{1,2}; ¹Helen Wills Neurosci. Inst., ²Psychology, Univ. of California, Berkeley, Berkeley, CA; ³Mol. Biophysics and Integrated Bioimaging, Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract: Large scale changes in brain structure occur in aging and Alzheimer's disease (AD) and particularly reflect sulcal vulnerability to age-related atrophy and AD-related amyloid- β ($A\beta$) deposition. Recent work has shown that the morphology of tertiary sulci — small sulci that emerge last in gestation — is associated with individual differences in cognitive development and symptoms of neuropsychiatric disorders, but these sulci have not been investigated in aging. Here we extend these findings on tertiary sulci to the aging brain.

We manually defined sulci on cortical surface reconstructions of T1 MRI scans in FreeSurfer and extracted the cortical thickness of each sulcus. We defined sulci in medial parietal cortex (MPC) in 72 younger adults (YA; 18-35 years old) from the Human Connectome Project, 72 cognitively normal older adults (CN; ≥ 65 years old) from the Alzheimer's Disease Neuroimaging Initiative (ADNI), and 72 age-matched older adults with AD from ADNI: 4,341 sulci total (9-13 per hemisphere: 8 non-tertiary, 1-5 tertiary). We also defined lateral prefrontal (IPFC) sulci in a subset (36 YA and 21 CN): 2,076 total (~18 per hemisphere: 8 non-tertiary, 9-11 tertiary). 16 of the 21 CN participants had $A\beta$ PET scans; half are positive ($A\beta+$). We ran 3-way mixed-design age (YA/CN) or disease (CN/AD) or $A\beta$ status ($A\beta-/A\beta+$) x sulcal type (non-tertiary/tertiary) x hemisphere ANOVAs for thickness of MPC or IPFC sulci. LASSO regression was used for regularization to predict Mini Mental State Exam (MMSE) scores in the CN group from the thickness of all consistent MPC sulci.

In MPC, tertiary sulci show more age- and AD-related thinning compared to non-tertiary sulci (group x sulcal type interaction $p < .0001$ for both age and disease models), while IPFC sulci also thin with age ($p < .0001$), but without an interaction with sulcal type ($p > .2$). Preliminary results suggest this vulnerability of MPC tertiary sulci to atrophy may be related to AD pathology: $A\beta+$ CN participants trend toward having thinner sulci compared to $A\beta-$ CN in MPC tertiary sulci only (group x sulcal type interaction: $p = .05$ in MPC, $p > .5$ in IPFC). Additionally, the best model prediction of MMSE scores relies on the thickness of only one sulcus — the inframarginal sulcus, a newly-identified tertiary sulcus and the only tertiary sulcus found in all participants in MPC. This is the first study to investigate tertiary sulci in aging and AD. Results show tertiary sulci in MPC are especially vulnerable to atrophy in aging that is associated with cognitive decline and may be related to $A\beta$ deposition. While the effect of $A\beta$ on widespread cortical atrophy is mixed, our results suggest $A\beta$ may have more direct effects on specific types of sulci.

Disclosures: S.A. Maboudian: None. E.H. Willbrand: None. W.J. Jagust: None. K.S. Weiner: None.

Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 445.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG056782
NIH AG071243
NIH MH125928
NIH AG07694
NIH AG033570
NIH AG062251
NIH AG060238

Title: Alterations in inter- and intrahemispheric hippocampal functional connectivity in the App NL-G-F mouse model of Alzheimer's disease

Authors: *Z. MORRISSEY¹, J. GAO¹, L. ZHAN², W. LI¹, I. FORTEL¹, T. SAIDO³, T. SAITO⁴, A. BAKKER⁵, S. MACKIN⁶, O. A. AJILORE¹, O. LAZAROV¹, A. LEOW¹;

¹Univ. of Illinois at Chicago, Chicago, IL; ²Univ. of Pittsburgh, Pittsburgh, PA; ³RIKEN Ctr. for Brain Sci., Wako, Japan; ⁴Dept. of Neurocognitive Sci., Nagoya City Univ. Grad. Sch. of Med., Nagoya, Japan; ⁵Psychiatry and Behavioral Sci., Johns Hopkins Univ., Baltimore, MD; ⁶Univ. of California San Francisco, San Francisco, CA

Abstract: Alzheimer's disease (AD) is the most prevalent form of dementia. However, the underlying early pathological changes of AD are not fully understood. It is thought that alterations in the brain begin years before the presentation of clinical symptoms, which makes it challenging to identify potential early biomarkers or intervention. In addition, previous evidence suggests that both aging and AD contribute to changes in the default mode network, interhemispheric communication, and excitation-inhibition balance, which are important for maintaining healthy cognitive function. In order to better understand early functional alterations in AD across age, we performed *in vivo* resting-state functional magnetic resonance imaging (rs-fMRI) in mice to measure inter- and intrahemispheric hippocampal functional connectivity (FC) in early (4.5 months), middle (10-months) and late (15+ months) age groups using a 9.4 T MRI system. Here, we used the App NL-G-F mouse model of familial AD (FAD) that harbors the Arctic, Beyreuther/Iberian, and Swedish FAD mutations. These mice exhibit A β plaque deposition starting at approximately 4 months of age and begin to show memory deficits at approximately 6 months of age. We observed a significant increase in interhemispheric hippocampal FC with age in both genotypes. However, in App NL-G-F mice, we observed an early increase in interhemispheric FC compared to wild-type mice, followed by reduced interhemispheric FC after middle age, with the left-right CA3 in particular showing the largest increase with age. Furthermore, we observed reduced intrahemispheric FC with age for both genotypes. However, while wild-type mice showed consistently higher intrahemispheric hippocampal FC in the right hippocampus relative to the left, we observed a reduced asymmetry between the left and right hemispheres in App NL-G-F mice. Together, our results suggest that FAD mutations lead to changes in hippocampal FC that are detectable before the accumulation of amyloid plaque deposition and memory impairment, and that changes in inter- and intrahemispheric FC are potential early biomarkers in the pathological development of AD.

Disclosures: Z. Morrissey: None. J. Gao: None. L. Zhan: None. W. Li: None. I. Fortel: None. T. Saido: None. T. Saito: None. A. Bakker: None. S. Mackin: None. O.A. Ajilore: None. O. Lazarov: None. A. Leow: None.

Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 445.05

Topic: C.02. Alzheimer's Disease and Other Dementias

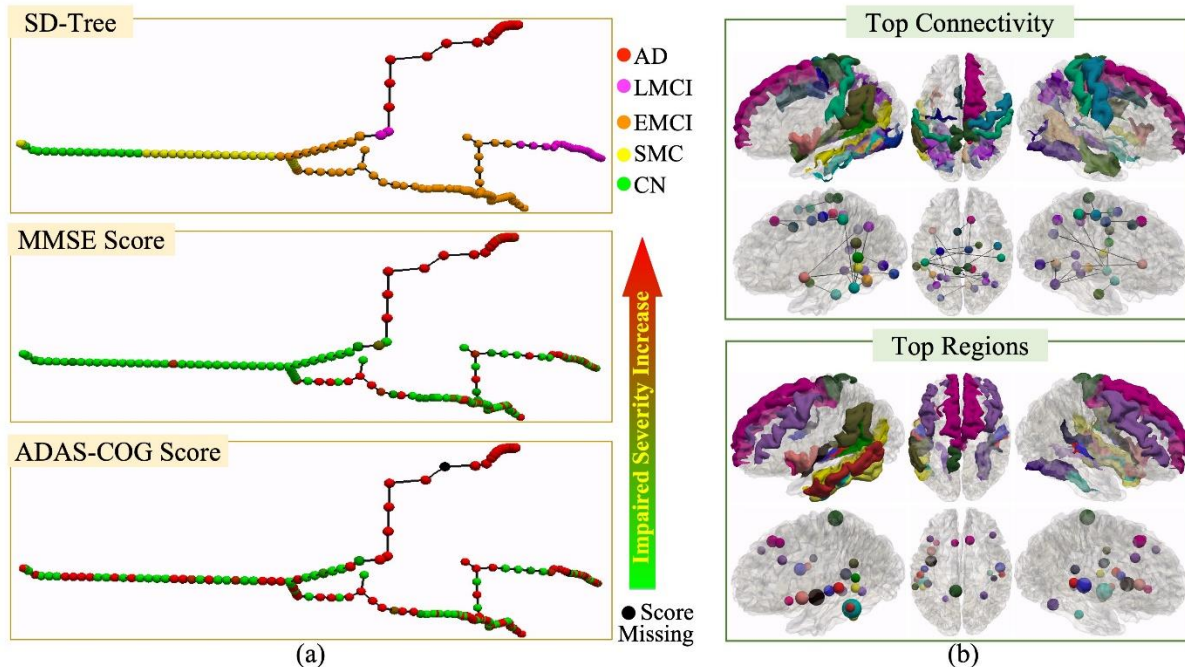
Support: R01AG075582

Title: Modeling Alzheimer's progression by supervised deep tree

Authors: *L. ZHANG¹, L. WANG^{2,1}, X. YU¹, Y. LYU¹, D. ZHU¹;

¹Dept. of Computer Sci. and Engin., ²Dept. of Mathematics, Univ. of Texas at Arlington, Arlington, TX

Abstract: Alzheimer's disease (AD) is the most common cause of dementia whose spectrum spans from clinically asymptomatic to severely impaired. Effectively representing the AD progression is extremely critical for deepening our understanding of AD development as well as facilitating the intervention and diagnosis of AD. However, existing progression models of AD mainly designed to determine and compare the order of specific biomarkers. The continuous nature of AD development and transition states between successive AD related stages have been overlooked. In this work, we propose a supervised deep tree model (SDTree) to integrate AD progression and individual prediction. Specifically, taking individual vectorized functional connectivity as input, we model the progression of AD as a tree embedded in the latent space using nonlinear reversed graph embedding (Wang and Mao, 2017). The predictive model is learned jointly with the tree structure learning to discriminate multiple clinical groups. We use resting state fMRI data of gender and age-matched 170 subjects from ADNI datasets (CN/SMC/EMCI/LMCI/AD) to evaluate our model. The learned SDTree is shown in Fig. 1(a) and MMSE scores and ADAS-COG scores are mapped on the tree to show the general tendency of the disease progression. The results show that SDTree can not only perform effective prediction for patients at any stages of AD development (76% accuracy for five clinical groups), but also provide richer status information by examining the projecting locations within a wide and continuous AD progression process. Moreover, we use Laplacian Score to find functional connections and brain regions that contribute most to the AD progression as shown in Fig. 1(b), which may reflect the functional alterations along with the disease progression. Fig. 1. (a): For each tree, each bubble represents one subject and is color-encoded by clinical groups/scores. (b): Top functional connections and regions contribute most to the AD progression.



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Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 445.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIA U54 AG054349

Title: Multifactorial analysis reveals time sensitive progression in Alzheimer's Disease mouse models

Authors: *N. RAHIMZADEH¹, B. NOARBE², A. JULLIENNE³, V. SWARUP⁴, A. OBENAU²;

¹Ctr. For Complex Bio Systems, ²Pediatrics, ³Inst. for Memory Impairments and Neurolog. Disorders (UCI MIND), ⁴Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Magnetic resonance imaging (MRI) has been pivotal in monitoring the progression of Alzheimer's Disease (AD). Since there are no current cures for the disease, prevention is of the utmost essence in reducing the burden on patients as well as healthcare systems. Although human studies have allowed us to understand the physiology and symptomatic features of this disease, performing longitudinal studies on mouse models can provide a gateway into deciphering the molecular and structural effects of the disease over time. Non-invasive

monitoring allows us to pinpoint regions that may be contributing to the disease in advance thereby enabling therapeutic interventions. We utilized diffusion magnetic resonance imaging (dMRI) data in the 5xFAD mouse AD model that harbors five human genetic mutations known to be associated with AD. We used the diffusion MRI and its associated metrics axial diffusivity (AxD), radial diffusivity (RD), mean diffusivity (MD), fractional anisotropy (FA), T2 (relaxation time), and volumetrics. Data was collected from male and female 5xFAD mice at 4, 8, and 12 months of age from 40 regions in the brain including cortical, limbic, and white matter regions. The aim of this study was to infer features that principally contribute to AD temporal progression. We employed a data-driven approach with principal component analysis (PCA) and multiple factor analysis (MFA) to identify the principal contributors in AD initiation and progression. Our PCA results show that the first and second PCA contain a cumulative variance of 64.52%. 8 months contributes the most to dimensions 1 and 2 collectively (54.19%). Our results suggests that 8 months age identifies a significant shift in AD progression. AD, FA, and MD dMRI metrics contribute the most to the first dimension at 57.32% while volumetric changes account for 80.73% of variation in dimension 2. Similar analyses at 4 and 12 months of age also show that diffusivity metrics drive the first dimensions, whereas the volumetric and T2 data contribute to the second dimension. RD and AD report disease progression in the cortical region while FA monitor changes in the white matter regions, specifically the dorsal fornix, cingulum, and corpus callosum. In summary, a data driven approach using longitudinal dMRI data can identify regions and MRI metrics that could be predicative of ensuing AD.

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Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

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Program #/Poster #: 445.07

Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH R01AG060731-A1
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Title: Brain Volumes in Hypertensive Alzheimer's Disease Rat Model

Authors: *R. ROYCHOUDHURY¹, Y. CHEN^{3,1}, Z. FERNANDEZ¹, C. QIAN¹, M. GIFANI⁴, J. HUBERT¹, A. M. DORRANCE², S. E. COUNTS⁴, D. C. ZHU¹;
¹Radiology, ²Pharmacol. & Toxicology, Michigan State Univ. Col. of Osteo. Med., East Lansing, MI; ³High-field Magnetic Resonance, Max Planck Inst. for Biol. Cybernetics, Tübingen,

Germany; ⁴Translational Neurosci., Michigan State Univ. Col. of Human Med., Grand Rapids, MI

Abstract: Hypertension is a known risk factor in Alzheimer's disease (AD) development and progression. Rat models of AD have increasingly been used to study AD neurodegenerative progression. Developing clinically relevant models of AD that reflect the comorbidities observed in patients such as hypertension is the logical next step. Moreover, the use of neuroimaging techniques has not been widely used to assess the translational significance of a hypertensive AD rat model. To address this limitation and understand whether a hypertensive AD rat model mimics the changes in brain volume seen in comorbid AD and hypertension, we took MRI scans (7 T Bruker scanner) over the whole brain using a FLASH sequence (0.2 mm × 0.2 mm × 0.2 mm spatial resolution) of 30 male and female hypertensive +/- AD rats and evaluated the differences in brain volumes between AD+ and AD- animals. The neuroimaging software AFNI (Analysis of Functional Neuroimages) and customized algorithms in Matlab were used to map brain regions of interest, and subsequently calculate the brain volumes in units of mm³. We found an increase in overall brain volume in AD+ hypertensive male rats (N=6) vs. AD- hypertensive male rats (N=8), as well as in AD+ hypertensive female rats (N=8) compared to AD- hypertensive female rats (N=8). We are also analyzing the hippocampal volumes and plan to present these data. Our data suggest that a hypertensive AD rat model is effective in portraying the synergistic role that hypertension has on the neurodegenerative effect of AD.

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Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

Location: SDCC Halls B-H

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Program #/Poster #: 445.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RO1 AG046266

Title: Accelerated aging process with neurodegeneration in the cerebral cortex of cognitively impaired aged marmosets (*Callithrix jacchus*)

Authors: *C. FREIRE-COBO¹, E. ROTHWELL², M. VARGHESE¹, W. G. M. JANSSEN¹, A. LACREUSE³, P. R. HOF¹;

¹Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; ²Psychology, Univ. of Massachusetts - Amherst, Easthampton, MA; ³Psychological and Brain Sci., Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Aging is a major risk factor for developing late-onset sporadic Alzheimer's disease (AD). The investigation of neurobiological and neuropathological changes that affect synaptic

integrity and function in aging is key to understanding why the aging brain is vulnerable to AD. With a naturally short lifespan, the common marmoset (*Callithrix jacchus*) is a suitable primate model for longitudinal studies of neurocognitive aging. However, the relationships between pathological hallmarks of AD and cognitive aging have not been established in this species. Cognitive performance was assessed in a longitudinal study, spanning midlife to early old age. Marmosets could be categorized as cognitively impaired or non-impaired across aging, and these categories aligned with a variety of neuropathological changes. We analyzed astrocytes and microglia expressing glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule 1 (Iba1), respectively; cluster of differentiation (CD68) for identifying microglial lysosomes and the presence of soluble and insoluble forms of amyloid beta (MOAB-2 antibody). In addition, we performed iontophoretic dye-filling of pyramidal neurons, followed by confocal imaging of dendritic segments and three-dimensional reconstruction for the morphological analysis of dendritic spines. We found increased astrogliosis, increased microglial activation, and differences in their resting and reactive cell phenotypes and cell morphology. Also, although not statistically significant, we observed a different degree of amyloid-beta deposition between cognitively impaired and non-impaired marmosets. Furthermore, we observed age-related alterations in dendritic spine morphology in pyramidal neurons of layer 3 of the dorsolateral prefrontal cortex and CA1 field of the hippocampus. Cognitively impaired marmosets showed a decrease in total spine density, specifically driven by the mushroom spines. This was accompanied in impaired animals by changes in their spine head morphology with a rightward shift in the frequency distribution of thin spine heads, towards larger sizes, which also may imply a selective vulnerability of small, thin spines. Overall, our data suggest that an accelerated aging process, accompanied by gliosis and neurodegeneration, takes place in cognitively impaired aged marmosets, which affects the function of dendritic spines in cortical areas involved in cognition. This points to mechanisms of neuronal vulnerability to aging in marmosets that confirm this anthropoid primate species as a valuable model for the development of early interventions to preserve cognition across aging.

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Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

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Program #/Poster #: 445.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG065819

Title: Sex differences in functional network topological measures in the APP-PS1 mouse model of amyloidosis

Authors: *Z. SIMON¹, M. FEBO², P. CHAKRABARTY¹;

¹Univ. of Florida Dept. of Neurosci., ²Univ. of Florida Dept. of Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: Increased amyloid beta (A β) plaque deposition is linked to synaptic communication deficits in Alzheimer's disease (AD). Functional deficits observed at the synaptic level are thought to alter neural communication across regions playing roles in cognition and affect. There is also growing evidence of a greater risk of AD in women than men, but the extent to which this involves differential responses to amyloidosis remains to be investigated. In the present work, we tested the hypothesis that amyloid deposition alters functional network topology, particularly markers of network integration, in male and female mice. Male and female mice (9-month-old) harboring human mutation amyloid precursor protein (Swedish APP KM670/671NL) and presenilin-1 (PS1) (the APP-PS1 [line 85] mouse) (Tg) and wildtype littermate controls (nTg) were tested on 3 consecutive days on a contextual fear conditioning (CFC) paradigm. On day 1, mice received CFC training for 15 minutes using a series of 4 trials in the presence of an unconditioned stimulus (US) foot shock (0.90mA, 1 second) following a 20-second conditioned stimulus (CS) tone (60dB). On day 2, mice received CS but not US in a 'recall' session and on day 3 the recall session was repeated with visual contextual cues modified. Following CFC, mice were scanned on an 11.1 Tesla scanner under combined dexmedetomidine/isoflurane sedation using the following parameters: single shot echo planar images with echo time of 15 ms and repetition time of 1.5 seconds (600 total repetitions; 0.3mm² in plane resolution, 0.7 mm slice thickness n 17 slices). Images were processed and analyzed using nodes created on the common coordinate framework mouse atlas (version 3) and graph theory algorithms in brain connectivity toolbox. First, all mice developed robust CS freezing behavior and expressed this behavior during both recall sessions. Percent time freezing and number of freezing bouts did not differ between Tg vs. nTg male and female mice, although in males a non-significant trend towards lower recall expression was observed. In addition, we observed significant differences in several measures of functional connectivity in APP-PS1 mice that were driven by effects of amyloid in female mice. Compared to sex-matched controls, female Tg mice had lower clustering, assortative mixing and node strength, and these differences were not observed in Tg vs nTg males. The results suggests that amyloid plaque burden reduces integration of neural activity (as measured by low clustering and assortativity) in female but not male APP-PS1 mice.

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Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

Location: SDCC Halls B-H

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Program #/Poster #: 445.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG065819

UF AI Catalyst

Title: Applied graph theory for biomarker identification in Alzheimer's disease

Authors: ***P. L. KOTLARZ**¹, M. D. HASTROM¹, L. DA SILVA¹, J. C. NINO¹, M. FEBO²;
¹Dept. of Materials Sci. and Engin., ²Dept. of Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that affects 6.5 million Americans and over 50 million people worldwide. Using graph theory, the brain can be represented and analyzed as a network made up of brain regions (nodes) and the connections between them (edges). Connectomic analysis can then be implemented to understand network changes in AD to identify connectivity-biomarkers via functional MRI. We investigated the brain's functional connectivity in control elderly patients (n = 51) and in elderly patients with Mild Cognitive Impairment (MCI) (n = 58), the neurodegenerative step before AD. Participants were part of the ADNI study (ages: 61-96; n=134, 50% female; initial visit). All resting-state functional MRI scanners were obtained on a Siemens Prisma Fit 3-Tesla scanner using EPI with 197 image repetitions, TR=3seconds, TE=30ms, and 3.4mm isotropic voxel resolution. Undirected, weighted matrices were constructed from 44,550 pairwise correlations (edges) between fMRI signals from 300 regions (nodes). Matrices were analyzed independently, normalized, and thresholded at 5% intervals from 5% to 30%. Global quantifiers were used to evaluate the network as a whole and nodal quantifiers were used to examine specific sub-regions. Quantifiers were calculated using the Brain Connectivity Toolbox in MATLAB. The statistical significance of the difference in quantifiers from the control and MCI human brains was evaluated using the Wilcoxon-rank sum test since the quantifier data was non-parametric. Significant differences were identified in mean eigenvector centrality at thresholds of 15%, 20%, 25%, and 30%. Notable differences were also found in diameter, largest cluster size, maximized modularity, mean betweenness centrality, mean participation coefficient, and radius. The calculated values point to functional network differences between healthy and mild cognitive impairment, a traditionally difficult differential diagnosis in early stages. These results can potentially lead to point of care screening of fMRI scans for functional connectivity quantifiers that would enable providers to evaluate a patient's risk for developing AD and promote early medical intervention.

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Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 445.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG065819
UF AI Catalyst

Title: Differences in the anatomical distribution of low versus high strength nodes in normal aging versus young individuals

Authors: *M. D. HAGSTROM¹, P. L. KOTLARZ¹, Y. CRUZ-ALMEIDA², J. C. NINO¹, M. FEBO³;

¹Dept. of Materials Sci. and Engin., ²Dept. of Community Dent., ³Dept. of Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: There is a large and growing literature establishing functional differences between normal cognitive aging, mild cognitive decline, and Alzheimer's disease (AD). However, much less is known on normal aging differences in functional connectivity between young and elderly subjects without signs of cognitive or affective dysregulation. Investigating the normal developmental course of functional network organization for a wide range of ages is needed to better interpret neural changes specifically linked to neurologic conditions that affect the aged, such as AD. In the present study, we assessed functional connectome measures of node strength in brains of young (20-40 y/o) and older individuals (>60 y/o) using a high-density node sampling strategy for the resting state fMRI signal. We focus on node strength because it is well established that functional connectivity between regions of distinct cognitive and sensorimotor networks are weaker in older individuals with cognitive dysfunction relative to matched controls. Data were obtained from the ADNI (aged controls 65+ years, n=51) and ABIDE (young controls 20-40 years, n=21) repositories, and the local University of Florida NEPAL study (with controls subjects of both age ranges, n=87). All resting state functional MRI scans were collected at different sites, and as part of different studies, on either a Siemens or General Electric 3-Tesla scanner with the following parameters: echo planar images with 197 image repetitions, TR=2-3seconds, TE=30ms. Graph theory-based calculations were applied to weighted undirected matrices constructed from 44,550 pairwise correlations between fMRI signals from 300 regions (Yeo parcellation). Using this highly dense sampling of nodes, we observed a novel pattern of distribution of node strength that varied across distinctly known networks between young and older adults. Young control individuals from the ABIDE study had high strength nodes in regions of the default mode network. Older adults (n=51) had low strength nodes in default mode regions, but significantly high strength nodes in regions of the somatomotor network. We are currently comparing these results from two different databases to in house NEPAL study data collected on broader age range. The present unexpected results suggest distinct salient functional network features in aged and young individuals that might correspond to distinct cognitive strategies inherent to brain functional organization in these two age groups. The data are consistent with emerging work from the animal literature showing distinct patterns of synchronized activity between motor and cognitive areas that vary with age.

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Poster

445. Clinical and Preclinical Studies in Alzheimer's Disease

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Program #/Poster #: 445.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Stanford ADRC Grant #P30AG066515

Title: Contribution of genetic polymorphisms to variable sleep disturbances in Alzheimer's disease

Authors: *A. HEATH^{1,2}, M. W. MCNERNEY², J. YESAVAGE^{1,2};
¹Stanford Univ., Palo Alto, CA; ²VA, Palo Alto, CA

Abstract: Alzheimer's disease (AD) presents itself as a highly heterogeneous disorder. Many diagnosed express variable symptom base which may further contribute to cognitive decline or be protective against the neurodegeneration caused by this disorder. One such factor is sleep. Aspects of sleep architecture are known to be heritable and influenced by genetic polymorphisms. Therefore, we hypothesized that genetic differences which underlie sleep disturbances influence the progression of AD. This study used existing data collected through the National Alzheimer's Coordinating Center's Uniform Data Set (NACC). Our preliminary analysis assessed the effect of sleep disturbances defined by the nighttime behaviors measure in the Neuropsychiatric Inventory Questionnaire (NITE). We used linear and logistic regression models controlling for sex, race and education level to assess the influence of NITE scoring on measures of the CDR® Dementia Staging Instrument and age of onset of cognitive decline in those diagnosed with AD. We found that at the final visit sleep disturbances were associated with a higher rating on the CDR and were also associated with an earlier onset of cognitive decline. As it is well known that sleep and AD have a bidirectional relationship we next wanted to observe if sleep disturbances in those which were cognitively normal at baseline and would eventually be diagnosed with AD influenced the progression of the disorder. We found sleep disturbances at baseline were associated with faster cognitive decline on the CDR scale, however, had no significant impact on the MMSE. In order to determine whether genetic differences underlie these sleep effects, we will examine GWAS SNP data and use VNTRseek to genotype over 100,000 tandem repeats of interest in sequencing data from the NACC cohort. We have previously used this method to identify VNTRs associated with AD in specific ethno-racial subgroups. However, this method has yet to be tested on a variable phenotype such as sleep. In conclusion, we found that differences in sleep affect outcomes and the progression of AD, even abnormal sleep behaviors present in the cognitively normal stage. Sleep remains one of the only modifiable factors that could easily influence the progression of AD. By identifying genetic associations which could allow for early detection of sleep disturbances which may accelerate the progression of this disorder we will open up avenues to apply early interventions and monitor the progression of this symptom closely.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

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Program #/Poster #: 446.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R21AG063012
TAME-AD Grant

Title: Investigating caspase-2 induced neuronal death and microglia activation in a mouse stereotaxic injection model of Alzheimer's Disease

Authors: *C. CHEN¹, M. ARNES FERNANDEZ², E. JACOTOT³, E. E. KONOFAGOU⁴, M. L. SHELANSKI⁵, C. M. TROY⁶;

¹Pathology & Cell Biol., Columbia Univ. Irving Med. Ctr., New York, NY; ²Pathology & Cell Biol., Columbia Univ. Irving Med. Ctr., New York City, NY; ³Inserm, Paris, France; ⁴Biomed. Engin. and Radiology, Columbia Univ., New York City, NY; ⁵Office for Res., ⁶Pathology & Cell Biol. and Neurol., Columbia Univ., New York, NY

Abstract: Over the past two decades, reported Alzheimer's Disease (AD)-related deaths have increased more than 145%, constituting a significant disease burden in the United States. As such, the development of therapies to alleviate the progressive and painful cognitive decline is more critical than ever. Studies of the brains of AD patients have shown elevated expression of caspase-2, indicating it as a potential target of intervention. Our lab has previously demonstrated the necessity of caspase-2 in A β ₁₋₄₂ induced cell death *in vitro* and *in vivo*. In this study, we investigate the efficacy of a caspase-2 inhibitor in the mitigation of damage in a mouse model of AD. This model employs the stereotaxic injection of oligomeric A β ₁₋₄₂ into the CA1 region of the hippocampus, where substantial neuronal loss is observed over the progression of AD in humans. Prior to injection, focused ultrasound (FUS) with lipid microbubbles (LM) was used to open the blood brain barrier and then either a Pen1-siRNA to caspase-2 or a Pen1-siLuciferase control was administered to each mouse intranasally. Brains were collected 24-hours post-injury for immunohistochemical analyses. Immunohistochemistry revealed marked changes in microglia number and morphology in hippocampi injected with A β ₁₋₄₂, with distinct differences in those treated with a caspase-2 inhibitor. While microglia-mediated neuroinflammation was previously believed to be secondary to AD disease progression, recent studies have suggested that microglia activation may have critical involvement in the pathogenesis of neurodegenerative diseases. When activated, microglia take on a wide range of morphologies which each play different—and sometimes opposing—roles in mediating inflammation. The exact mechanisms and pathways by which various microglia phenotypes contribute to neurodegeneration remains somewhat unclear. We conducted skeleton and single-cell fractal analysis of Iba-1 staining, which showed differences in microglia circularity, lacunarity, and end-point density between experimental groups. The activation of microglia is typically associated with caspases-3, -7, and -8, in separate pathways from caspase-2. This makes the observed differences in microglia morphology with the inhibition of caspase-2 even more intriguing. Future experiments will further investigate interaction between the caspase-2 apoptotic pathway and microglia behavior through immunohistochemistry and western blot, as well as study the progression of A β ₁₋₄₂ induced injury over longer time points.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

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Program #/Poster #: 446.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Eicosapentaenoic acid can decrease protein arginine methyltransferase 4 in the Alzheimer's disease.

Authors: *C. T. CITADIN¹, M. S. B. UDO², G. CLEMONS³, W. C. CARR², C. H. ACOSTA⁴, H. LIN⁵;

¹Cell. Biol. and Anat., Lsuhs- Shreveport, Shreveport, LA; ²Neurol., LSU Hlth. Sci. Ctr., Shreveport, LA; ³Cell. Biol. and Anat., Louisiana State Univ. Shreveport Hlth. Scien, Shreveport, LA; ⁴Cell. Biol. and Anat., ⁵Neurol., LSU Hlth. Sci. Ctr. Shreveport, Shreveport, LA

Abstract: Dietary supplementation of fish oils containing eicosapentaenoic acid (EPA) may convey neuroprotection throughout the aging process, resulting in improved outcomes in Alzheimer's disease (AD). We recently discovered that protein arginine methyltransferase 4 was enhanced in 1) patients with AD and related dementias (ADRD), 2) in post-mortem ADRD brain, 3) 3xTg mouse brain. In the 3xTg-AD mouse, this was further characterized by cognitive deficits and attenuated cerebral blood flow. Additionally, treatment with EPA (50 μ M) reduced PRMT4 protein expression in Bend.3 (brain endothelial cells) in vitro. Altogether, this led us to further determine if EPA can regulate PRMT4, which is thought to be important in ADRD. We treated 3xTg-AD mice with an equivalent of a daily human dose of 500 mg of fish oil containing 70% EPA for 5 weeks (P.O.). PRMT4 protein relative expression was reduced in the cortex after 5 weeks treatment (6.971 ± 0.180) as compared to control (8.761 ± 0.516). These results lead us to believe that omega-3 fatty acids can modulate PRMT4 levels to improve ADRD and associated pathologies related to ADRD such as learning/memory, cerebral blood flow dynamics, and the blood brain barrier. We will investigate into the mechanism(s) of EPA-mediated PRMT4 in AD.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Inhibition of 15-hydroxyprostaglandin dehydrogenase protects 5xFAD mice from Alzheimer's disease-like pathology

Authors: *Y. KOH^{1,3,4}, M. SHIN^{2,4,3}, E. F. VAZQUEZ-ROSA^{5,3,4}, P. SRIDHARAN^{6,3,4}, Z. BUD^{7,3,4}, S. BARKER^{1,3,4}, K. FRANKE^{4,3}, C. J. CINTRÓN-PÉREZ^{2,3,4}, H. LI¹, D. DAWSON¹, H. FUJIOKA⁸, S. FINK¹, M. E. FLANAGAN⁹, J. READY¹⁰, S. MARKOWITZ¹, A. PIEPER^{2,3,4};

¹Dept. of Pathology, ²Dept. of Psychiatry, Case Western Reserve University, Sch. of Med., Cleveland, OH; ³Harrington Discovery Institute, Univ. Hosp. Cleveland Med. Ctr., Cleveland, OH; ⁴Geriatric Res. Educ. and Clin. Centers, Louis Stokes Cleveland Veterans Affairs Med. Ctr., Cleveland, OH; ⁵Dept. of Psychiatry, Case Western Reserve Univ., Cleveland, OH; ⁶Dept. of Neurosci., Case Western Reserve Univ. Sch. of Med., Cleveland, OH; ⁷Case Western Reserve University, Sch. of Nursing, Cleveland, OH; ⁸Case Western Reserve University, Cryo-Electron Microscopy Core, Cleveland, OH; ⁹Northwestern Univ., Chicago, IL; ¹⁰Dept. of Biochem., UT Southwestern Med. Ctr., Dallas, TX

Abstract: As the most prevalent form of dementia, Alzheimer's disease (AD) represents a global health challenge due to its currently irreversible and progressive neuropsychiatric impairment. Sadly, there is still no neuroprotective medicine that halts or slows nerve cell death in patients suffering from AD. The 15-hydroxyprostaglandin dehydrogenase (15-PGDH) enzyme catalyzes inactivation of various eicosanoids, including prostaglandin D2 and E2 (PGD2 and PGE2). Previous research has shown that elevating PGE2 by inhibiting 15-PGDH results in potentiated stem cell responses in peripheral organs, and PGD2 is known to increase endothelial cell barrier function. Whether inhibiting 15-PGDH is protective in the brain, however, has not previously been investigated. Here, we report pathologically elevated levels of 15-PGDH in the brains of both human AD patients and 5xFAD mice. Pathological features of AD include impaired survival of young hippocampal neurons arising from neural stem cells, and degradation of the blood-brain barrier (BBB). Given the role of PGE2 and PGD2 in stem cell and endothelial cell biology, respectively, we tested the protective efficacy of genetic inhibition of 15-PGDH in the 5xFAD mouse model of AD. We found that brain 15-PGDH activity was pathologically elevated in these mice at 6 months of age, and further increased at 12 months. However, 15-PGDH deficient 5xFAD mice showed significantly reduced 15-PGDH activity at all ages, which correlated with protection of the BBB and elevation of the net magnitude of hippocampal neurogenesis. Spatial memory deficits in 5xFAD mice were also prevented by elimination of 15-PGDH activity, as measured by performance in the Morris water maze test. In addition, axonal degeneration was also prevented in 5xFAD mice by elimination of 15-PGDH activity. Notably, all protective effects occurred without impacting accumulation of amyloid β plaques in the brain. Thus, 15-PGDH inhibition represents a novel non-amyloid based therapy for AD, and possibly other forms of neurodegeneration as well.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Cathepsin B pH-dependent cleavage and inhibitor properties at cytosolic and lysosomal conditions: implications for its role in Alzheimer's disease and traumatic brain injury

Authors: ***M. C. YOON**¹, **A. SOLANIA**³, **Z. JIANG**¹, **M. P. CHRISTY**², **S. PODVIN**¹, **C. MOSIER**¹, **C. B. LIETZ**¹, **G. ITO**¹, **W. H. GERWICK**², **D. W. WOLAN**³, **G. HOOK**⁴, **A. J. O'DONOGHUE**¹, **V. HOOK**¹;

¹Skaggs Sch. of Pharm. and Pharmaceut. Sci., ²Scripps Inst. of Oceanography, UCSD, La Jolla, CA; ³Departments of Mol. Med. and Integrative Structural and Computat. Biol., The Scripps Res. Inst., La Jolla, CA; ⁴American Life Sci. Pharmaceuticals, Inc., La Jolla, CA

Abstract: Evidence for participation of cathepsin B (CTSB) in Alzheimer's disease (AD) and traumatic brain injury (TBI) is supported by studies in the field showing that (1) elevation of CTSB in AD patients that correlates with cognitive dysfunction, and (2) CTSB gene knockout or inhibition in AD and TBI animal models results in improved behavioral deficits and reduced neuropathology. Studies have demonstrated that lysosomal leakage occurs in AD and TBI to result in translocation of CTSB from its normal location in acidic lysosomes to the cytosol of neutral pH, where the enzyme activates cell death and inflammation. While CTSB is active in the cytosol, the ~400-fold change in proton concentration from acidic lysosomes of pH 4.6 to the neutral pH 7.2 of the cytosol raises the question of whether CTSB may display pH-dependent substrate cleavage and inhibitor properties. To answer this question, we assessed the cleavage profiles of CTSB at acidic pH 4.6 compared to neutral pH 7.2 by multiplex substrate profiling by mass spectrometry (MSP-MS) with iceLogo analysis, and found different cleavage preferences for amino acid P2 residues adjacent to the P1-P1' cleavage site. Based on the observed pH-dependent cleavage preferences at neutral pH 7.2, a neutral pH-selective substrate was designed and demonstrated as Z-Arg-Lys-AMC. Selective peptide substrates can be modified with the AOMK warhead to generate peptidic inhibitors. Therefore, the modified Z-Arg-Lys-AOMK was

synthesized and was found to potently inhibit CTSB with nM potency at neutral pH 7.2 that was 100-fold more selective for inhibition at pH 7.2 over acidic pH 4.6. The Z-Arg-Lys-AOMK inhibitor was found to be specific for CTSB over other cysteine cathepsins, and is cell permeable. These results show that CTSB possesses pH-dependent cleavage properties that can lead to design of a potent, neutral pH inhibitor of this enzyme. It will be of interest in future studies to assess inhibition of cytosolic neutral pH CTSB in AD and TBI animal models.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 446.05

Topic: C.02. Alzheimer's Disease and Other Dementias

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María Zambrano Excellence Program from the Ministry of Science and Innovation and the University of Valladolid (V.T.)
Internationalization program of Junta de Castilla y León, Spain, CL-EI-2021-09 IBGM (V.T.)

Title: The NAD⁺ Precursor Nicotinamide Riboside Preserves Mitochondrial Integrity and is Protective in Experimental Models of Parkinson's Disease and Alzheimer's Disease

Authors: N. Y. CALINGASAN¹, J. CHILUWAL¹, A. A. STARKOV^{1,2}, G. E. GIBSON^{1,3}, L. NÚÑEZ^{4,5}, C. VILLALOBOS⁴, M. BEAL¹, ***V. TAPIAS**^{1,4,5};

¹Feil Family Brain and Mind Res. Inst., Weill Cornell Med., New York, NY; ²Pediatrics, Robert Wood Johnson Med. Sch., New Brunswick, NJ; ³Burke Neurolog. Inst., White Plains, NY;

⁴Excellence Unit Inst. of Genet. and Mol. Biol. (IBGM) – CSIC, Valladolid, Spain; ⁵Biochem. and Mol. Biol. and Physiol., Univ. of Valladolid, Valladolid, Spain

Abstract: Impaired mitochondrial function has been associated with the etiopathogenesis of neurodegenerative diseases, including Parkinson's disease (PD) and Alzheimer's disease (AD). Sustained alterations in mitochondrial electron transport chain complexes lead to mitochondrial dysfunction and downstream neurodegenerative processes. Variations in nicotinamide adenine dinucleotide (NAD⁺) content have been related with aging and play a key role in both health and

life span. Nicotinamide riboside (NR), a source of vitamin B3, is a precursor of NAD⁺ that induces mitochondrial biogenesis and potentiates the activity of sirtuins, which are a family of NAD⁺-dependent protein deacetylases that dynamically regulate transcription, metabolism, and cellular stress response. Our results demonstrated that treatment with NR has beneficial properties in PD and AD both *in vitro* and *in vivo*. Particularly, long-term dietary supplementation with NR improved behavioral phenotype and increased NAD⁺ brain content in the Tg19959 transgenic mouse model of AD, thereby improving the cardinal features of neurodegenerative diseases, including mitochondrial damage, oxidative insult, formation of advanced glycation end products (AGEs), and inflammation. We have also provided new insights into the molecular mechanism underlying the neuroprotective effects of NR, which upregulates Pgc-1 α , Sirt1, Sirt3, and Sod2 gene expression and increases protein levels and concomitant mitochondrial biogenesis and function. Our findings provide important evidence that NR might be beneficial to the treatment of neurodegenerative diseases.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: P50 AA017823
P50 AA017823-S1
T32 AA 025606

Title: Sex-dependent effects of adolescent ethanol exposure on hippocampal-dependent memory impairment in a transgenic model of Alzheimer's Disease & TgF344-AD >, which can be rescued by exercise

Authors: *N. L. REITZ¹, F. SUMMERS¹, S. E. VENTRELLO¹, P. T. NUNES¹, L. M. SAVAGE²;

¹Binghamton Univ., Binghamton, NY; ²Psychology-Behavioral Neurosci., SUNY - Binghamton Univ. Behavioral Neurosci., Binghamton, NY

Abstract: Both Alzheimer's Disease (AD) and ethanol consumption in adolescence are widely prevalent and both result in a loss of cholinergic projections from the basal forebrain as well as the emergence of hippocampal-dependent cognitive impairments. Alcohol-related brain damage (ARBD) during a critical developmental timepoint, such as adolescence, may interact with AD-related pathologies to accelerate disease progression later in life. Women are more likely to develop AD and show more rapid cognitive decline/progression of brain atrophy once diagnosed, yet most preclinical work in the field of AD has been in male animals. The current study

investigates whether voluntary exercise can recover memory deficits caused by early-life pathology due to ethanol consumption in adolescence. To study this, the current project utilized a transgenic rat model of AD (TgF344-AD) and an adolescent intermittent ethanol (AIE) exposure model of binge drinking. TgF344-AD and wildtype F344 rats (male and female) were exposed to an intragastric gavage of water (control) or 5g/kg of 20% ethanol (AIE) for a 2 day on/off schedule throughout adolescence (PD27-57). At 6 months old, rats either remained in their home cage (control) or were placed in a voluntary wheel running apparatus for 4 weeks. At 7 months old, all rats were tested on spatial working memory and pattern separation. Female TgF344-AD rats that had been exposed to AIE have impaired spatial memory performance at 7 months of age, compared to TgF344-AD control rats and both wildtype groups. This spatial working memory deficit was recovered by 4 weeks of voluntary wheel running exercise. Selectively, TgF344-AD female rats, both control and AIE, were impaired on a pattern separation task at 7 months old. Voluntary exercise recovered this pattern separation impairment in control TgF344-AD female rats, but provided only a trend towards improvement for AIE-exposed TgF344-AD females. Male rats were not impaired on either spatial working memory or pattern separation as a result of AIE or AD genotype. Furthermore, voluntary exercise did not alter memory performance in male rats, regardless of AIE or AD status. This indicates that although adolescent ethanol exposure produces a sex-dependent emergence of memory impairments in a rodent model of AD by 7 months, voluntary exercise may recover these deficits.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

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Program #/Poster #: 446.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS 5R01NS117968
NIA 1R56AG073734

Title: Derivatives of MK886 modulate the proteasome and enhance neurite outgrowth

Authors: *E. E. LIAO¹, M. YANG², A. R. BRAUN¹, D. M. FERGUSON², J. N. SACHS¹;
¹Biomed. Engin., ²Medicinal Chem., Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Proteasomal degradation of intrinsically disordered proteins (IDPs), such as tau, is a critical component of proteostasis in both ageing and neurodegenerative diseases. We previously identified MK886 as a lead compound capable of modulating tau fluorescence lifetime FRET (FLT-FRET) oligomerization and rescuing SHSY5Y cytotoxicity induced by P301L tau overexpression. In this study, we synthesized a series of MK886 analogs and investigated their mechanism of action (MOA). Our assays probed the proteasomal activity, which is one of the

primary MOAs of MK886, autophagic activity by monitoring LC3, and tau cellular FLT-FRET oligomerization. We also developed a pathologic neurite outgrowth model using differentiated SHSY5Y neurospheres expressing wildtype tau to determine the analogs effect on neuronal development and degeneration. The MK886 derivatives had distinct effects on the proteasome and using these assays, we identified essential substituents of MK886 that are required for compound activity. Furthermore, we found that induction of proteasomal activity by MK886 and a few of its derivatives were able to overcome tau induced deficiencies in neurite outgrowth.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

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Program #/Poster #: 446.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the National Institute on Aging R01AG063750 (PV)

Title: Treatment with AV-001, an Angiotensin-1 mimetic peptide, improves dementia outcomes in middle-aged rats

Authors: *H. GAO, L. CULMONE, E. FINDEIS, A. ZACHAREK, B. POWELL, J. LANDSCHOOT-WARD, M. LU, M. CHOPP, P. VENKAT;
Henry Ford Hlth. Syst., Detroit, MI

Abstract: Background: Vascular dementia (VaD) is a complex neurodegenerative disease affecting cognition and memory. We previously found that in middle-aged rats subjected to a multiple microinfarction (MMI) model of VaD, treatment with AV-001, a Tie2 receptor agonist, significantly improves short-term memory, long-term memory, as well as improves preference for social novelty compared to MMI rats. In this study, we tested the early therapeutic effects of AV-001 on inflammation and glymphatic function in rats subjected to VaD. **Methods:** Male, middle-aged Wistar rats (10-12m) were assigned to Sham, MMI, and MMI+AV-001 groups. MMI was induced by injecting 800±200, 70-100µm sized, cholesterol crystals into the internal carotid artery. AV-001 treatment (1µg/Kg, i.p.) was initiated at 1 day after MMI and administered once daily. At 14 days after MMI, inflammatory factor expression was evaluated in cerebrospinal fluid (CSF) and brain. Immunostaining was used to evaluate white matter integrity, perivascular space (PVS) and perivascular Aquaporin-4 (AQP-4) expression in the brain. An additional set of rats were prepared to test glymphatic function. At 14 days after MMI, 50µl of 1% Tetramethylrhodamine (3 kD) and FITC conjugated dextran (500 kD) at 1:1 ratio was injected into CSF. Rats (3-4/group/time point) were sacrificed at 30mins, 3h, and 6h from the start of tracer infusion and brain coronal sections (80µm) were cut and Laser scanning confocal microscopy was used to evaluate tracer movement in brain. **Results:** Treatment of MMI with

AV-001 significantly improves white matter integrity in the corpus callosum at 14 days after MMI. MMI induces significant dilation of PVS, reduces AQP-4 expression and impairs glymphatic function compared to Sham rats. AV-001 treatment significantly reduces PVS, increases perivascular AQP-4 expression and improves glymphatic function compared to MMI rats. . MMI significantly increases while AV-001 decreases the expression of inflammatory factors (tumor necrosis factor- α (TNF- α), chemokine ligand 9) and anti-angiogenic factors (endostatin, plasminogen activator inhibitor-1, P-selectin) in CSF. MMI significantly increases while AV-001 reduces brain tissue expression of endostatin, thrombin, TNF- α , toll like receptor-4 (TLR-4), and interleukin-6 (IL-6). **Conclusions:** AV-001 treatment of MMI significantly reduces PVS dilation and increases perivascular AQP-4 expression which may contribute to improved glymphatic function compared to MMI rats. AV-001 treatment significantly reduces inflammatory factor expression in the CSF and brain which may contribute to AV-001 treatment induced improvement in cognitive function.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 446.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MoST 110-2321-B-001-011
AS-BRPT-110-11

Title: Equilibrative nucleoside transporter 1 (ENT1) as a novel therapeutic target to rescue Alzheimer's disease pathology and cognitive impairment

Authors: C.-Y. LIN^{1,2}, C.-P. CHANG^{1,2}, K.-C. WU^{1,3}, C.-W. WU^{1,2}, C.-J. LIN^{3,1}, *Y. CHERN^{2,1};

¹Biomed. Translation Res. Center, Academia Sinica, Taipei, Taiwan; ²Inst. of Biomed. Sciences, Academia Sinica, Taipei, Taiwan; ³Sch. of Pharmacy, Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Alzheimer's disease (AD) is the most prominent neurodegenerative disease in aging societies. The major pathogenic features of AD include extracellular beta-amyloid deposition, intracellular Tau tangles, neuroinflammation, oxidative toxicity and energy dysfunction, which cause neuritic dystrophy, synapse loss, cognitive impairment, and memory loss. ENT1 is a bidirectional transporter that transports adenosine in a concentration-dependent manner. We have developed an orally active ENT1 inhibitor (designated J4) that rescues cognitive decline and impaired spatial memory in amyloid- and tau-based mouse models. Treatment of symptomatic APP/PS1 and Thy-Tau22 mice with J4 ameliorated mitochondrial mass and energy production accompanied by the reduction of A β and tau deposition, oxidative stress, and neuroinflammation.

In addition, therapeutic efficacy examined by PET images (for amyloid-, Tau-, and mitochondria) and FDG-PET were established in preclinical study, which can be further used as potential biomarkers clinically for Alzheimer's disease. Our findings indicate that J4 is a novel and promising new chemical entity for treating AD by inhibiting ENT1 and modulating adenosine homeostasis.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Upregulation of endothelial *Picalm* with a drug selected from an FDA-approved drug screen ameliorates amyloid pathology and improves functional outcomes in mice

Authors: *K. KISLER, A. P. SAGARE, D. LAZIC, S. BAZZI, E. LAWSON, Y. WANG, C.-J. HSU, E. ZUNIGA, A. R. NELSON, Z. ZHAO, B. V. ZLOKOVIC;
Physiol. and Neuroscience, Zilkha Neurogenetic Inst., USC, Los Angeles, CA

Abstract: *PICALM*, phosphatidylinositol binding clathrin assembly protein, is one of the most significant susceptibility factors for Alzheimer's disease (AD). In mice and humans, *PICALM* is highly expressed in brain endothelial cells, and involved in clathrin-mediated endocytosis, trafficking, and clearance of amyloid- β ($A\beta$) from the brain across the blood-brain barrier. *PICALM* brain endothelial levels are reduced in AD, and *PICALM* loss from endothelium in mice diminishes $A\beta$ clearance from brain and exacerbates $A\beta$ pathology, which could be reversed by reintroducing endothelial *Picalm* expression using gene therapy. This suggests that therapeutic strategies that upregulate *Picalm* expression in brain capillaries could alleviate brain $A\beta$ pathology and improve functional outcomes. To identify a drug to increase *Picalm* levels, we screened a library of 2007 FDA-approved drugs with a luciferase reporter assay. After excluding neurotoxic drug hits, secondary mRNA screen confirmed drug T-65 as the lead hit. This drug elevated *Picalm* mRNA and protein levels by 2-3 fold in a human endothelial cell line and *in vivo* in mouse brain capillaries. Treatment of *Picalm*-hemizygous 5XFAD mice with T-65 increased brain capillary *Picalm* levels, reduced brain $A\beta$ load, improved behavior, cerebral

blood flow responses and blood-brain barrier integrity. Endothelial-specific genetic deletion of *Picalm* abolished all beneficial effects of the drug, confirming that it acts on endothelial PICALM. Together these data indicate that PICALM upregulation in endothelium by T-65 could be a promising new treatment to reduce A β pathology in AD.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 446.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Ganaxolone can attenuate the progression of AD pathology and modulate cognitive deficits in 3xTg-AD mice model of Alzheimer's disease

Authors: *V. TESIC¹, M. S. B. UDO¹, C. T. CITADIN², G. CLEMONS³, C. H. ACOSTA⁴, W. C. CARR⁵, H. LIN⁵;

¹Neurol., LSU Hlth. Sci. Ctr., Shreveport, LA; ²Cell. Biol. and Anat., Lsuhs- Shreveport, Shreveport, LA; ³Cell. Biol. and Anat., Louisiana State Univ. Shreveport Hlth. Scien, Shreveport, LA; ⁴Cell. Biol. and Anat., ⁵Neurol., LSU Hlth. Sci. Ctr. Shreveport, Shreveport, LA

Abstract: Alzheimer's disease (AD) is the most common chronic neurodegenerative disorder, affecting over 6 million Americans. By 2050, this number is projected to rise to nearly 13 million. AD is characterized by the accumulation of hyperphosphorylated tau protein in neurofibrillary tangles and amyloid- β (A β) in senile plaques, associated with progressive cognitive deficits. Targeting novel pathways in AD is a critical unmet need as there is no effective treatment available to prevent, attenuate, or reverse the disease. Aberrant function of brain's major inhibitory system, γ -Aminobutyric acid (GABA)-ergic system, is increasingly recognized as an important factor in early AD pathogenesis. A β -induced neurotoxicity impairs GABAergic neuron activity and downregulates postsynaptic GABA_A receptors. The reduction of GABA_A-current was found to be larger in younger AD patients, suggesting that GABAergic impairment is an early event in the AD brain. Modulation of GABA_A function may serve as a preventive strategy for AD, as GABA-ergic system disturbances precede cognitive impairment. To activate GABAergic system, we used neurosteroid ganaxolone (GNX), a selective, high-affinity, positive modulator of GABA_A receptor. We conducted experiments on young, pre-symptomatic (4-6 months) and aged, cognitively impaired (9-12 months) 3xTg-AD mice model of AD, as they manifest age-dependent neuropathology that includes both β -amyloid plaques and neurofibrillary tangles. Our preliminary *in vivo* data show that short term (1 week) GNX treatment modulates AD-related pathology in age and sex dependent manner, as indicated by the level of A β and phosphorylated tau. GNX treatment also changed cerebral blood flow in 3xTg-

AD female mice, as evaluated by laser speckle. Behavioral testing for assessing cognitive deficits, such as novel object recognition and Barnes maze, showed attenuation of cognitive impairment in aged 3xTg-AD females, without affecting performance in young 3xTg animals. Our data suggest that potentiation of GABAergic signaling can attenuate the progression of AD pathology and modulate cognitive deficits in 3xTg-AD mice.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 446.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NS101967
AG077396

Title: Non-electrophilic Bach1 inhibitor attenuates progression of Alzheimer's Disease-like pathology in the APP/PS1 mouse model.

Authors: *D. DUTTA^{1,2}, M. AHUJA^{1,2,7}, N. KAIDERY^{1,2}, R. KARIM⁸, J. MEINTS⁸, O. ATTUCKS⁹, S. SHARMA^{3,4}, M. LEE⁸, B. THOMAS^{1,2,5,6};

¹Darby Children's Res. Inst., ²Dept. of Pediatrics, ³Dept. of Biochem., ⁴Hollings Cancer Ctr., ⁵Dept. of Neurosci., ⁶Dept. of Drug Discovery, Med. Univ. of South Carolina, Charleston, SC; ⁷Dept. of Pharmaceut. Sci., Univ. of Buffalo, Buffalo, NY; ⁸Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN; ⁹vTv Therapeut. LLC, High Point, NC

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia affecting 50 million people worldwide with no known cure. Nuclear-factor-erythroid 2-related factor 2 (Nrf2) is a key transcription factor that orchestrates a multifaceted response to modulate multiple etiological pathways involved in AD. A decline in the expression of Nrf2 and alteration of the Nrf2-related pathways are observed in humans and animal models of AD. Consequently, activation of the Nrf2 pathway represents a promising therapeutic approach in AD. Unfortunately, canonical Nrf2 activators are electrophiles as they not only react with cysteines on Kelch-like-ECH-associated protein 1 (Keap1) to activate Nrf2 but non-specifically react with thiol groups on a variety of cellular proteins resulting in side effects. BTB and CNC homology 1 (Bach1) is a known transcriptional repressor of the Nrf2 pathway. Using a drug library screen we identified a substituted benzimidazole (HPPE) as a Bach1 inhibitor that was validated as a non-electrophile. Cohorts of APP/PS1 transgenic mice were administered with HPPE for 45 days starting at 11 months and 15 months of age. We report that daily administration of HPPE (20 mg/kg twice a day, 12 h apart) treatment attenuated cognitive deficits in the APP/PS1 mice behaviorally assessed using Barnes maze and novel

object recognition tests. Quantitative neuropathological analyses using unbiased stereology showed that HPPE treatment significantly reduced amyloid pathology (A β deposits), microglial activation (Iba1), and attenuated the progressive loss of cortical tyrosine hydroxylase afferents in the APP/PS1 mice compared to vehicle treated controls. Functional genomics analysis demonstrated that the neuroprotective effects of Bach1 inhibition was due to upregulation of Bach1-targeted pathways that are associated with both Nrf2-dependent antioxidants response element (ARE) and Nrf2-independent non-ARE genes. Our findings suggest that HPPE treatment after the onset of AD pathology can reverse/slow further progression of AD-like pathology and that Bach1 inhibition is a promising therapeutic approach.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 446.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A new translational model to successfully treating AD

Authors: *J. COLIN¹, A. ALLOUCHE¹, M. BALDONI^{1,2}, E. LAGER¹, H. SCHROEDER², J.-F. BISSON¹, N. VIOLLE¹;

¹ETAP-Lab, Vandoeuvre-lès-Nancy, France; ²INSERM U1256, NGERE, Faculté de Médecine, Vandoeuvre-lès-Nancy, France

Abstract: Alzheimer's disease (AD) is a complex disease involving numerous mechanisms in line with processes of biological aging. A growing body of evidence suggests that one of the key mechanisms in the physiopathology of AD is the role of amyloid-beta oligomers (A β O).

Moreover, even though aging is the main risk factor for AD, little is known about the susceptibility of aging brain for A β O neurotoxicity. Here, we examined how AD is linked to aging and we compared the behavioural, biochemical and histological effects of A β O in both young and aged mice.

A β O were prepared in-house from human A β ₁₋₄₂ monomers. The oligomeric preparations were characterized by SDS-page and dot-plot assays. Young (3-month-old) and aged (18-month-old) wild-type mice received a single intracerebral injection of A β O. Memory performances were assessed for 2 weeks and hippocampal protein levels and brain immunohistochemical staining were assayed at the end of the experiment.

Naive old and young mice had similar memory performances despite significantly lower synaptic marker expressions in aged mice. A β O induced significant synaptic losses in both young and aged mice, but only aged mice showed significant memory impairments in episodic and spatial memory tasks. Interestingly, A β O produced more cerebral inflammation in aged mice than in young mice and promoted neuronal apoptosis as well as functional alterations of astrocytes.

Cognitive symptoms in aged mice were reversed by Donepezil.

In conclusion, aging dramatically changes the susceptibility for A β O neurotoxicity in mice. It confirms that the specificities of the aged brain should be considered to improve the translational value of AD models. Indeed, efforts to find disease-modifying treatments have met with limited success possibly because aging was not considered. Our non-inherited AD model is a new tool for preclinical testing of both disease modifying and symptomatic drugs.

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Poster

446. Alzheimer's Disease: Pharmacological Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 446.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Lineage negative stem cells of female Yoga practitioners reverse amyloid beta induced memory loss in mouse model

Authors: *P. NADHOLTA, A. ANAND;

Post Grad. Inst. of Med. Educ. and Res., PGIMER: Post Grad. Inst. of Med. Educ. and Res., Chandigarh, India

Abstract: Amnesia is major clinical problem among elderly and is associated with neuro-degeneration and memory loss during old age. There is no reliable treatment available for amnesia despite of several clinical trials. In this explorative study we aimed to determine the efficacy of Umbilical cord blood (UCB) derived lin-ve ste cells (SCs) of Yoga practitioner mothers during pregnancy in reversal of Amyloid beta (A β) induced amnesia and simultaneously the pathways involved with this reversal. The efficacy of the UCB has already been studied in enhancing memory in such memory loss models. We want to see whether the UCB from Yoga practitioner mothers has more stemness and effects in memory reversal or not. This study included both the human population (Pregnant women aged b/w 18 to 35, n=56) and the animal model (Swiss albino, 6-8 weeks old). Memory loss was performed via stereotactic administration of A β in the hippocampal region of mice brain. UCB was collected from pregnant women who were given Yoga intervention in the 2nd and 3rd trimesters, Mononuclear cells were isolated, and Lin+ve, and Lin-ve populations were separated to scrutinize the stemness of Lin-ve SC through flow cytometry for markers such as CD34 and CD117. A β was injected in hippocampus stereotactically to create memory loss and after 21 days of A β administration, Lin-ve SCs were transplanted at the site of injury. Neurobehavioral tests (Morris Water Maze & Passive avoidance) were used in this study to examine memory loss and memory reversal. Neuro-behavioral analysis after 10 days of transplantation showed improved memory in the A β injury model. Yoga can be adopted as a lifestyle during pregnancy for improved outcomes, and Lin-ve

SCs derived from these practitioners can be considered a good source of memory reversal in amnesia and dementia patients however, more studies are warranted in the arena.

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Poster

447. Parkinson's Disease Animal and Cellular Models

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Program #/Poster #: 447.01

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation

Title: Characterizing the relationship between L-DOPA-induced dyskinesia and psychosis-like behaviors in a bilateral rat model of Parkinson's disease

Authors: *N. LIPARI, A. CENTNER, J. GLINSKI, S. COHEN, C. BISHOP;
State Univ. of New York, Binghamton, State Univ. of New York, Binghamton, Binghamton, NY

Abstract: Parkinson's Disease (PD) is most commonly characterized by severe dopamine (DA) depletion within the substantia nigra (SN) leading to a myriad of motor and non-motor symptoms (NMS) including cognitive and affective dysfunction. Parkinson's disease associated psychosis (PDAP), is a prevalent non-motor symptom (NMS), which significantly erodes patients' and caregivers' quality of life, yet remains vastly understudied. PDAP has been shown both in treatment-naïve and late-stage Parkinson's disease (PD) as well as a treatment-related side effect. The gold standard therapy for many PD symptoms is Levodopa (L-DOPA), however, chronic treatment leads to L-DOPA-induced dyskinesia (LID) in some patients and exacerbates NMS. Given the high incidence of LID in later phases of PD and a clinical correlation between motor and NMS, this study sought to characterize the relationship between PDAP and LID in a bilateral medial forebrain bundle 6-hydroxydopamine hydrobromide (6-OHDA) lesion rat model. To assess PDAP, prepulse inhibition (PPI), a well-validated assay of sensorimotor gating was employed. First, in experiment 1 we tested whether a bilateral lesion with or without chronic L-DOPA treatment induced PPI dysfunction. Rats were also monitored for LID development, using the abnormal involuntary movements (AIMs) test to examine PPI and LID associations. Given growing evidence of the serotonin (5-HT) system's involvement in LID and PDAP, in experiment 2, Vilazodone (VZD), a serotonin 1A receptor (5-HT_{1A}) partial agonist was administered to test its potential efficacy in reducing LID and PPI dysfunction. Results indicate that bilateral DA lesions produced motor deficits and chronic L-DOPA induced moderate AIMs. Interestingly, rats that developed more severe AIMs were also more likely to display sensorimotor gating problems. Moreover, VZD treatment dose-dependently reduced L-DOPA-induced AIMs without altering L-DOPA efficacy, but VZD's effects on PPI were limited. Altogether, this project established the bilateral 6-OHDA lesion model for accurately portraying

LID and PDAP-like behaviors, uncovered their potential relationship, and supported the utility of VZD for reducing LID.

Disclosures: N. Lipari: None. A. Centner: None. J. Glinski: None. S. Cohen: None. C. Bishop: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.02

Topic: C.03. Parkinson's Disease

Support: MOST-111-2636-B-002-021

Title: Early dysbiosis and dampened gut microbe oscillation precede motor dysfunction and neuropathology in a mouse model of alpha-synucleinopathy

Authors: *A. HSIEH¹, F. LIANG², C.-Y. CHEN^{2,4}, Y.-P. LI², S.-K. CHEN^{2,3}, C.-H. LIN⁵;
¹Grad. Inst. of Brain and Minds Sci., Natl. Taiwan Univ., Taipei, Taiwan; ²Dept. of Life Sci.,
³Genome and Systems Biol. Degree Program, Natl. Taiwan Univ., Taipei, Taiwan; ⁴Biodiversity Res. Ctr., Academia Sinica, Taipei, Taiwan; ⁵Dept. of neurology, Natl. Taiwan Univ. Hosp., Taipei, Taiwan

Abstract: The pathological hallmark of Parkinson's disease (PD), neuronal α -synuclein accumulations named Lewy body, has been identified within the gut enteric nervous system early in the disease process. Studies have shown different gut microbiomes in PD patients compared to healthy controls. However, when the gut microbiota shift toward dysbiosis in the PD process remains unclear. Here, we investigate the gut microbiota in PD rodent models using 16s rRNA next-generation sequence, and their locomotor function and neuropathology longitudinally. Compared to non-transgenic littermate controls, the altered gut microbiota of the *SNCA* p.A53T mice can be detected starting at 2 months old, while motor deficits were observed as early as 8 months old. Notably, the diurnal oscillation of the gut microbiome was dampened throughout PD progression starting from 4 months old. Similar changes in altered gut microbiota were also observed in another PD genetic mouse model carrying the *LRRK2* p.G2019S mutation at 2 months old. Finally, using metagenomic sequencing, we found that *Parabateroides Merdae* and *Ruminococcus torques* were enriched in human PD patients. Interestingly, genera *Parabateroides* and *Ruminococcaceae* were also enriched in both PD mouse models. These findings revealed the altered gut microbiota communities and oscillations preceding the occurrence of neuropathy and motor dysfunction in the PD process. Furthermore, a common host and gut microbe interaction may be conserved in mammals such as humans and rodents.

Disclosures: A. Hsieh: None. F. Liang: None. C. Chen: None. Y. Li: None. S. Chen: None. C. Lin: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

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Program #/Poster #: 447.03

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1F99NS129168-01

Title: Neuromelanin pigmentation in mouse locus coeruleus leads to severe noradrenergic dysfunction, degeneration, and immune response

Authors: *A. IANNITELLI¹, D. WEINSHENKER²;

¹Emory Univ., ²Sch. of Medicine, Dept. of Human Genet., Emory Univ., Atlanta, GA

Abstract: Cytosolic neuromelanin (NM) pigment granules contain catecholamine metabolites, lipid droplets, protein aggregates and heavy metals. NM is uniquely produced in dopamine neurons of the substantia nigra (SN) and norepinephrine neurons of the locus coeruleus (LC) that are selectively vulnerable in Parkinson's disease (PD). NM accumulation is thought to serve a neuroprotective role by sequestering toxins and reactive metabolites, but may eventually pose a threat by overwhelming cellular machinery, and the release of NM-bound toxins during degeneration could accelerate the dysfunction and loss of neighboring cells. Causal relationships between NM and PD have been difficult to study because NM is not produced endogenously in rodents, the canonical model systems for uncovering mechanisms underlying PD. It was recently shown that viral-mediated expression of human tyrosinase (hTyr), the enzyme responsible for melanin production in skin, can drive NM formation in the rodent SN. We adapted this approach to express NM-like pigmentation in the LC, as noradrenergic dysfunction has been implicated in the early, non-motor symptoms of PD. TH-Cre transgenic mice were injected with either a Cre-dependent AAV5-hTyr virus or mCherry control bilaterally into the LC. hTyr expression in the LC resulted in a time-dependent accumulation of pigment granules in mice comparable to endogenous primate NM as assessed by histology and electron microscopy. NM-expressing mice displayed LC-sensitive behavioral deficits that reflect non-motor PD symptoms, including abnormal sleep latency, contextual fear conditioning, and novelty-suppressed feeding. By 10-weeks, complete degeneration of LC neurons as a result of pigmentation was evident, coupled with a robust infiltration of reactive astrocytes in the region. Pigment granules persisted despite cell body loss, suggesting extracellular localization. Translating Ribosome Affinity Purification (TRAP) is ongoing to determine the NM-induced molecular changes associated with LC dysfunction and degeneration. This novel mouse model of NM will allow us to identify mechanisms underlying the selective vulnerability of LC neurons and the consequences of their deterioration in PD.

Disclosures: A. Iannitelli: None. D. Weinshenker: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.04

Topic: C.03. Parkinson's Disease

Title: To assess and evaluate gut microbiome diversity in two mice strains with differentially susceptible to MPTP and their crossbreds

Authors: *Y. HAOREI^{1,2}, V. DJ⁵, C. SAGAR³, T. RAJU⁴, P. ALLADI⁴;

¹Natl. Inst. of Mental Hlth. and Neuro Scie, Zoology Department, Hansraj College, Univ. of Delhi, Delhi, India; ²Dept. of Neuropathology, Natl. Inst. of Mental Hlth. and Neuro Sci., Bangalore, India; ³Dept. of Neuropathology, Natl. Inst. of Mental Hlth. and Neuro Sci., Benaluru, India; ⁴Natl. Inst. of Mental Hlth. and Neuro Sci., Bengaluru, India; ⁵Dept. of Neurosci., New Haven, CT

Abstract: BACKGROUND: It is long known that aging in the brain and PD is associated with a number of non-motor changes, especially pertaining to the digestive system. There are a community of microbes residing in the distal gut.

Colonization occurs at the time of

birth. The microbes undergo diversification and changes. In PD the composition of these

complex systems has been

reported to be altered which is associated with PD. We deemed it important to investigate if the bacteria in the gut may

have any role in susceptibility to PD

METHODS: Bacterial 16S rRNA hypervariable regions V3-V4 were extracted and amplified from the fecal matter of parent strains(C57BL/6J and CD-10) and their crossbreds (F1X1 and F1X2) using V3V4F and V3V4R primers. Following

metagenomic analysis, the top 10 of the bacterial genus were picked from heat map distribution and their abundance

was correlated between different groups in normal conditions.

RESULTS: The gut microbes in MPTP-susceptible C57BL/6J had more genera belonging to the Streptococcus, Bacteroides andLachnospiraceae family whereas its presence is relatively lower in MPTP-resistant CD-1 and the crossbreds. The CD-1

and the crossbreds seemed to harbor more microbes and genera belonging to the Prevotellacea family. The F1X1 and

F1X2 crossbreds showed the highest abundance of bacteria including those Prevotellacea and Lachnospiraceae which

are reportedly shown to be reduced in human PD patients when compared to normal subjects.

The maximum

concentration of bacteria of the Prevotella spp have seen in crossbreds F1X2 which is the least susceptible group

among all strains tested for MPTP

CONCLUSIONS: The evaluation of the gut microbes in different mice strains depicted diverse

microbes in each of them. The resistant mice strains specifically harbor more of the Prevotella family microbes. The bacteria of this genus are known to be reduced in human PD patients when compared to normal subjects. The resident microbes and their secretions and metabolites released may have functional relevance in terms of modulation host susceptibility to diseases. It is tempting to assume that there could be some functional network between the presence of the bacterial community in the gut, the types of glial cells and the individuals' risk of suffering from PD.

REFERENCES: Scheperjans et al.,(2015). Gut microbiota are related to Parkinson's disease and clinical phenotype. *Movement Disorders*, 30(3), 350-358.

Sampson et al., (2018). Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell*, 167(6), 1469-1480.e12

Disclosures: Y. Haorei: None. V. Dj: None. C. Sagar: None. T. Raju: None. P. Alladi: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

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Program #/Poster #: 447.05

Topic: C.03. Parkinson's Disease

Support: Wallenberg Academy Fellowship
StraNeuro Bridging Grant
Karolinska Institute Principal Researcher
Vetenskapsrådet Starting Grant

Title: Involvement of striato-pallidal DARPP32 in sleep regulation

Authors: *C. A. PISANÒ, D. DE CASTRO MEDEIROS, E. SANTINI, G. FISONE;
Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: *Aim:* DARPP32 is highly enriched in the striatal formation and has been intensively studied for its role in the regulation of the activity of the striato-nigral (dMSNs) and striato-pallidal projection (iMSNs) neurons, belonging to the direct and indirect pathway. The present study investigates the involvement of DARPP32 in different types of striato-pallidal driven behaviors, including locomotion and motor coordination, sleep, and cognition. Moreover, since DARPP32 is a key player in dopaminergic transmission, we checked the effect of cell specific manipulations of DARPP32 on sleep-related disturbances observed in Parkinson's disease (PD). *Methods:* *Animals:* we used transgenic mice with a specific depletion of DARPP32 in iMSN. To reproduce PD, mice received a unilateral injection of 6-hydroxydopamine (6-OHDA) in the right medial forebrain bundle. *Behavioral tests:* The motor phenotype was studied using the open-field

and rotarod test. The novel object recognition test and the water Y maze were used to examine cognition and memory. *Sleep analysis:* sleep architecture was analyzed by EEG and EMG recording using a MATLAB script. *Results:* Depletion of DARPP32 in iMSN increases basal exploratory behavior in the open-field. This effect is accompanied by a significant increase in wakefulness and decrease in non-rapid eye movement (NREM) sleep during the active period of the 24hr light-dark cycle. Cognitive and learning behaviors are not affected by DARPP32 depletion in iMSN. In the mouse PD model, DARPP32 deficiency in iMSN reduces the increase in NREM observed during the active period, which is indicative of excessive daytime sleepiness (EDS) in PD patients. *Conclusion:* Our findings show that DARPP32 deficiency in iMSNs increases motor behavior and reduces sleep during the active period of the sleep-wake cycle. Moreover, the normalization of EDS in lesioned mice suggests that targeting the indirect pathway represents a promising approach to improve sleep disorders in PD patients.

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Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.06

Topic: C.03. Parkinson's Disease

Title: Co-administration of rutin and capsaicin improved 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced behavioural deficits, inflammatory and oxidative changes in mice model of Parkinson's disease

Authors: *D. E. BABATUNDE^{1,2}, J. A. ADEDIJI^{3,4}, M. O. ADEYEMO¹, Y. Z. ABDULRAZAQ¹, P. G. AYUBA¹, O. OLABIYI¹, G. O. OMOTOSO²;

¹ANATOMY DEPARTMENT, COLLEGE OF HEALTH SCIENCES, BOWEN UNIVERSITY IWO, IWO, Nigeria; ²Anat. department, FACULTY OF BASIC MEDICAL SCIENCES, UNIVERSITY OF ILORIN, ILORIN, NIGERIA, Ilorin, Nigeria; ³Pharm., Visiting Researcher, Sch. of Applied Sciences, Univ. of Huddersfield, United Kingdom, University of Huddersfield, United Kingdom, United Kingdom; ⁴Hlth. Information, Ctr. for Training Community Hlth. Officers, Univ. of Benin Teaching Hosp., Benin, Nigeria

Abstract: **Abstract** Parkinson's disease (PD) is a neurodegenerative disorder affecting dopaminergic neurons in the brain. Intraperitoneal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a known neurotoxicant, is commonly used to model symptoms of Parkinson's disease in experimental mice. Flavonoids like Capsaicin and Rutin have been reported to possess anti-inflammatory and anti-oxidative properties which are hallmarks of PD. This study aims to investigate the role of Capsaicin and Rutin on MPTP induced hippocampal and nigrostriatal/dopaminergic neurotoxicity in female swiss mice. 32 female mice were divided into four (4) groups: (A; Control), (B; Rutin +Capsaicin Only), (C; MPTP only), (D;

Rutin+Capsaicin+MPTP) were treated for 5 days after which neurobehavioral tests like, pole test, hanging wire test (both for motor function and neuromuscular strength), Y-maze and open field test (both for cognition and exploratory skills) were conducted. Biochemical levels for inflammatory and oxidative biomarkers like Nitric Oxide, Catalase, Superoxide Dismutase, and Malondialdehyde were assayed. Immunohistochemical staining (α -synuclein) and histological staining (Hematoxylin & Eosin, and Cresyl Violet) procedures were conducted on the hippocampus and substantia nigra tissues harvested. One-way ANOVA and post hoc Bonferroni's test were used for statistical analysis at $p < 0.05$. Co-administration of Rutin+Capsaicin significantly increased the levels of glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase when compared with the MPTP only group. MPTP-induced increase in malonaldehyde and nitric oxide level were significantly reduced by the co-administration of Rutin+Capsaicin when compared with the MPTP only group. Immunohistochemical and histological micrographs showed that MPTP-distorted histoarchitecture of the hippocampus and substantia nigra in MPTP only group were improved in other groups. Behavioural tests showed reduced motor and non-motor symptoms of PD in MPTP only groups compared to the Rutin+Capsaicin and control groups. Given that rutin and capsaicin were reported to individually have anti-inflammatory and anti-oxidative properties, this research work has shown the Co-administration of rutin and capsaicin as a promising drug-lead, but the mechanism(s) of action need to be studied further. **Keywords;** Capsaicin, Rutin, MPTP, Oxidative stress, Swiss mice, Parkinson's disease

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Poster

447. Parkinson's Disease Animal and Cellular Models

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Topic: C.03. Parkinson's Disease

Support: MJFF-13711
MJFF-021946
MJFF-17492,
MJFF-021913
MJFF-15838
MJFF-15985

Title: The Michael J. Fox Foundation's development and distribution of novel alpha synuclein viral vectors to study Parkinson's disease

Authors: *E. M. CLARK¹, N. K. POLINSKI¹, B. CASEY¹, T. BJÖRKLUND², F. P. MANFREDSSON³, J. B. KOPRICH⁴, S. MARSHALL⁵, J. EBERLING¹;

¹The Michael J. Fox Fdn. for Parkinson's Res., New York, NY; ²Mol. Neuromodulation,

Wallenberg Neurosci., Lund, Sweden; ³Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; ⁴Atuka Inc., Toronto, ON, Canada; ⁵GeneDetect, Sarasota, FL

Abstract: Alpha synuclein (aSyn) plays an important role in Parkinson's disease (PD) with pathological changes of the protein observed in PD patients and mutations/multiplications in the gene leading to PD. Commonly used rodent models overexpress wildtype and mutant forms of aSyn, and have been helpful in understanding molecular mechanisms and the role of aSyn in PD pathogenesis. However, the lack of comparable phenotypes makes it challenging to reproduce PD in animal models. Therefore, it is important to have preclinical tools that best suit the scientific questions we want to answer to further our understanding of aSyn biology in order to develop and evaluate potential therapies for targeting aSyn aggregation. The Michael J. Fox Foundation for Parkinson's Research (MJFF) sponsors the development of resources for PD research and drug development communities that endeavors to provide researchers with easy access to rigorously validated preclinical tools for their studies. The MJFF preclinical tools portfolio currently includes novel viral vector tools that utilize human aSyn to serve as a platform for PD model development. Recently, MJFF developed a novel adeno-associated virus (AAV) vector that can be used to overexpress human wildtype (WT) aSyn in different species. Extensive validation of this viral vector six weeks post-intranigral injection in rat shows dose-dependent degeneration of the nigrostriatal system and corresponding motor impairments, producing an ideal platform for studying PD biology and therapeutic interventions. We also showcase data from an MJFF-generated virus that overexpresses the A53T mutant form of aSyn, including behavioral deficits and nigrostriatal degeneration at 6 weeks post-intranigral injection in rat. Finally, we highlight a different set of viral vectors that express an SNCA miR designed to reduce expression of human or mouse aSyn. Ultimately, these research tools aim to address field-wide challenges in the PD preclinical tools and reagents landscape and to overall accelerate Parkinson's disease research.

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Poster

447. Parkinson's Disease Animal and Cellular Models

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Ministry of Science and Innovation (MICINN), Spain (PID2020-116339RB-I00 to M.V.)
Ministry of Science and Innovation (MICINN), Spain (SAF2016-77541-R to N.P.)

Title: Transcriptomic changes linked to age-dependent neuromelanin accumulation in transgenic neuromelanin-producing parkinsonian mice

Authors: *N. PEÑUELAS¹, A. LAGUNA¹, H. XICOY¹, E. MARKIDI¹, M. VILA^{1,2,3};
¹Vall d'Hebron Res. Inst., Neurodegenerative Dis. Res. Group, Vall d'Hebron Res. Inst. (VHIR)-Network Ctr. for Biomed. Res. in Neurodegenerative Dis. (CIBERNED)-Autonomous Univ. of Barcelona, Barcelona, Spain; ²Catalan Inst. for Res. and Advanced Studies (ICREA), Barcelona, Spain; ³Aligning Sci. Across Parkinson's (ASAP) Collaborative Res. Network, Chevy Chase, MD

Abstract: Neuromelanin (NM)-containing neurons preferentially degenerate in Parkinson's disease (PD). We previously reported that intracellular NM levels in the Substantia Nigra pars compacta (SNpc) may set a threshold for the initiation of PD in a rodent model based on the unilateral viral vector-mediated nigral overexpression of melanin-producing enzyme tyrosinase. Next, we generated a new NM-producing mouse model (tgNM) based on the tissue-specific constitutive expression of human tyrosinase under the tyrosine hydroxylase promoter. TgNM mice exhibit major age-dependent PD features, including motor and non-motor behavioral alterations, inclusion body formation, neuronal degeneration in lower brainstem areas (Locus Coeruleus, LC) together with neuronal dysfunction in higher brainstem areas (SNpc and ventral tegmental area, VTA). To identify the specific molecular events that result from progressive NM accumulation and that may underlie PD selective vulnerability, we performed genome-wide transcriptomic characterization of the main pigmented PD-relevant areas in tgNM mice. Anatomically defined SNpc, VTA and LC brain regions were isolated by laser capture microdissection from tgNM and wild-type (wt) mice at different ages across mouse lifespan (3, 12 and 20 months of age). Differential expression analysis between tgNM and wt brain regions together with gene-set enrichment analysis (GSEA) led to the identification of altered biological pathways preceding overt neurodegeneration; i.e., immune system, mitochondrial function and RNA metabolism (ncRNA processing, transcription, and translation). Targeted-based validation of specific identified candidate RNA species was performed in microdissected samples by quantitative real-time PCR. Overall, the transcriptomic profiles identified here may contribute to our understanding of selective vulnerability in PD and brain aging, and point to key biological pathways and molecular targets that are affected in prodromal and early PD stages.

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Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

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Program #/Poster #: 447.09

Topic: C.03. Parkinson's Disease

Title: Evaluation of Anti-Parkinson's activity of *Trachyspermum ammi* on Rotenone induced Parkinson's disease in *Drosophila melanogaster*.

Authors: *A. SHANKAR¹, V. DATE²;

¹Dept. of Pharmacol. & Therapeut., ²Seth GS Med. Col. & KEM Hosp., Mumbai, India

Abstract: Evaluation of Anti-Parkinson's activity of *Trachyspermum ammi* on Rotenone induced Parkinson's disease in *Drosophila melanogaster*. Arun Shankar Nair Institute- Department of Pharmacology & Therapeutics, Seth GS Medical College & KEM Hospital, Parel, Mumbai- 400012.

Background and rationale: The study was designed to validate the claims in Ayurveda regarding the efficacy of Ayurvedic drugs in neurodegenerative disorders. It was decided to conduct an efficacy study using a herbal drug which has anti-oxidationve effect. The herbal plant selected was *Trachyspermum ammi*. Parkinson's disease has symptom descriptions in Ayurveda and modern medicine. The latter offers only the symptomatic therapy by replacing dopamine, the neurotransmitter involved, but does not slow down or reverse the loss of dopaminergic neurons.**Methods:** Rotenone, in a final concentration of 125 μ M, was induced for inducing Parkinson's disease in *Drosophila melanogaster*. Table concentration of *Trachyspermum ammi* was selected on the basis of viability assays carried out in our lab. The group serving as negative control will not have the study drugs in the cornmeal medium and the flies from positive control will be fed with L-dopa dissolved in the medium in the concentration of 1 mM. The flies in the bottle will be maintained for a period of 7 days at 25°C. On the 8th day, they will be subjected to climbing assay. Malondialdehyde and Glutathione estimation will be carried out to check for the anti-oxidative properties of the drug. The brain tissue of *Drosophila melanogaster* will be dissected and be subjected to immunohistochemistry against Tyrosine hydroxylase.**Results:** Climbing assays showed a significant reduction in the climbing/ motor ability between the control and disease control groups. There was an improvement in the levels of MDA and GSH in the study group as compared to the disease control group. There was a significant improvement in the climbing ability of flies fed with l-dopa and study drug. Immunohistochemistry showed a significant reduction of dopaminergic neurons in the disease control group and a significant reduction in the loss of dopaminergic neurons in the study group. **Conclusion:** *Trachyspermum ammi* was effective in reducing the rotenone induced dopaminergic loss of neurons.**Ethics statement:** NA.**Acknowledgement:** I would like to acknowledge SAIF dept., IIT Mumbai and our lab members for their support.

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Poster

447. Parkinson's Disease Animal and Cellular Models

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Program #/Poster #: 447.10

Topic: C.03. Parkinson's Disease

Support: Aligning Science Across Parkinson's

Title: Intestinal *Citrobacter Rodentium* Infection In Pink1 Knockout Mice Leads To Regional Blood-Brain-Barrier Dysfunction

Authors: *S. MUKHERJEE¹, V. GROUZA², M. YAQUBI², A. EVEN³, A. TCHUNG³, M. TUZNIK², S. RECINTO², M.-J. BOURQUE³, P. ROSA-NETO², H. MCBRIDE², S. GRUENHEID², J. STRATTON², D. RUDKO², L.-E. TRUDEAU³;

¹Univ. de Montréal, Montreal, QC, Canada; ²McGill Univ., Montreal, QC, Canada; ³Univ. de Montréal, Montreal, QC, Canada

Abstract: Research in the past decade established a strong link between immune system activation and the development of Parkinson's disease (PD). In a recent study, we showed that repeated gastrointestinal infection with *Citrobacter rodentium* can lead to PD-like symptoms in Pink1 KO mice and to immune cell entry in the brain. Whether such mild infections are sufficient to increase blood brain barrier (BBB) permeability and to cause brain inflammation in unclear. Pink1 WT and KO mice were infected with *Citrobacter rodentium* and at days 13 and 26 post infection, we conducted gadolinium-enhanced MR imaging to identify signs of BBB permeability. We also quantified expression of endothelial tight junction proteins and dopamine metabolites along with investigating markers of brain inflammation. Using MRI, we obtained support for the hypothesis that increased blood-brain barrier breakdown in Pink1 KO infected mice occurs 26 days after infection in the striatum, dentate gyrus, and thalamus. This has been verified using sensitive statistical methods applied to the T1 relaxation time probability distributions in each anatomical ROI of the in vivo mouse brain images. We found no change in systemic inflammatory markers among control and infected mice, suggesting that no long-term peripheral inflammation persisted. However, preliminary data suggest that chronic microglial and astrocyte activation occurs at day 26 post infection. These results support the hypothesis that increased immune cell entry in the brain after gastro-intestinal infection may result in part from regional increases in BBB permeability. Further studies are required to examine the links between immune cell entry in the brain of these mice and the appearance of dopamine neuron dysfunction. Our observation of increased microglial activation suggests that a chronic state of brain inflammation could also mediate some of the dysfunctions observed in these mice.

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Poster

447. Parkinson's Disease Animal and Cellular Models

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Topic: C.03. Parkinson's Disease

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Ministry of Science and Innovation (MICINN), Spain (SAF2016-77541-R to N.P.)

Title: Brain-wide age-dependent neuromelanin accumulation in tyrosinase-expressing transgenic mice recapitulates multisystem motor and non-motor features of Parkinson's disease

Authors: A. LAGUNA¹, N. PEÑUELAS¹, M. GONZALEZ-SEPULVEDA¹, C. GUILLARD-SIRIEIX¹, A. NICOLAU¹, M. LORENTE¹, J. COMPTE¹, A. PARENT¹, T. CUADROS¹, J. ROMERO-GIMÉNEZ¹, L. MIQUEL-RIO², G. PUJOL¹, E. MARKIDI¹, B. RODRÍGUEZ-GALVÁN¹, L. GIMÉNEZ-LLORT³, A. BORTOLOZZI², I. CARBALLO-CARBAJAL¹, *M. VILA^{1,4,5};

¹Vall d'Hebron Res. Inst. (VHIR) - Ctr. for Networked Biomed. Res. on Neurodegenerative Dis. (CIBERNED) - Autonomous Univ. of Barcelona (UAB), Barcelona, Spain; ²Dept. of Neurochemistry and Neuropharmacology, IIBB-CSIC, August Pi i Sunyer Biomed. Res. Inst. (IDIBAPS) - Ctr. for Networked Biomed. Res. on Mental Hlth. (CIBERSAM), Barcelona, Spain; ³Dept. of Psychiatry and Forensic Medicine, Autonomous Univ. of Barcelona, Barcelona, Spain; ⁴Catalan Inst. for Res. and Advanced Studies (ICREA), Barcelona, Spain; ⁵Aligning Sci. Across Parkinson's (ASAP) Collaborative Res. Network, Chevy Chase, MD

Abstract: In Parkinson's disease (PD) there is a preferential degeneration of neuromelanin (NM)-containing neurons, especially neurons from the Substantia Nigra pars compacta (SNpc) and Locus Coeruleus (LC). However, NM pigmentation in the human brain is not limited to these areas but is also present, at varying degrees, in most catecholaminergic neuronal groups. We have previously shown that intracellular NM levels in SNpc may set a threshold for the initiation of PD in a rodent model based on the unilateral viral-vector mediated nigral overexpression of melanin-producing enzyme tyrosinase. Here, we generated a new transgenic NM-producing mouse model (tgNM) based on the tissue-specific constitutive expression of human tyrosinase under the tyrosine hydroxylase promoter that mimics the bilateral pigmentation within the whole human brain (i.e. catecholaminergic groups A1-A14) in order to assess the potential multisystem effects of NM accumulation. In parallel to progressive NM intracellular buildup, tgNM mice exhibited major PD features in an age-dependent manner,

including both motor and non-motor behavioral alterations, inclusion body formation, neuroinflammatory changes, neuronal degeneration in lower brainstem areas (i.e. medullary nuclei and LC) together with neuronal dysfunction in higher brainstem areas (i.e. SNpc and VTA). In addition to dopaminergic and noradrenergic deficits, these animals also exhibited alterations in cholinergic and serotonergic systems [i.e. Pedunculopontine Nucleus (PPN), Nucleus Basalis of Meynert (NBM), Dorsal Raphe (DR)], as reported in PD. Overall, we found that modelling human-like NM accumulation in mice leads to a progressive multisystem neuronal dysfunction and neurodegeneration impacting brain and body functions, which may be relevant to PD pathology and brain aging.

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Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.12

Topic: C.03. Parkinson's Disease

Title: The gut microbiome influences mitochondrial function and motor symptoms in α -synuclein overexpressing mice

Authors: *L. HECKE MORAIS¹, L. STILES⁴, M. FREEMAN², J. BOKTOR³, M. LADINSKI³, J. JONES³, F. GAO³, T.-F. CHOU³, J. TRINH⁵, S. MAZMANIAN¹; ¹Caltech, ²Div. of Biol. & Biol. Engin., ³Caltech, Pasadena, CA; ⁴UCLA Metabolomics Ctr., David Geffen Sch. of Med. at the Univ. of California, Los Angeles, CA; ⁵Inst. of Neurogenetics, Univ. of Lübeck, Lübeck, Germany

Abstract: Gut microbiome-brain interactions have been implicated in a wide range of neurological conditions, including Parkinson's disease (PD). Motor dysfunction in PD is primarily associated with the selective dysfunction and loss of nigrostriatal dopaminergic neurons, potentially due to their relatively high energetic demand in comparison to other neurons. Defects in mitochondrial function may underlie vulnerability to neurodegeneration through impaired cellular respiration and accumulation of oxygen reactive species. While the etiology of PD is incompletely understood, most cases are believed to have a strong environmental contribution. The gut microbiome is altered in PD stool samples compared to household or population controls. Accordingly, our group has previously demonstrated that the gut microbiome exacerbates motor deficits, promotes neuroinflammation and α -synuclein (α Syn) brain pathology in mice. Various metabolites produced by gut bacteria have the potential to modulate host metabolism, but a link between the microbiome and brain mitochondrial function

remains unknown. Using α -syn overexpressing (ASO), we investigated the influence of the microbiome on mitochondrial function and motor performance. Herein, we reveal that the presence of a microbiome alters mitochondrial morphology and mitochondrial complex I and II respiration in the mouse brain. Furthermore, striatal gene and protein expression patterns suggest a role for the microbiome in regulating mitochondrial protein metabolism and oxidative stress. Motor testing of mice with or without microbiomes uncovered associations with striatal oxidative stress and enhanced progression of PD-like symptoms. These data demonstrate that the microbiome influences mitochondrial functions in the brain of α Syn overexpressing mice, and suggest gut microbial deviations in humans may be environmental risks for PD.

Disclosures: L. Hecke Morais: None. L. Stiles: None. M. Freeman: None. J. Boktor: None. M. Ladinski: None. J. Jones: None. F. Gao: None. T. Chou: None. J. Trinh: None. S. Mazmanian: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.13

Topic: C.03. Parkinson's Disease

Support: Contera Pharma A/S

Title: Chemogenetic activation of microglia in the substantia nigra pars compacta causes parkinson's disease like symptoms

Authors: *M. M. NIELSEN¹, S. L. FRANDBSEN¹, B. C. LUZON¹, K. V. CHRISTENSEN², S. RASMUSSEN², A. A. JENSEN¹, A. M. KLAWONN¹;

¹Univ. of Copenhagen, Univ. of Copenhagen, Copenhagen, Denmark; ²Contera Pharma, Servier, Horsholm, Denmark

Abstract: Parkinson's Disease (PD) is the second most common neurodegenerative disorder and is primarily characterized by movement disturbances, loss of Dopaminergic neurons in the Substantia Nigra Pars Compacta (SNc), accumulation of alpha-Synuclein protein aggregates and neuroinflammation. Microglia is the immune cell of the brain and is more densely present in the SNc compared to neighboring brain regions. In this study, we aimed to investigate the role of microglia activation in the development of PD symptomatology in mice. To activate SNc microglia, we used a viral approach for expressing the stimulatory Gq Designer Receptor Exclusively Activated by Designer Drugs (DREADD) in 8-12 weeks old male Cx3cr1Cre mice. We tested different adeno-associated viral (AAV) strategies for transducing SNc microglia (AAV-DJ8, AAV-TM6, AAV9) and found that AAV9 was the most effective serotype, as visualized with fluorescent immunohistochemistry targeting microglia marker Iba1, Tyrosine Hydroxylase for dopaminergic neurons and red fluorescent reporter proteins visualized using confocal microscopy. Cx3cr1Cre mice underwent bilateral stereotaxic injections in the SNc with

AAV9-based vectors containing either the DREADDs-Gq construct or a fluorescent reporter control-construct. Three weeks after viral injections, the mice were evaluated for changes in motoric and affective measures on day 1, 4, 7 and 10 of microglia activation. Some of these behaviors can be viewed on the poster “Microglia activation in Substantia Nigra Pars Compacta of mice results in a similar Parkinson’s Disease like Phenotype as α -Synuclein overexpression”. Here are selected data demonstrating that acute (day 1) microglial activation causes PD-like motor impairments in mice. Mice received the DREADDs ligand Clozapine-N-Oxide (CNO) 2mg/kg i.p. 45 minutes prior to the behavioral assays. Acute microglia activation results in decreased baseline locomotion and amphetamine induced locomotion in a home-cage environment compared to wild type control animals. In line with this, microglia-DREADDs mice performed significantly worse on the rotarod compared to the control group, indicating decreased motor coordination. Acute microglial activation also significantly decreases effort-based and motivated movement such as marble burying and grooming in the splash test. These findings suggest that acute chemogenic activation of SNc microglia causes a distinct PD-like phenotype characterized by movement dysfunction.

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Poster

447. Parkinson's Disease Animal and Cellular Models

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Topic: C.03. Parkinson’s Disease

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Title: Low-dose, oral insecticide exposure impairs gastrointestinal function and disrupts nigrostriatal dopamine circuitry in mice

Authors: *A. C. WHITE¹, B. PFLUGER¹, L. D. BLACKMER-RAYNOLDS¹, T. C. CHEN¹, J. CHANG¹, S. D. KELLY¹, W. CAUDLE², T. R. SAMPSON¹;

¹Cell Biol., Emory Univ., Atlanta, GA; ²Envrn. Hlth., Emory Univ., Decatur, GA

Abstract: Pesticide exposure increases the risk of developing Parkinson’s disease (PD). PD is a progressive motor neurodegenerative disease attributable to the death of the dopamine (DA) neurons in the nigrostriatal dopamine pathway, resulting in gradual loss of motor function. Decades before motor symptom onset, some individuals with PD experience a range of

gastrointestinal (GI) disturbances. Emerging associations between PD, pesticide exposure, and GI abnormalities have led to the idea that some incidences of PD may be triggered from within the gut after oral pesticide exposure. Pyrethroids, a class of commonly used insecticides, target the nervous system and disrupt DA signaling pathways. Since ingestion is an important route of pyrethroid exposure, we predicted that low-dose oral exposure to the pyrethroid deltamethrin would induce GI dysfunction and disrupt nigrostriatal DA circuitry in mice. Wildtype mice of both sexes were orally gavaged once weekly with deltamethrin or vehicle control in a chronic exposure paradigm. GI function was assessed by measuring fecal output, fecal water content, total intestinal transit time, and microbiome composition measured by 16S rRNA sequencing. Motor function was assessed using a battery of behavioral assays including pole test, wire hang, sticker removal, and open field activity. Tyrosine hydroxylase (TH) and dopamine transporter (DAT) gene and protein levels were quantified in gut and brain tissues via qPCR and western blot to determine effects on the DA pathway. Our results indicate that oral deltamethrin exposure triggers intestinal behaviors indicative of constipation in males, but not females. Motor behaviors were altered in deltamethrin-treated mice of both sexes, and oral deltamethrin exposure significantly changed levels of proteins important for DA synthesis (TH) and reuptake (DAT) compared to controls. Together, these findings suggest that epidemiologically-relevant exposure to deltamethrin in adulthood leads to altered dopamine handling in the gut and brain, rendering the system more vulnerable to PD-like phenotypes.

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Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.15

Topic: C.03. Parkinson's Disease

Support: The Owens Foundation

Title: Retromer stabilization by molecular chaperone rescues Parkinson's disease phenotype

Authors: *S. ELEUTERI, T. ZHANG FANG, M. PERSICO, D. K. SIMON;
Neurol., Harvard Med. School, BIDMC, Boston, MA

Abstract: Retromer stabilization by molecular chaperone rescues Parkinson's disease phenotype

Simona Eleuteri¹, Tracy Shi Zhang Fang¹, Michele Persico¹, David K Simon¹

1. Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline avenue Boston, MA 02215 USA Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting 1% of people over 65. The loss of dopaminergic (DA) neurons in the *substantia nigra* (SN) resulting in degeneration of the nigrostriatal tract and

dopamine deficiency is a key pathological feature that contributes particularly to the motor features of PD. The neuropathological hallmark of PD is the occurrence of intracellular inclusions, Lewy bodies and Lewy neurites mainly consisting of aggregated α -Synuclein (α -Syn). VPS35 has been genetically associated with familial PD, and the VPS35-D620N mutation leads to late-onset autosomal dominant PD. The VPS35 gene encodes a component of the heteropentameric retromer complex, which mediates retrograde transport of cargo proteins from endosomes to the trans-Golgi network (TGN) and plasma membrane. VPS35 has a variety of functions within neurons, many of which are potentially relevant for the pathophysiology of PD; in particular, VPS35 controls: i) the regulation and maintenance of neuronal signaling events (i.e. downregulation of some receptors, synaptic plasticity, trafficking of proteins in dendritic spines), ii) mitochondrial dynamics (fusion and fission) and, iii) the accumulation and clearance of pathological forms of α -Synuclein. Previously we identified and patented a retromer stabilizer (**2a**), able to increase retromer-subunit levels by almost 2-fold in cell line and in the PD-related brain areas (*striatum* and *substantia nigra*). Here we validate the pharmacological approach by **2a** in a PD mouse model overexpressing unilaterally A53T- α -Syn by viral vector and we show that the administration of **2a** compound by daily IP for 100 days rescues behavioral defects and PD phenotype.

Disclosures: S. Eleuteri: None. T. Zhang Fang: None. M. Persico: None. D.K. Simon: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.16

Topic: C.03. Parkinson's Disease

Support: Aligning Science Across Parkinson's

Title: Comparison of the ability of the pathogen-associated molecular patterns LPS and Poly(I:C) to trigger Parkinson's disease-like pathology in Pink1 and Parkin KO mice

Authors: *A. EVEN, S. MUKHERJEE, S. PAQUEREAU-GABOREAU, M.-J. BOURQUE, A. TCHUNG, L.-E. TRUDEAU;
Univ. de Montréal, Montreal, QC, Canada

Abstract: Comparison of the ability of the pathogen-associated molecular patterns LPS and Poly(I:C) to trigger Parkinson's disease-like pathology in Pink1 and Parkin KO mice
Authors: Amandine EVEN^{1,3}, Sriparna MUKHERJEE^{1,3}, Soraya PAQUEREAU-GABOREAU^{1,3}, Marie-Josée BOURQUE^{1,3}, Alex TCHUNG^{1,3}, Louis-Eric TRUDEAU^{1,2,3,1}
Department of pharmacology and physiology, Faculty of medicine, Université de Montréal²
Department of neurosciences, Faculty of medicine, Université de Montréal³ SNC and CIRCA research groups

Disclosures: Amandine EVEN: None, Sriparna MUKHERJEE: None, Soraya PAQUEREAU--

GABOREAU: None, Marie-Josée BOURQUE: None, Alex TCHUNG: None, Louis-Eric TRUDEAU: None

Abstract : The main motor symptoms of Parkinson's disease (PD) are caused by the loss of dopamine (DA) neurons. Although growing evidence links inflammation to disease onset, the underlying mechanisms are unclear. An early onset form of PD is associated with mutations of Pink1 or Parkin, proteins involved in regulating mitophagy and repressing the presentation of mitochondrial antigens to the immune system. In Pink1^{-/-} mice, repeated infections with the intestinal bacterium *Citrobacter Rodentium* leads to the establishment of an autoimmune response, associated with a disruption of DA neuronal terminals and the late and transient onset of motor disorders. To identify strategies to simplify and enhance damage to the DA system in this model, we examined if the intestinal infection could be replaced by repeated injections of LPS and/or Poly(I:C), pathogen-associated molecular patterns mimicking bacterial or viral danger signals. Pink1^{-/-} or Parkin^{-/-} mice, aged 3 months, were injected once a week for 4 weeks with these agents or vehicle control and examined 3 months later. Initial results show that while repeated administration of LPS induces an adaptation, reflected by a temporary loss of body weight which attenuates with each injection, this was not the case with administration of Poly(I:C). Suggestive of an impairment in motor circuits in these mice, initial behavioral profiling performed blindly revealed the appearance of motor impairments 3 months after the last injection in Pink1^{-/-} or Parkin^{-/-} mice but not in WT controls. This was observed both for LPS or Poly(I:C) or after an alternate administration of both. An examination of the integrity of the DA system in these mice is ongoing, as is the respective contribution of innate or adaptive immune responses. We hope these experiments will contribute to the establishment of a simple and robust model in which inflammation and genetic mechanisms interact to promote the development of PD-related pathology.

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Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.17

Topic: C.03. Parkinson's Disease

Title: Innate regional and hemispheric differences of dopaminergic neurons in the substantia nigra of naïve adult rats

Authors: *Y. PI, R. DRISCOLL, C. EHRENFELS, R. MASSOL, M. ARNOLD;
Translational Biol., Biogen Inc, Cambridge, MA

Abstract: Rats are a common species used for Parkinson's disease (PD) studies and drug development but large variations in neuromotor behavior and total dopaminergic (DA) neurons in the substantia nigra (SN) are often encountered. We previously noticed substantial differences

in the density of DA neurons in some regions of the SN among different rats as well as between the left and right SN of individual adult Sprague Dawley rats. The goal of this study was to comprehensively evaluate DA neurons in the SN of each hemisphere of different rat strains and determine if similar variations exist across different strains. Methods: Following assessment of neurological behavior, brains from four strains of naïve adult rats, Fisher, Long Evans, Sprague Dawley, and Wistar, were sectioned. DA neurons and microglia were co-immunolabeled with anti-tyrosine hydroxylase (TH) and anti-Iba1 antibodies, respectively. TH+ neurons and Iba1+ microglia within each SN were quantified with a customized algorithm using the imaging software IMAB. Results: Fisher male rats exhibited the lowest neurological activities while Sprague Dawley, Long Evans, and Wistar behaved similarly. A lower number of TH+ neurons was observed on one side of the SN in all four strains. Regional lower density of TH+ neurons was often noticed within the same side of SN as compared to that in the contralateral side. Similar differences were also observed in female Sprague Dawley rats. Wistar rats showed the lowest incidence of regional and hemispheric differences in the density of TH+ neurons in the SN while Fisher rats exhibited the lowest number of TH+ neurons in the SN. Although there were some variations in the Iba1-positive cells between the two SN, similar symmetry of Iba1+ cell density was observed in all four strains. Taking those results together, regional and hemispheric differences in total TH+ neurons within SN are not concurrently associated with any change in microglial homeostasis but a result of developmental differences in the SN in those naïve rats. Conclusions: Regional and hemispheric differences in DA neurons occur in the SN of some naïve adult rats, suggesting the necessity of using stereology to quantify DA neurons throughout the entire SN to accurately assess potential treatment effects. Wistar rats exhibited the most symmetry of DA neurons between the left and right SN and would be superior to other strains for PD or dopamine-related disorder studies and preclinical drug development.

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Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.18

Topic: C.03. Parkinson's Disease

Support: North Carolina Funding

Title: Substantia nigra inflammation in a mouse model of COVID-19

Authors: *J. EELLS¹, S. M. AKULA², S. SRIRAMULA³;

¹Anat. & Cell Biol., ²Immunol. and Microbiology, ³Pharmacol. & Toxicology, East Carolina Univ. Sch. of Med., Greenville, NC

Abstract: Currently, SARS-CoV-2, the virus causing COVID-19, has infected an estimated 60% of the population in the United States. Although the long-term consequences of these infections are not known, one potential effect is an increased risk for developing neurodegenerative diseases like Parkinson's disease. To assess this risk, we have used transgenic mice with human angiotensin converting enzyme 2 expressed via the K18 promoter (K18-hACE2) infected with a low dose (4000 TCID₅₀) of SARS-CoV-2. We found that 20-30% of these mice develop severe disease. However, the rest of the mice survive and develop only a mild to moderate infection. In our previous experiments, using these mice recovered from mild/moderate SARS-CoV-2 infection (38d post infection), the combination of SARS-CoV-2 infection and MPTP induced significantly greater loss of nigrostriatal dopamine neurons and activation of microglia in the substantia nigra compared to either treatment alone. However, at this time point, no difference in microglia activation in the substantia nigra was observed following SARS-CoV-2 infection alone, however elevated microglia activation was observed in other areas of the brain. Our current hypothesis is that SARS-CoV-2 infection causes transient inflammation in the substantia nigra that damages dopamine neurons causing an increased sensitivity to the oxidative stress induced by MPTP. In this model, mice always develop severe disease within 10 days post infection. Therefore, to assess damage to dopamine neurons shortly after recovery from mild/moderate infection, K18-hACE2 mice were infected with a low dose (4000 TCID₅₀) of SARS-CoV-2 (n=10) or a sham infection (n=8) and euthanized 10 days later. Two mice developed severe disease and were euthanized. In the olfactory bulb or prefrontal cortex, virus antigens and replicating virus, based on dsRNA immunofluorescence, were observed in mice with severe disease but was not detected in mice following mild/moderate disease. This suggests that SARS-CoV-2 does not enter the brain in this moderate disease model and is not the cause of dopamine neuron damage. Continuing experiments will examine the presence of virus, microglia and astrocyte activation, and expression of inflammatory markers in the substantia nigra of these mice. Early damage to the substantia nigra following SARS-CoV-2 infection could account for a potential increased risk for Parkinson's disease. Due to the prevalence of SARS-CoV-2 infection, it is critical to understand how this virus affects the brain to prevent this damage.

Disclosures: J. Eells: None. S.M. Akula: None. S. Sriramula: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.19

Topic: C.03. Parkinson's Disease

Support: AG057931

Title: Gba1-l444p^{-/+} mice exhibit sleep abnormalities correlated to behavioral differences and, after sleep disruption, exhibit motor deficits

Authors: *J.-P. WIEGAND¹, R. D. BRINTON²;

¹Ctr. for Innovation in Brain Sci., ²Univ. of Arizona, Univ. of Arizona, Tucson, AZ

Abstract: Over 80% of REM sleep behavior disorder (RBD) diagnoses convert to Parkinson's Disease within 15 years. Mutations in the GBA1 gene are the predominant genetic risk factor for Parkinson's Disease. However, the role of sleep or GBA1 in disease progression is not fully understood. 16 male GBA1-L444P (8 GBA1-L444P^{-/-} and 8 GBA1-L444P^{+/-}) 6.58 ± .32mo mice were switched from 14:10 to 12:12 light cycle and subsequently recorded in piezoelectric sleep cages for 2 months (ending at 8.58mo ± .32mo). Significant differences were found in piezoelectric sleep measures, including higher percentages of sleep, sleep bout lengths, and diurnal wake ratios. This suggests that GBA1-L444P^{+/-} does confer sleep abnormalities. A follow-up study examined cognitive, motor, metabolic, and sleep outcomes in 7.79 ± 1.12mo wild-type (8m/6f) and heterozygous (5m/9f) GBA1-L444P mice (after transfer to 12:12 light cycle) through open field, gait analysis, body composition, fasting blood glucose, and sleep analysis via piezoelectric housing (2mo after light cycle transfer). Outcome parameters were analyzed using two-way sex by genotype ANOVAs. GBA1-L444P^{+/-} mice exhibited lower travel distance and speed than GBA1-L444P^{-/-} controls, though anxiety-like measures of center zone time and fecal boli counts were comparable. However, little to no difference was observed in gait analyses. Lower fasting blood glucose levels were observed in GBA1-L444P^{+/-} mice. Previously found significant differences in piezoelectric sleep measures were largely confirmed, including higher percentages of sleep and increased bout lengths in GBA1-L444P^{+/-} mice, however differences were reversed in diurnal wake ratios. Significant correlations between sleep measures and open field measures, combined with literature on the effects of circadian rhythm on behavioral performance, suggest that sleep impairments may drive behavioral differences in GBA1-L444P^{+/-} mice. These mice were then subjected to sleep disruption for 2 months (randomized vibration stimulus frequency: 50-80Hz, amplitude: .5, with a randomized duration: .5-2s, delayed 5s after sleep detection) and behaviorally tested again: significant differences were found in gait analyses via CatWalk in GBA1-L444P^{+/-} mice including maximum variation and hindpaw max contact. These results suggest that sleep dysfunction may underlie the initial stages of familial Parkinson's disease.

Disclosures: J. Wiegand: None. R.D. Brinton: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

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Program #/Poster #: 447.20

Title: WITHDRAWN

Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.21

Topic: C.03. Parkinson's Disease

Title: Characterization of IGF-1 receptor expression in the hemiparkinsonian rat brain

Authors: M. DEVINE¹, A. ULRICH², *C. KIESSLING²;

¹Neurosci., King's Col., Wilkes Barre, PA; ²King's Col., WILKES BARRE, PA

Abstract: Parkinson's disease (PD) is characterized by progressive loss of nigrostriatal dopamine (DA) neurons. Recent evidence shows peripheral hormones can modify DA neuron activity in the CNS. The insulin-like growth factor 1 (IGF-1) signaling pathway in particular is a major regulator of the aging process and has been implicated in the pathogenesis of PD. PD patients frequently show reduced IGF-1 serum levels compared to healthy controls; low IGF-1 levels early in PD are associated with worse disease progression. IGF-1 is neuroprotective for DA neurons. Midbrain DA neurons manufacture and release IGF-1, and DA-neuron derived IGF-1 mediates future synthesis and release of DA. Nigrostriatal DA loss seen in PD can be mimicked in rats using the toxin 6-hydroxydopamine (6-OHDA). Work in rats shows that infusions of IGF-1 prior to 6-OHDA reduces DA neuron loss, however little work has characterized the extent of changes seen in the IGF-1 system in late-stage models of the disease. Therefore, the current work characterized 6-OHDA-induced changes in IGF-1 receptor expression in a late-stage rat model of PD. Sprague-Dawley rats received sham or 6-OHDA infusions into the left medial forebrain bundle and were evaluated for motor deficits using the forepaw adjusting steps test prior to brain harvesting for western blot evaluation of the dopamine marker tyrosine hydroxylase (TH) and IGF-1 receptors in the striatum, prefrontal cortex, hippocampus, and hypothalamus. As expected, 6-OHDA produced severe stepping deficits and unilateral TH loss relative to sham lesions. IGF-1 receptor expression was reduced in the striatum, hippocampus, and hypothalamus, but did not significantly change in the prefrontal cortex. Reductions in IGF-1 receptor levels likely reflects the severe loss of midbrain DA neurons produced by 6-OHDA. While previous evidence suggests targeting the IGF-1 system may reduce the loss of DA neurons in PD, future work is warranted evaluating whether IGF-1 interventions remain a viable in late stages of the disease.

Disclosures: M. Devine: None. A. Ulrich: None. C. Kiessling: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

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Program #/Poster #: 447.22

Topic: C.03. Parkinson's Disease

Support: NSERC PGS-D

Title: Phenomic characterization of Orthologs of Parkinson's Disease-Associated Genes in *C. elegans*

Authors: *J. LIANG¹, C. H. RANKIN²;

¹Univ. of British Columbia Grad. Program In Neurosci., Univ. of British Columbia Grad. Program In Neurosci., Vancouver, BC, Canada; ²Univ. British Columbia, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Parkinson's Disease (PD) is a neurodegenerative disease of the central nervous system that is pathologically characterized by the death of dopaminergic (DA) neurons in the brain. Our current understanding of the genetic contributions of PD has been expanded by advances made in genome wide association studies (GWAS) in the past decade, but efforts in the functional characterization of newly identified risk loci have lagged behind the rate of new discoveries. To address this issue, I will establish a pipeline for *in vivo* characterization of orthologs of newly identified PD risk loci. *C. elegans* is ideal because: 1) *C. elegans* have orthologs to many PD-associated and biologically relevant genes. 2) We benefit from having access to repositories of *C. elegans* strains harbouring loss-of-function mutations in almost every gene in the nematode genome. 3) Our lab developed the **Multi-Worm Tracker (MWT)** for high-throughput characterization of behavioural and morphological phenotypes in populations of freely behaving animals carrying mutations in disease associated genes in real time and have proven its efficacy in a similar project targeting another neurological disease. Phenotyping strains with mutations in orthologs of PD-linked will yield unique phenotypic profiles for each disease-linked gene, and further analyses in similarity of these phenotypes may yield further insights on novel gene interactions or molecular pathways enriched involved in PD. A list of 180 mutant strains harbouring mutations in 83 *C. elegans* genes that are orthologous to 53 PD-linked genes, a majority of which are previously uncharacterized in biological and disease relevance will be phenotyped across an array of 30 behavioural and morphological features ranging from the Basal Slowing Response, a DA-dependent behaviour, and habituation, the simplest form of learning, to baseline morphology and behaviour including but not limited to locomotion speed and animal width and length. Based on the hypothesis that genes functioning in the same molecular pathway will lead to similar or strongly opposite phenotypic profiles when mutated compared to those that are not, I will perform hierarchical clustering of phenomic profiles to predict novel gene interactions and molecular pathways involved in PD. This research will characterize PD-linked genes in a dopamine-dependent behaviour, establish high-throughput genotype-to-phenotype characterization of newly identified risk genes for PD, and identify new functional interactions and gene networks to inform future disease modelling efforts and further our understanding of the biological processes underlying PD.

Disclosures: J. Liang: None. C.H. Rankin: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.23

Topic: C.03. Parkinson's Disease

Title: Intra-gastric administration of low dose rotenone post colitis accelerate brain neuropathology and motor impairment in Parkinson's disease

Authors: *N. SHARMA¹, H. KUMAR², A. KHAIRNAR¹;

¹Natl. Inst. of Pharmaceut. Educ. and Research-Ahmedabad, Gandhinagar, India; ²Dept. of Pharmacol. and toxicology, Natl. Inst. of Pharmaceut. Educ. and Res., Gandhinagar, India

Abstract: Background and Objective: Parkinson's disease (PD) etiopathogenesis is best explained by the aberrant buildup and characteristic spreading pattern of alpha-synuclein (α -Syn) aggregates from the gut to the brain, which is caused by gastrointestinal (GI) inflammation and local exposure to neurotoxin in the gut. This study was therefore designed to explore the exacerbation of proinflammatory intestinal milieu in a progressive mouse model of PD.

Methods: 10 months old C57BL/6 male mice were given 1% Dextran Sodium Sulphate (DSS) to induce chronic colitis. After colitis-induction, mice received intra-gastric rotenone (undetectable in blood or brain) for 8 weeks, followed by testing for Parkinsonian behavior and GI phenotypes of inflammation. At 8 weeks, intestine, brain stem, and midbrain tissue were isolated and examined for misfolded α -Syn, inflammatory markers, and dopaminergic neuronal loss in their synaptically connected nervous structures. **Results:** In 8 weeks, local rotenone exposure worsened disease severity with subsequent loss of barrier junction proteins (Zona occludens-1, Occludin, and Claudin-1) and raised pro-inflammatory gene expressions (TNF-alpha, IL-6, IL-1B, CCL2), as well as the substantial buildup of α -Syn in the colon. It also worsened motor impairment and α -Syn induced ChAT⁺ and TH⁺ neuronal death in the dorsal motor nucleus of the vagus (DMV) and the Locus coeruleus (LC). Furthermore, we found that colitis exacerbated rotenone-induced α -Syn pathology, which extended upward and resulted in dopaminergic neuron loss and astroglia activation in the substantia nigra and striatum. Interestingly, rotenone and DSS alone animals showed α -Syn driven neuronal loss limited to the DMV and Locus coeruleus, respectively. **Conclusions:** These results strongly suggest that long-term pesticide exposure in conjunction with a proinflammatory intestinal milieu can exacerbate the progression of PD.

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Poster

447. Parkinson's Disease Animal and Cellular Models

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Topic: C.03. Parkinson's Disease

Support: ECR Grant 787679
Michael J. Fox Foundation MJFF-019033

Title: Alpha-synuclein fibrils amplified from MSA- and PD-patient brain spread after intracerebral injection into mouse brain

Authors: *S. ZHANG¹, K. DAUER², T. STROHÄKER³, L. TATENHORST², L. CALDI GOMES¹, B. CHUL JUNG⁴, W. S. KIM⁵, S.-J. LEE⁴, S. BECKER⁶, F. LIESCHE-STARNECKER⁷, M. ZWECKSTETTER³, P. LINGOR⁸;

¹Tech. Univ. of Munich, Munich, Germany; ²Univ. Med. Ctr. Goettingen, Goettingen, Germany; ³German Ctr. for Neurodegenerative Dis., Goettingen, Germany; ⁴Neurosci. Res. Inst., Seoul Natl. Univ., Seoul, Korea, Republic of; ⁵The Univ. of Sydney, Camperdown, Australia; ⁶Max Planck Inst. for Multidisciplinary Sci., Goettingen, Germany; ⁸Dept. of Neurol., ⁷Klinikum rechts der Isar, Munich, Germany

Abstract: Parkinson's disease (PD) and multiple system atrophy (MSA) are both alpha-synucleinopathies with alpha-synuclein (α -syn) aggregation pathology. Different strains of α -syn possess unique properties and are suggested to cause distinct clinical and pathological manifestations even within the same disease entity. To study individual α -syn seeding and spreading patterns, we injected α -syn fibrils amplified from brain homogenates of two MSA patients and two PD patients into the brains of C57Bl6/J mice. In total, 39 mouse brains were analyzed after being divided into five groups: one control group (monomeric α -syn n=9) and four treatment groups (PD1 n=8, PD2 n=7, MSA1 n=7, MSA2 n=8). Animals were injected with 5 μ g of α -syn monomers or fibrils in 2 μ l solution at the age of 12 weeks into the right striatum and sacrificed at 90 days post-injection. Brains were cryosectioned and histologically analyzed in 6 different areas. Immunofluorescence staining showed significant differences in the amount of pS129- α -syn-positive signal with different levels of α -syn spreading between the five groups. Fibrils of one of the two MSA patients with a more aggressive disease course triggered the strongest α -syn pathology, followed by comparable pS129- α -syn induction by the other MSA and one PD patient material. We also detected an increased microglia activation by staining against Iba1 correlating with the load of pS129- α -syn aggregates. However, we did not observe differences in numbers of dopaminergic neurons in the substantia nigra pars compacta or varied co-localization of α -syn in oligodendrocytes between the different groups. Our finding that strong α -syn spreading was induced in the brains injected with α -syn fibrils amplified from MSA patient brain homogenate is in accordance with the rapidly progressive disease course and overall poor prognosis of MSA compared to PD. Together, our results highlight the seeding and spreading of α -syn pathology induced by α -syn fibrils from patient brain homogenates and provide evidence that heterogeneous spreading between different patients may be driven by individual patient immanent factors.

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Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.25

Topic: C.03. Parkinson's Disease

Title: *Tinospora cordifolia* improves oxidative stress-mediated mitochondrial dysfunction against rotenone-induced PD mice via Pink1/Parkin signaling pathway

Authors: *H. DILNASHIN¹, S. P. SINGH²;

¹BANARAS HINDU UNIVERSITY, Varanasi, India; ²Biochem., Banaras Hindu University/ Fac. of Sci., Varanasi, India

Abstract: *Tinospora cordifolia* improves oxidative stress-mediated mitochondrial dysfunction against rotenone-induced PD mice via Pink1/Parkin signaling pathway Hagera

Dilnashin*, Surya Pratap Singh Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi-221005 (U.P.), India.

Abstract Background: Neuroprotection targeting mitochondrial dysfunction has been proposed as an important therapeutic strategy for Parkinson's disease. *Tinospora cordifolia* has emerged as a novel medicinal plant that protects neurons from oxidative stress. In this study, we investigated the neuroprotective effects of *Tinospora cordifolia* and the underlying mechanisms in classic rotenone-induced Parkinsonism. **Material and Methods:** Mice were divided into four experimental groups: control, rotenone (2 mg/kg body wt., subcutaneous), *Tinospora cordifolia* extract (TCE, 200 mg/kg body wt., oral) + rotenone, and TCE only]. Mice were pre-treated with TCE for a week and then simultaneously injected with ROT for 35 days. **Results:** TCE administration significantly improved locomotor performance and increased tyrosine hydroxylase (TH) expression in the substantia nigra pars compacta (SNpc) of rotenone-intoxicated mice. Furthermore, TCE improved mitochondrial dysfunction via counteracting the decline in mitochondrial electron transport chain complex activity evoked by ROT. Similarly, TCE suppressed ROT-induced imbalance of Bax/Bcl-2 ratio and activation of caspase-3. Furthermore, TCE also significantly decreased the expressions of caspase-3, caspase-9, and increased pink1 and parkin expression. **Discussion and Conclusion:** The Bax/Bcl-2 ratio, mitochondrial dysfunction, and expression of caspase-3, and caspase-9 were seen to be significantly increased on rotenone intoxication. The expression of pink1 and parkin are also decreased in ROT group leading to mitochondrial dysfunction. However, TCE was potent in protecting the neurons against rotenone-induced cytotoxicity through the regulation of oxidative stress-mediated mitochondrial dysfunction and apoptosis in the mouse model of PD. Taken together, our results suggested that TCE attenuated rotenone-induced oxidative stress, through the regulation of mitochondrial functions. **Keywords:** *Tinospora cordifolia* extract; Parkinsonism; Mitochondrial Dysfunction; Apoptosis; Rotenone.

Disclosures: H. Dilnashin: None. S.P. Singh: None.

Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.26

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation

Title: Potential differential effects of the serotonin 5-HT_{2A/2C} receptor agonist DOI on prepulse inhibition in naïve and bilateral parkinsonian rats

Authors: *E. WHEELIS¹, K. ELDER¹, E. KLAYMAN¹, R. ASLAM², N. HUNT³, N. LIPARI¹, C. R. BISHOP¹;

¹Binghamton Univ., Binghamton, NY; ²Westchester Community Col., Valhalla, NY; ³Monroe Community Col., Rochester, NY

Abstract: Parkinson's Disease (PD) is characterized by the profound loss of nigrostriatal dopamine neurons, leading to motor dysfunction and various non-motor symptoms such as PD-associated psychosis (PDAP), some of which may be related to increased serotonin (5-HT) neurotransmission and stimulation of 5-HT_{2A} receptors. One feature of psychosis is a deficit in sensorimotor gating which can be tested with prepulse inhibition (PPI). Because some 5-HT_{2A}R antagonists and reverse agonists are known to reduce PDAP, we tested whether 2,5-Dimethoxy-4-iodoamphetamine (DOI), a potent serotonin 2A/2C receptor (5-HT_{2A/2C}R) agonist and known hallucinogen, differentially induces PPI deficits in rats. In experiment 1, in a within-subjects counterbalanced design, naïve male and female rats (N=8) underwent a dose-response curve for DOI (0.0, 0.25, 0.5, 1.0 mg/kg) and PPI was tested during each condition. Seventy trials were presented in a pseudo-random order of 10 prepulse trials presented alone for 3 conditions (70,75, 80 dB), and a startle stimulus (110 dB) presented alone for 10 trials and directly following each of the prepulses for 10 trials. Results showed a dose-dependent effect of lowering the startle amplitude (p = 0.0003), with the greatest reductions at 0.5 and 1.0 mg/kg DOI. There was a trend towards reduced PPI (p = 0.066), which indicates a modest hallucinogenic effect of DOI in naïve rats. Ongoing studies are examining whether 5-HT_{2A/2C}R stimulation with DOI can differentially compromise PPI and induce additional behavioral hallucinogenic reporters like head twitch responses in a bilateral 6-hydroxydopamine rat model of PD. Successful completion of this work will provide novel evidence for a key mechanism underlying PDAP pathogenesis and support the targeted treatment for this pervasive problem in PD.

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Poster

447. Parkinson's Disease Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 447.27

Topic: C.03. Parkinson's Disease

Title: Analysis of brain oxidative stress in an α -synucleinopathy animal model

Authors: *P. A. MARTÍNEZ¹, A. MORALES², D. M. FONG⁴, F. PEREZ³, L. O. SOTO⁵;
¹CELL BIOLOGY, Escuela Superior de Medicina, IPN, MEXICO CITY, Mexico;
³Neurofarmacología Mol. y Nanotecnología, ²Inst. Nacional de Neurología y Neurocirugía, Mexico, Mexico; ⁴Ctr. de Investigación y de Estudios Avanzados del IPN, SAN PEDRO ZACATENCO, Mexico; ⁵Edificio A4 lab 4, Facultad de Estudios Superiores Iztacala, Mexico city, Mexico

Abstract: "Analysis of brain oxidative stress in an α -synucleinopathy animal model "

Authors: **Paola A. Martínez-Gómez***, Adriana Morales-Martínez, Daniel Martínez-Fong, Francisca Pérez-Severiano, Luis O. Soto-Rojas.

Parkinson's disease (PD) is the most common α -synucleinopathy, characterized by misfolding of the α -synuclein (α -syn) protein, as well as its aggregation and spread in different brain nuclei. The mechanisms by which this misfolded protein could trigger neurodegeneration in several brain areas are still unclear. Recently, our working group developed an animal model by single intranigral administration of the neurotoxic β -Sitosterol- β -D-glucoside (BSSG), which develops α -synucleinopathy in neuroanatomical interconnected brain nuclei with the *substantia nigra pars compacta* (SNpc). We investigated whether a single intranigral BSSG administration (6 μ g BSSG/ μ L DMSO) in Wistar rats causes oxidative stress in certain brain nuclei that develop α -synucleinopathy. The study period comprised 15 to 60 days after the injury. Bilateral concentrations of reactive oxygen species (ROS) and lipid peroxidation (LP) of the brain nuclei of interest were determined: olfactory bulb (BO), SNpc, striatum, and hippocampus (Hi). Depending on the brain nucleus evaluated, different effects were found: 1) in both BO increased ROS and only LP on the ipsilateral side; 2) in the SN, ipsilateral increase in ROS and bilateral increase in LP; 3) in the striatum, increased ROS bilaterally, but not LP; 4) in Hi, bilateral increase in ROS and LP ipsilateral to the lesion. Our results show that the single and intranigral BSSG administration causes oxidative damage in brain nuclei that develop α -synucleinopathy. This approach will make it possible to identify pathological mechanisms associated with α -synucleinopathy and validate new therapies.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

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Topic: C.03. Parkinson's Disease

Support: NIH MH116003
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Title: Analysis of glutamate delta 1 receptor mediated intracellular signaling in the striatum

Authors: *P. CHETTIAR¹, D. T. MONAGHAN², S. M. DRAVID¹;
¹Creighton Univ., omaha, NE; ²Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Analysis of glutamate delta 1 receptor mediated intracellular signaling in the striatum

AuthorsP. CHETTIAR¹, D. T. MONAGHAN², S.M. DRAVID¹¹Department of Pharmacology and Neuroscience, Creighton University School of Medicine, USA; ²Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, USA.

DisclosuresP. CHETTIAR: None. S.M. DRAVID: None. D.T. MONAGHAN: None.

AbstractThe glutamate delta (GluD1 & GluD2) receptors form the delta family of ionotropic glutamate receptors. GluD1 is widely expressed in the cerebral cortex, striatum, hippocampus, nucleus accumbens, lateral septum, bed nucleus stria terminalis, lateral habenula, and central nucleus of the amygdala, and cerebellar cortex. In contrast, GluD2 is enriched at PF-PC synapse in cerebellum and is also expressed in the forebrain. GluD1 is involved in the organization and maintenance of both excitatory and inhibitory synapses by forming a transsynaptic bridge via cerebellin and neuroligin. GluD1 ablation results in deficits in social and emotional behaviors as well as altered learning and memory. Previously, we have demonstrated that deletion of GluD1 from the striatum results in impaired behavioral flexibility and repetitive behaviors. We also found that thalamostriatal dysfunction may underlie these deficits. The role of GluD1 in downstream signaling is however poorly understood. To better understand GluD1 mediated signaling we performed GST fusion protein tagged pull down assay. Among the pulldown proteins we identified several mitochondrial proteins suggesting relationship of GluD1 with mitochondrial function. These results are relevant to our recent ultrastructural analysis of GluD1 expression where we found that GluD1 expression was not only restricted to putative axo-dendritic and axo-spinous glutamatergic synapses but was also in intracellular location specifically localized to mitochondria. To further explore this finding, we conducted immunoprecipitation using GluD1 specific antibody as well as proteome analysis. Results suggest that GluD1 may play a role in mitochondrial function which may be relevant to neurological diseases that involve altered metabolic signaling.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 448.02

Topic: C.03. Parkinson's Disease

Support: 5 R01 NS101958
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Title: Estrogen-related receptor gamma modulation influences mitochondrial and lysosomal gene expression and vulnerability to synucleinopathy in dopaminergic neurons

Authors: S. N. FOX¹, L. J. MCMEEKIN⁷, C. H. SAVAGE², K. L. JOYCE¹, S. M. BOAS¹, M. S. SIMMONS⁷, L. PEREBOEVA³, C. B. FARMER⁴, J. RYAN⁸, K. BECKER⁹, J. AUWERX¹⁰, S. SUDARSHAN⁵, J. MA¹¹, A. LEE⁸, R. C. ROBERTS¹², D. K. CROSSMAN⁶, A. KRALLI¹³, *R. M. COWELL^{7,1};

¹Cell, Development, and Integrative Biol., ²Med., ³Pediatrics, ⁴Psychiatry & Behavioral Neurobio., ⁵Urology, ⁶Genet., Univ. of Alabama at Birmingham, Birmingham, AL; ⁷Neurosci., Southern Res., Birmingham, AL; ⁸Neuroinitiative, LLC, Jacksonville, FL; ⁹Ctr. for Neurodegenerative Sci., Van Andel Inst., Grand Rapids, MI; ¹⁰Swiss Federal Inst. of Technol., Lausanne, Switzerland; ¹¹Van Andel Res. Inst., Grand Rapids, MI; ¹²Psychiatry and Behavioral Neurobio., Univ. of Alabama, Birmingham, Birmingham, AL; ¹³Physiol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Many studies implicate mitochondrial dysfunction as a key contributor to cell loss in Parkinson Disease (PD). Previously published gene expression analyses from laser-captured dopaminergic (DAergic) neurons from the substantia nigra of patients with Lewy-body pathology revealed a deficiency in nuclear-encoded genes for mitochondrial respiration. The functional consequences of these changes and their role in disease progression and pathology are still unknown. The coordinated downregulation of a large number of nuclear-encoded mitochondrial genes suggests the disruption of a central regulator for mitochondrial gene expression. In fact, most affected genes are putative targets for the transcription factor estrogen-related receptor gamma (*Esrrg*/ERR γ), and several recent studies indicate that in addition to regulating mitochondrial genes, ERR γ can modulate dopaminergic phenotype and neurogenesis. Here, we determine the dependence of DAergic neurons on ERR γ for gene expression, survival, and regulation of motor function and explore how cell type-specific manipulation of ERR γ expression influences DAergic neuron vulnerability in the pre-formed fibril model of synucleinopathy. We demonstrate that deletion of ERR γ from adult DAergic neurons is sufficient to cause a levodopa-responsive PD-like phenotype with reductions in mitochondrial gene expression and number, that partial deficiency of ERR γ hastens synuclein-mediated toxicity, and that ERR γ overexpression reduces inclusion load, delays synuclein-mediated cell loss, and maintains striatal dopaminergic innervation. In contrast to adulthood deletion, developmental deletion caused a reduction in classic transcriptional markers for DAergic neurons without causing overt cell loss. While ERR γ deletion did not fully recapitulate the transcriptional alterations observed in postmortem tissue, it caused reductions in genes involved in synaptic and mitochondrial function and autophagy. Altogether, these experiments suggest that ERR γ -deficient mice could provide a model for understanding the regulation of transcription in DAergic neurons and that amplifying ERR γ -mediated transcriptional programs should be considered as a strategy to promote DAergic maintenance in PD.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

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Program #/Poster #: 448.03

Topic: C.03. Parkinson's Disease

Support: NIH/NIEHS ES027245
NIH/NINDS NS100090

Title: Mitochondrial neurotoxic stress impairs the nuclear pore complex: implications for Parkinson's disease pathogenesis

Authors: *Z. RIAZ¹, A. CHARLI², H. JIN¹, V. ANANTHARAM¹, A. KANTHASAMY¹, A. G. KANTHASAMY¹;

¹Physiol. and Pharmacol., Univ. of Georgia, Athens, GA; ²Biomed. Sci., Iowa State Univ., Ames, IA

Abstract: Environmental exposure to neurotoxic pesticides, especially the mitochondrial complex I inhibitor rotenone, is a key etiological factor in the development of sporadic Parkinson's disease (PD). However, the precise mechanisms driving neurotoxic pesticide-induced dopaminergic (DAergic) neurodegeneration are not well understood. Loss of the nucleocytoplasmic permeability barrier and disruption of the nuclear pore complex are emerging as crucial pathogenic mechanisms of neurodegenerative diseases. We recently found that mitochondria-impairing pesticides induce phosphorylation and loss of nuclear Lamin B1, thereby compromising nuclear membrane integrity. The involvement of channel-forming nucleoporins (NUPs) in mitochondrial dysfunction-related neurodegeneration has not been investigated. Here we characterize the alterations in NUPs in both rotenone-induced and transgenic mitochondrial dysfunction models of PD. Our immunoblot results show that rotenone exposure induced a significant loss of the scaffold and central channel NUPs 107 and 62 as well as a notable reduction in the nuclear basket NUPs 50 and 153 in N27 DAergic neuronal cells. To validate our findings on how NUP levels respond to rotenone-induced mitochondrial stress, we generated a CRISPR/Cas9-based stable mitochondrial transcription factor A (TFAM) knockdown DAergic cell culture model of mitochondrial dysfunction. The results were consistent with the rotenone-induced model. We then examined these NUPs in MitoPark transgenic mice, an *in vivo* model of mitochondrial impairment. Double immunohistochemical staining for TH and NUPs in substantia nigra sections revealed reduced NUP levels, with altered staining patterns, in the surviving TH neurons in MitoPark mice compared to age-matched littermate controls. Interestingly, mass spectrometry analysis of the phosphopeptide-enriched nuclear fractions from

rotenone-exposed and control N27 cells revealed that rotenone induces NUP153 hyperphosphorylation. This increase in NUP153 phosphorylation was also confirmed by immunoprecipitation and proximity ligation assays. To establish the functional relevance of mitochondrial stress-induced nuclear pore alteration, we are studying nucleocytoplasmic transport using a reporter system with both GFP-tagged nuclear export and RFP-tagged nuclear localization signals in a single construct (Martens et al., 2015). Collectively, our data offer new mechanistic insights into PD pathogenesis by identifying nuclear pore complex dysregulation as one potential mechanism for mitochondrial dysfunction-induced DAergic neuron loss.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

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Program #/Poster #: 448.04

Topic: C.03. Parkinson's Disease

Title: Dysregulation of lysosomal pathways induces mitochondrial signature, an early driver of pathogenesis in Parkinson's disease

Authors: *K. ENNIS, L. LEE, A. CHEONG, A. BLAZIER, F. RAPINO, D. OFENGEIM, N. HAGAN;
Sanofi, Cambridge, MA

Abstract: Parkinson's disease (PD) is a multifactorial complex neurodegenerative disease which represents a significant burden to affected individuals. Elucidating the genetic and environmental factors that contribute to disease pathophysiology is critical for developing effective disease-modifying therapies. Recent genome-wide association studies (GWAS) have identified risk loci harboring disease-susceptibility variants in addition to causative genes driving pathogenesis. A number of these causative genes have implicated both mitochondrial function (Parkin, DJ-1, and PINK1) and endo-lysosomal pathways (LRRK2 and GBA) as critical determinants of disease onset and progression. To explore the interplay of mitochondrial and lysosomal dysfunction in PD, a human tri-culture system consisting of iPSC derived motor neurons, microglia, and astrocytes, was developed to model the complex CNS micro-environment *in vitro*. These tri-cultures were then treated with α -synuclein pre-formed fibrils (PFFs) to simulate PD pathogenesis *in vitro*. Following PFF treatment, a robust signature of mitochondrial function was observed in the tri-culture after 4- and 24-hours as measured by RNA sequencing suggesting deficits in mitochondrial respiration may be an early driver of PD phenotypes. To investigate how lysosomal pathway dysregulation may contribute to this mitochondrial signature, we characterized mitochondrial respiration in PD-relevant dopaminergic neurons, in monoculture as well as in the tri-culture system. Specifically, iPSC-derived dopaminergic neurons harboring PD mutations linked to endo-lysosomal pathways such as SNCA (A53T), LRRK2 (G2019S), and

GBA (N370S) displayed significant alterations in mitochondrial respiration. Future efforts will investigate how deficits in PD- related mitochondrial genes (Parkin, DJ-1, and PINK1) disturb lysosomal function and influence disease progression. Taken together, PD- relevant mutations in endo-lysosomal genes notably alter mitochondrial respiration suggesting a complex interconnection between the mitochondria and lysosome which contributes to the pathogenesis of disease.

Disclosures: **K. Ennis:** A. Employment/Salary (full or part-time);; Sanofi. **L. Lee:** A. Employment/Salary (full or part-time);; Sanofi. **A. Cheong:** A. Employment/Salary (full or part-time);; Sanofi. **A. Blazier:** A. Employment/Salary (full or part-time);; Sanofi. **F. Rapino:** A. Employment/Salary (full or part-time);; Sanofi. **D. Ofengeim:** A. Employment/Salary (full or part-time);; Sanofi. **N. Hagan:** A. Employment/Salary (full or part-time);; Sanofi.

Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 448.05

Topic: C.03. Parkinson's Disease

Title: Mitochondrial dysfunction, an in vitro model of neurodegenerative disorders: a focus on multiple endpoints, humanisation, and drug discovery platforms to enable new therapeutic discovery

Authors: ***T. ROSENSTOCK**¹, **K. WHITE**^{1,2}, **T. PHILLIPS**¹, **S. THOMSON**¹, **N. MIRZA**¹; ¹Signature Discovery, Nottingham, United Kingdom; ²Sch. of Life Sci., Univ. of Nottingham, Nottingham, United Kingdom

Abstract: Mitochondrial dysfunction occurs in neurodegenerative disorders (NDs), and there is considerable R&D effort to develop treatments aimed at improving the function of these organelles. It is therefore important to have validated and reproducible measures of mitochondrial (dys)function, that can reliably inform basic disease understanding and R&D projects. Various aspects of mitochondrial function can go awry in NDs, and these might be differentially modulated by drugs. Therefore, it is desirable to be able to measure multiple endpoints in an *in vitro* model of mitochondrial (dys)function. To achieve these goals, we exposed SHSY5Y cells to Rotenone (mitochondrial complex I inhibitor), commonly used to chemically-induce Parkinson's disease (PD). We evaluated cell death, mitochondrial membrane potential (MMP), oxidative stress, Ca²⁺ homeostasis, and oxygen consumption rate (OCR), in addition to ATP, ADP, Pyruvate, Lactate, NAD⁺, and NADH levels. Moreover, we examined whether Cyclosporin A (CSA), Mn(III)tetrakis(4-benzoic acid)porphyrin Chloride (MnTBAP), or N-Acetyl Cysteine (NAC) could counteract Rotenone's effects. Exposure of SHSY5Y cells to Rotenone (24hrs) concentration-dependently increased cell death, with an IC₅₀ of 335 nM (p<0.001). Rotenone also concentration-dependently impaired MMP, oxidative stress, and Ca²⁺ homeostasis measured using specific fluorescent probes (TMRE, H2DCF-DA and Fluo-4-AM,

respectively). At 200nM Rotenone decreased OCR ($p < 0.05$), ATP, ADP, Pyruvate, Lactate and NAD⁺/NADH. Importantly, we demonstrated that CSA (1 and 10 μ M), MnTBAP (2 and 20 μ M), and NAC (100 μ M and 1mM) all reversed cell death ($p < 0.001$). The effects of these compounds on cell viability could be due to their reversal of Rotenone's effect on MMP, calcium homeostasis, oxidative stress, or a combination of these. We show that MnTBAP and NAC significantly reduced Rotenone-elicited oxidative stress. CSA seems to inhibit cell death primarily through changes in MMP, affecting consequently calcium and redox homeostasis. These data concur with previous reports, however the intention with the current data is to understand the specific mitochondrial endpoints differentially affected by drugs. Further, we are transferring this mitochondrial dysfunction model to human iPSC-derived neurons carrying known PD mutations and implementing a miniaturised 384-well plate format. This approach to humanising, miniaturizing, and automating assays will improve the translational value and speed of CNS-drug discovery in identifying novel drugs combined with a deeper understanding of their mechanism by measuring multiple biological endpoints.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 448.06

Topic: C.03. Parkinson's Disease

Title: Genome-wide CRISPR screen in human PARK2 deficient dopaminergic neurons identifies genes involved in alternative mitophagy

Authors: *T. NISHIMURA, H. L. LI, C. A. HIGHFILL, K. NAGASAWA, Y. KUNISADA, Y. TSUJIHATA;
Takeda Pharmaceut. Co., Kanagawa, Japan

Abstract: PARK2/Parkin is the gene responsible for the early-onset familial Parkinson's disease (PD). Patients with PARK2 mutation are characterized by the mitochondrial dysfunction and loss of dopaminergic neurons (DAn) in the substantia nigra. As PARK2 is a key component of mitophagy, the mitochondria-specific autophagy, defect in mitochondrial quality control by mitophagy is thought to be the main etiology of PD with PARK2 mutations. In this context, induction of PARK2-independent mitophagy to maintain mitochondrial health in the patients would be a beneficial therapeutic approach. To discover novel drug targets which address this compensatory mitophagy mechanism, we performed a whole genome CRISPR screening with PARK2-null human iPS cell-derived DAn. We established PARK2-null human iPS cells stably expressing Cas9 and differentiated them into DAn. Cell imaging-based, quantitative and high throughput screens with mito-SRAI, a novel mitophagy fluorescent probe, were utilized. We confirmed successful differentiation of those cells to DAn, Cas9-mediated knockout of target genes, and PARK2-dependent mitophagy deficit under mitochondrial stress condition. With these cells, we first discovered 167 candidate genes which induce PARK2-independent mitophagy (alternative mitophagy) specifically under mitochondrial stress condition. Some of those hit genes were causal genes of monogenic disorders associated with mitochondria, indicating the compensatory mitophagy induction in response to those genes deprivation. After prioritizing hit genes with such human genetics information, we further validated remaining hit genes with siRNAs and with cell survival assays. We found that gene knockdown of a few hit genes of them showed protective effects against paraquat-induced cell death in PARK2-null DAn. Those include genes which encode molecules accessible by small molecules drugs. These results indicate complexed relationship between human genes and PARK2-independent alternative mitophagy, and that there are potential therapeutic targets for PD patients with PARK2 mutation and other diseases with impaired alternative mitophagy.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 448.07

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS R21 NS121959-01A1

Title: Development of an adeno-associated virus (AAV)-based tool to ablate astrocytic mitochondria in pre-specified subregions of the adult mouse brain

Authors: *D. A. AYALA¹, B. SEABERG¹, T. E. HUNTINGTON^{2,1}, A. J. MATARAZZO¹, M. RIMER¹, R. SRINIVASAN^{1,2};

¹Neurosci. and Exptl. Therapeut., Texas A&M Univ. Sch. of Med., Bryan, TX; ²Neurosci. and Exptl. Therapeut., Texas A&M Univ. Inst. for Neurosci., Bryan, TX

Abstract: The generation of ATP by mitochondria is critical for normal functioning of neural circuits in the brain. In this regard, studies show that astrocytes contribute ~20% of the total energy required by the brain. In addition, we have shown that mitochondria in astrocytes possess robust Ca²⁺ signals required for ATP generation (Huntington and Srinivasan, 2021, Cell Calcium) and that in a mouse model of early Parkinson's disease, astrocytic mitochondria become functionally compromised (Huntington *et al.* poster). Thus, an important question is whether or not the loss of mitochondria only in astrocytes can accelerate neurodegenerative processes in the brain. In this study, we exploited the fact that mitochondrial DNA (mtDNA) in mice possess two unique sites for the restriction enzyme PstI, which flanks genes involved in the mitochondrial oxidative phosphorylation cascade. We rationalize that deletion of PstI-flanked mtDNA will enable us to ablate mitochondrial function in astrocytes within pre-specified brain regions and allow us to assess the consequences of this manipulation on neurons in specific brain regions that are prone to neurodegeneration. To do this, we created an adeno-associated virus (AAV) that expresses PstI tagged to a mitochondrial localization signal, and under control of the astrocyte specific promoter, GfaABC1D (Mito-PstI). We also created a control AAV expressing GFP targeted to astrocytic mitochondria (Mito-GFP). Mito-PstI and Mito-GFP were stereotaxically injected into the dorsolateral striatum (DLS) of mice. Brain sections obtained from these mice demonstrated specific expression of GFP in astrocytic mitochondria of the DLS. When compared with Mito-GFP control mice, we observed a significant depletion in mtDNA content and a specific deletion of the PstI-flanked mtDNA sequence in mice injected with Mito-PstI. These data suggest that Mito-PstI is a viable tool to ablate mtDNA in pre-specified brain regions. We are currently assessing the effect of Mito-PstI on mitochondrial morphology and dynamics as well as mitochondrial Ca²⁺ signals in astrocytes of the adult mouse brain.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 448.08

Topic: C.03. Parkinson's Disease

Support: Greehey Family Foundation Endowment for Alzheimer's Research
Zachry Family Foundation Endowment for Alzheimer's Disease

Title: Expression of cell-death related mRNAs in single cell dopaminergic neurons in primary culture during an excitotoxic challenge

Authors: *L. PADILLA, O. MALDONADO, G. A. DE ERAUSQUIN;
Glenn Biggs Inst. for Alzheimer's, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Mesencephalic dopaminergic neurons (DN) in primary culture are selectively susceptible to excitotoxic injury mediated by activation of the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) glutamate receptors, through a pathway requiring loss of intracellular calcium homeostasis, opening a mitochondrial permeability transition, nuclear translocation of nuclear factor kappa B (NFkB) and phosphorylation of the transcription factor p53 leading to programmed neuronal death. Dopaminergic neurons development is involved in diseases like Parkinson's and Schizophrenia, making the study of this process very important for a better understanding of the molecular basis of this diseases which could lead to new therapeutical approaches. We evaluated the expression of mRNA in single dopaminergic neurons during key times for commitment to die after an excitotoxicity challenge. Primary mesencephalic mouse neurons were exposed to 30 μ M or 100 μ M AMPA during 3 or 6 hours. Dopaminergic neurons were labeled with 5,7, dihydroxytryptamine (DHT) and manually picked live under a fluorescent microscopy. The dopaminergic phenotype was confirmed by the expression of TH and Pitx3 mRNA by qRT-PCR. 18 different genes for proteins previously found to mediate key steps in excitotoxicity-induced programmed cell death were measured at 3 h (early expression of death-related proteins) and 6 h (commitment to die). At 3 h, dose-dependent effect on the expression of GAPDH and Nr4a2 was detected. As expected, expression of the death signalling p53 was also elevated. In addition, AMPA exposure decreased expression of CACNG2, a regulatory subunit of AMPA receptor type 1; Grin2C, a subunit of an NMDA glutamatergic receptor; and Pitx3, a transcription factor that regulates the differentiation of dopaminergic neurons. These results show that the commitment to die in dopaminergic neurons during an excitotoxic challenge is a tightly regulated process involving key components of the phenotype-specific cell death pathway.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 448.09

Topic: C.03. Parkinson's Disease

Support: Science and Engineering Research Board- India

Title: Neuronal mitochondrial responses require IP₃R and Parkin for viability and flight.

Authors: *A. SHARMA, S. B. MANJILA, A. BHAN, G. HASAN;
Natl. Ctr. for Biol. Sciences, Tata Inst. of Fundamental Res., Tata Inst. of Fundamental Res.,
Bangalore, India

Abstract: Endoplasmic Reticulum (ER)-mitochondrial cross talk at Mitochondrial associated membranes (MAMs) is indispensable for neuronal health and physiology. It regulates mitochondrial quality and output, calcium homeostasis, and lipid metabolism. Such functions are dysregulated in neurodegenerative diseases like Parkinson's disease (PD), Alzheimer's diseases (AD) and Amyotrophic Lateral Sclerosis (ALS). We are interested in understanding interaction of IP₃R and Parkin protein in maintaining MAMs and cellular homeostasis for efficient neuronal function during ageing. The IP₃R is an ER- resident Ca²⁺ channel and an important component of intracellular Ca²⁺ signaling. It interacts with various proteins at MAMs and transfers Ca²⁺ to mitochondria through MAMs, required for mitochondrial function. Failure to do so usually leads to impaired Ca²⁺ homeostasis and mitochondrial dysfunction. If mitochondrial homeostasis is perturbed beyond repair, dysfunctional mitochondria are detected, removed, and replaced by PINK1 and Parkin proteins, mutations in which increase the chances of hereditary PD. Ageing imposes oxidative and metabolic stress on neuronal machinery, thereby compromising its function. Hence, ageing neurons require intricate balance of healthy mitochondrial function and removal of non-functional mitochondria for operating efficiently. In this study we tested if mutant IP₃Rs lead to dysfunctional mitochondria and thus impact PD -like progression in Parkin mutant *Drosophila*. Our initial observations demonstrate a strong genetic interaction between Parkin and IP₃R mutants. Cellular assays using genetical encoded fluorescent sensors showed decreased ER-mitochondrial calcium transfer in heteroallelic combination of IP₃R and Parkin mutants. It was accompanied by increased ROS and defects in mitophagy. Preliminary results, thus, show that IP₃R and Parkin interaction is required to maintain cellular homeostasis in ageing neurons. Further experiments are being done to explore cellular and molecular basis of this interaction and its effect on mitochondrial health.

Disclosures: A. Sharma: None. S.B. Manjila: None. A. Bhan: None. G. Hasan: None.

Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 448.10

Topic: C.03. Parkinson's Disease

Title: Determining Parkinson's Disease causing variants in PRKN using a pooled FACS-based screen

Authors: *A. J. GILSRUD, J. A. THAYER, D. P. NARENDRA;
Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's Disease. The characteristic motor symptoms in PD are due to degeneration of dopamine neurons within a region of the midbrain called the substantia nigra pars compacta. Approximately 500,000 Americans are diagnosed with PD. 5-10% of Parkinson's disease cases are caused by genetic mutations, with loss of function mutations in PRKN (coding for the protein

Parkin) being the most common cause of autosomal recessive Parkinson's disease. The pathogenicity of many *PRKN* missense variants, however, remains unknown. Indeed, we recently found that approximately 1% of individuals carry a rare *PRKN* missense variant of unknown significance, complicating genetic counseling (Zhu et al., in press). To resolve this uncertainty, we generated a pooled cDNA library of all rare Parkin missense variants in publicly available databases as well as alanine substitutions of critical Parkin residues (599 variants in total). The Parkin variants in the library were additionally tagged with YFP. Therefore, the effect of each mutation on Parkin stability can be determined. Finally, we developed a novel FACS based screening approach to resolve the functional status of each variant, using a single-cell reporter of Parkin function. As an initial assessment of feasibility, we single cell sorted the high and low Parkin activity populations and grew up the resulting single cell clones. As expected, all (3/3) of the high single-cell clones had Parkin function, whereas 60% (3/5) of the low function single-cell clones did not. From one of these clones, we identified a novel loss of function Parkin variant. We are now developing a next-generation sequencing approach to test the library as a pool to differentiate benign mutations from loss of function mutations. The structural relationship of these variants will be assessed by mapping them onto the solved structures of Parkin in its auto-inhibited and active states. We anticipate the results of this study will help resolve the functional consequence of *PRKN* variants in the population and additionally reveal novel structural requirements for Parkin activity.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 448.11

Topic: C.03. Parkinson's Disease

Title: Novel Parkin activators improve mitochondrial quality control through mitophagy induction in wild-type and mutant Parkin-expressing cells

Authors: *N. YAMAGUCHI, K. NAGASAWA, M. HOMMA, T. KOJIMA, M. TANAKA, T. HIOKI;

Neurosci. Drug Discovery Unit, Res., Takeda Pharmaceut. Co. Limited, Fujisawa, Japan

Abstract: Impaired mitochondrial quality control is a key pathogenic factor in Parkinson's disease (PD). Parkin RBR E3 ubiquitin protein ligase, encoded by *PRKN* plays an essential role in mitochondrial quality control by catalyzing the transfer of ubiquitin to its mitochondrial substrates to induce the clearance of damaged mitochondria from lysosomes (mitophagy). Most *PRKN* missense mutations in autosomal recessive juvenile PD, defined as familial PD, result in decreased E3 ubiquitin ligase activity, which leads to the accumulation of damaged mitochondria, cellular dysfunction, and cell death. Parkin E3 activity is also impaired in some idiopathic PD without *PRKN* mutations. Therefore, Parkin activation might be a new strategy to

ameliorate deficient mitochondrial quality control in familial and sporadic PD. We identified novel compounds during screening *in vitro* and defined them as Parkin activators. These activators enhanced the phosphorylated ubiquitination of mitofusin1 (Mfn1), a Parkin substrate, under mitochondria-depolarized conditions induced by protonophores in human neuroglioma H4 cells stably expressing wild-type Parkin. We also established a mitophagy assay system using H4 cells that transiently express wild-type or mutant Parkin. Mitophagy induction was significantly reduced in cells expressing mutant Parkin (R275W and P437L) compared with cells expressing wild-type Parkin. Parkin activators restored mitophagy deficits in H4 cells expressing mutant Parkin to levels in wild-type cells expressing Parkin only when incubated with a protonophore. Moreover, mitophagy in fibroblasts derived from a healthy person and a patient with Parkin R275W/Q was enhanced by Parkin activator T-828 (10 μ M) when accompanied by a protonophore. These results indicate that small-molecule Parkin activators correct ubiquitination and mitophagy deficits. This leads to amelioration of impaired mitochondrial quality control in familial and sporadic forms of PD.

Disclosures: **N. Yamaguchi:** A. Employment/Salary (full or part-time);; Takeda pharmaceutical Company Limited. **K. Nagasawa:** A. Employment/Salary (full or part-time);; Takeda Pharmaceutical Company Limited. **M. Homma:** A. Employment/Salary (full or part-time);; Takeda Pharmaceutical Company Limited. **T. Kojima:** A. Employment/Salary (full or part-time);; Takeda Pharmaceutical Company Limited. **M. Tanaka:** A. Employment/Salary (full or part-time);; Takeda Pharmaceutical Company Limited. **T. Hioki:** A. Employment/Salary (full or part-time);; Takeda Pharmaceutical Company Limited.

Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 448.12

Topic: C.03. Parkinson's Disease

Support: DoD U.S. Army NETRP Grant W81XWH18-00443

Title: Administration of the humanin-like mitochondrial peptides SHLP2 and SHLP2-K4R provides neuroprotection against the dopamine-depleting neurotoxin MPTP.

Authors: *M. JAKOWEC, D. PHILIPS, G. PETZINGER, P. COHEN, S. KIM;
USC, Los Angeles, CA

Abstract: Mitochondria play several important metabolic roles in neurons including the generation of ATP through oxidative phosphorylation, regulation of calcium flux, buffering reactive oxygen species (ROS), and regulating autophagy. Proteins responsible for these mechanisms are encoded by both mitochondria and nucleus. Recently, a number of small mitochondrial encoded peptides including humanin, SHLP2, and others have been identified that play key roles in mitochondrial homeostasis. In fact, epidemiological studies have shown that an

allele of SHLP2 (encoding and isoform with the substitution K4R) provides a degree of protection from the onset of Parkinson's disease (PD). Furthermore, molecular studies indicate that SHLP2 and its related alleles interact with Complex I of the electron transport system and may enhance specific aspects of metabolism. Since many neurodegenerative disorders, like PD, demonstrate mitochondria dysbiosis and neuroenergetic dysfunction we set out to explore if SHLP2 and its K4R isoform could provide neuroprotection or neurorestoration in a rodent model of mitochondria dysfunction. The dopamine-depleting neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) selectively targets Complex I of mitochondria in dopaminergic midbrain neurons leading to the depletion of ATP, elevated ROS, and cell death. Daily single intraperitoneal injection of SHLP2 or SHLP2-K4R (3.2 mg/kg) to C57BL/6 mice one week prior to the administration of MPTP (single injection 20 mg/kg free-base) showed near complete neuroprotection based on measurement of the levels of striatal dopamine and expression of striatal tyrosine hydroxylase. To explore the potential role of these mitochondria proteins in providing neurorestoration mice were administered SHLP2 or SHLP2-K4R starting 5 days after MPTP-administration when toxin-induced cell death is complete. Mice were evaluated weekly for 4 weeks in motor (rotarod, open field) and cognitive (novel object recognition) behaviors. Behavioral tests indicated that SHLP2-K4R provided restoration of behavioral deficits. Molecular analyses were carried out to examine changes in dopamine neurotransmission and synaptogenesis within the basal ganglia. Findings from these studies suggest that pharmacological targeting of mitochondrial function through small-mitochondrial derived peptides may provide both neuroprotective and neurorestorative benefits in models of dopamine depletion and cell death.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 448.13

Topic: C.03. Parkinson's Disease

Support: NIH ES031124

Title: Environmentally relevant oral manganese exposure, mitochondrial bioenergetics, and behavior in mice.

Authors: *J. K. LEPP¹, P. T. KANG², K. M. CROUCHER^{1,3}, S. M. FLEMING^{1,3};
¹Pharmaceut. Sci., ²Integrative Med. Sci., Northeast Ohio Med. Univ., Rootstown, OH; ³Biomed. Sci. Grad. Program, Kent State Univ., Kent, OH

Abstract: Environmentally relevant oral manganese exposure, mitochondrial bioenergetics, and behavior in mice.

Josephine K. Lepp¹, Patrick T. Kang², Kristina M. Croucher^{1,3}, Sheila M. Fleming^{1,3}
¹Department of Pharmaceutical Sciences, ²Department of Integrative Medical Sciences,
Northeast Ohio Medical University, Rootstown, OH, ³Biomedical Sciences Graduate Program,
Kent State University, Kent, OH

Although Manganese (Mn) is an essential metal, excessive exposure to Mn can cause manganism, an age-related neurodegenerative condition. Manganism presents as a neurological syndrome with motor, neuropsychiatric, and cognitive abnormalities. Mn preferentially accumulates in the basal ganglia and intracellular Mn toxicity involves multiple mechanisms known to also be involved in other neurodegenerative conditions including mitochondrial dysfunction. The present study sought to determine the effect of an environmentally relevant regimen of Mn exposure on mitochondrial bioenergetics and behavior in mice. C57BL/6 mice received water (Ctrl) or 10mg/kg/day of MnCl₂ in water (Mn) for 10 months. Sensorimotor function, emotional reactivity, and cognitive function were measured. Striatum and cerebellum mitochondria were isolated and oxygen consumption rate (OCR) in response to substrates was measured in triplicate using a Seahorse XFp flux analyzer. In the presence of energetic substrates (glutamate, malate, and succinate), the OCR of mitochondria (5 µg/well) was sequentially measured after ADP, Oligomycin, FCCP, and Rotenone/Antimycin A injection. Behavioral analysis revealed Mn-treated mice showed alterations in emotional reactivity compared to controls. In the striatum but not cerebellum, the respiratory control ratio (RCR; State 3, ADP-stimulated/State 4_o, oligomycin-inhibited rates) was significantly different between Ctrl and Mn mice (Ctrl vs. Mn = 5.0±2.4 vs. 2.5±0.8, *n* = 6, *p* = 0.030). There was no significant difference between Ctrl and Mn mitochondrial State 3 respiration (39.1±7.8 vs. 37.9±5.6, *n* = 6), and the reduction of RCR was attributed to the increased State 4_o (Ctrl vs. Mn = 9.32±4.03 vs. 16.29±4.96 pmol O₂/min/µg protein, *n* = 6, *p* = 0.016). In conclusion, the data indicate chronic low dose manganese affects neuropsychiatric function and impairs striatal mitochondrial integrity via elevated State 4_o respiration.

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Poster

448. Parkinson's Disease Mitochondria Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 448.14

Topic: C.03. Parkinson's Disease

Title: Alterations in astrocytic mitochondria in the striatum of an AAV-alpha-synuclein model of early Parkinson's Disease

Authors: *T. E. HUNTINGTON, C. POLO, M. VALLE, R. SRINIVASAN;
Neurosci. & Exptl. Therapeut., Texas A&M Univ., Bryan, TX

Abstract: Parkinson's disease (PD) is caused by the loss of dopaminergic (DA) neurons in the nigrostriatal pathway. The dying back of dopaminergic axons terminating in the dorsal lateral striatum (DLS) is thought to be an early feature of PD, and this occurs prior to the onset of DA neuron loss in the substantia nigra pars compacta (SNc). One of the multi-faceted reasons for the dying back of axons may be a change in energy homeostasis within this region. While dysfunction in astrocytes and brain mitochondria have separately been tied to PD, astrocytic mitochondria in the DLS have not been studied in the context of PD. Here, we study astrocytic mitochondrial dysfunction in the DLS in a mouse model of early PD. To create this model, we performed unilateral stereotaxic injection of adeno-associated virus (AAV) overexpressing human alpha-synuclein (α -syn) in the substantia nigra pars compacta (SNc). At 5 months post AAV- α -syn injection, we did not observe a significant loss of SNc DA neurons in α -syn mice when compared to control mice, as measured by tyrosine hydroxylase expression. However, α -syn mice displayed significant increases in dopamine and major metabolite concentration in the DLS compared to control counterparts, which is thought to be a feature of early PD. When compared to mCherry overexpressing control mice, following 5 months of α -syn overexpression, we observed a significant increase in the duration of spontaneous calcium influx into DLS astrocytic mitochondria, along with significant changes in mitochondrial morphology and mitochondrial respiration. Given the well-established sex differences observed in clinical PD, we also assessed our model for any sex specific changes in astrocytic mitochondria. We found significant differences in astrocytic mitochondrial calcium fluxes and mitochondrial network morphology in the DLS of female α -syn mice when compared to male α -syn mice. Together these data suggest that significant pathological alterations in astrocytic mitochondria occur in an early model of PD and the underlying dysfunctions could be sex specific.

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Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 449.01

Topic: C.03. Parkinson's Disease

Support: AptaBio Therapeutics Inc (F7097)
NIH-R15N PI: Kim; 1R15NS121784

Title: A novel NOX inhibitor alleviates Parkinson's disease pathology in PFF-injected mice.

Authors: *K. OFORI¹, A. GHOSH¹, D. VERMA¹, G. CABRERA¹, D. WHEELER¹, S. MOON², S. LEE², Y. KIM¹;

¹Delaware State Univ., Dover, DE; ²AptaBio Therapeut. Inc, Heungdeok IT valley, Korea, Democratic People's Republic of

Abstract: Parkinson's disease (PD) is the most prevalent motor neurodegenerative disorder, resulting from the decline of dopaminergic neurons in the midbrain. Although numerous genetic mutations have been identified in PD pathology, a key pathological hallmark is mis-folded protein aggregation called Lewy body formation. Thus, it has been emphasized to develop therapeutics to halt the Lewy body formation to prevent dopaminergic neuronal loss in the nigrostriatal pathway. Despite the causes in up-stream, oxidative stress-mediated damage in down-stream often underlines PD pathology. Therefore, ultimately regulating oxidative stress can be an effective approach to prevent PD pathological progress. Here we assessed the efficacy of an exogenous reactive oxygen species (ROS) regulator, nicotinamide adenine phosphate (NADPH) oxidase (NOX) inhibitor, compound-11 which was synthesized by Aptabio Therapeutics. The compound is a safe and specific inhibitor for NOX-1, 2 and 4, based on our preliminary assessments. Using rat dopaminergic cells and alpha-synuclein preformed fibrils (PFF)-injected mouse model, we tested the novel NOX inhibitor as a potential therapeutic for PD. PFF is known to be a pathogenic form of alpha-synuclein leading to rapid protein aggregation for recapitulating PD pathology. In our *in vitro* assays, the novel compound enhanced cell viability and reduced cytotoxicity against PFF exposure at a wide range of concentrations (1 nM-10 μ M), but we confirmed that 1 μ M was an optimal concentration *in vitro*. We also found a significant reduction in ROS and protein aggregation in Thioflavin-T stain with the compound treatment in N27 cells. After 7-8 weeks of oral treatment (5 or 25 mg/kg), starting 3 months post-PFF injection using 12-month-old mice, we found that both doses of the compound treated mice (n=7/group) showed a significant reduction in motor deficits assessed by behavioral assays, such as grooming, nesting, rotarod, hindlimb clasp and pole test, in a blinded assessment. Further, in immunohistochemistry, the treatment reduced the level of protein aggregation and prevented or reversed dopaminergic neuronal loss in the striatum and Substantia Nigra, suggesting that the inhibition of NOX can be a viable option for developing potential therapeutics for PD.

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Poster

449. Parkinson's Disease Animal Models

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Program #/Poster #: 449.02

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1ZIAAG000936

Title: Restoring neuronal p38 γ MAPK by p38 α MAPK inhibition ameliorates synaptic degeneration in a mouse model of DLB/PD

Authors: *M. IBA¹, C. KIM¹, S. KWON¹, M. SZABO¹, C. PEER³, X. REED¹, R. A. RISSMAN⁴, M. R. COOKSON¹, C. OVERK⁴, W. WRASIDLO⁴, E. MASLIAH^{1,2};

¹Lab. of Neurogenetics, ²Div. of Neurosci., Natl. Inst. on Aging, Natl. Inst. of Hlth., Bethesda, MD; ³Clin. Pharmacol. Program, Natl. Cancer Institute, Natl. Inst. of Hlth., Bethesda, MD; ⁴Dept. of Neurosciences, UCSD Sch. of Med., La Jolla, CA

Abstract: Aggregation of presynaptic protein, alpha-synuclein (α -syn) is a hallmark of neurodegenerative diseases such as Parkinson's disease (PD) or dementia with Lewy bodies (DLB). We previously reported that p38 γ , one of mitogen activated protein kinases (MAPKs) are mis-localized in neuronal cell bodies in DLB patients and a DLB/PD mouse model although p38 γ locates in peripheral dendritic regions of the cells in healthy control or non-transgenic littermates mice (Iba et al., 2020). Activation of p38 α and mis-localization of p38 γ are associated with neuroinflammation and synaptic degeneration in DLB/PD, respectively. We hypothesized that p38 α might modulate the distribution of p38 γ , and inducing synaptic degeneration in models of DLB/PD. To investigate this hypothesis, we treated DLB/PD models in vivo and in vitro with SKF86002 which is a compound that attenuates inflammation by inhibiting p38 α/β and investigated the effects on p38 γ and neurodegenerative pathology. We found that inhibition of p38 α reduced neuroinflammation, ameliorated synaptic function, protected neurodegeneration, and improved motor function in the α -syn transgenic (tg) mice. Moreover, this compound promoted the re-distribution of p38 γ to the synapse and reduced the accumulation of α -syn in α -syn tg mice. Further, we found that in iPSC derived human neural cultures from familial PD (A53T α -syn), this compound reduced the accumulation of α -syn and promoted the re-distribution of p38 γ in neurons. p38 α inhibition ameliorated α -syn-induced neurodegeneration only when microglia were pre-treated with SKF86002, although direct treatment of SKF86002 to neurons did not affect to α -syn-induced neurotoxicity, which suggest SKF86002 treatment only inhibits microglial neurotoxicity. These results support that reducing neuroinflammation by targeting p38 α is a promising therapeutic approach to DLB and PD because it's effects on p38 γ MAPK and synaptic stability. We provide a novel pathogenic mechanism for initiation of synaptic degeneration in DLB and PD which includes the pathogenic crosstalk between microglial neuroinflammation and synaptic neurodegeneration via p38 α /p38 γ MAPK respectively.

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Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 449.03

Topic: C.03. Parkinson's Disease

Support: MWU College of Graduate Studies
MWU Arizona College of Osteopathic Medicine

Title: Folic Acid supplementation to parkin-null *Drosophila* improves lifespan, climbing behavior, and transiently reduces vulnerable dopaminergic neuron mitochondrial hydrogen peroxide levels and glutathione redox equilibrium.

Authors: *A. N. JUBA¹, P. KEOSEYAN², K. HOULIHAN¹, A. BABAOGHLI², J. NORRIS², M. VOSS², L. M. BUHLMAN¹;

¹Biomed. Sci., ²Arizona Col. of Osteo. Med., Midwestern Univ., Glendale, AZ

Abstract: Parkinson's disease is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta, leading to progressive decline in controlling coordinated movements. The dopaminergic protocerebral posterior lateral 1 (PPL1) region of the *Drosophila melanogaster* brain is functionally homologous to the substantia nigra, and loss-of-function *park*²⁵ (*Drosophila* ortholog of the human *PRKN* gene) mutants also lose PPL1 neurons as they age. We have previously shown that *park*²⁵ mutants exhibit swollen, fragmented PPL1 mitochondria subject to increased aging and transient decreases in mitophagy initiation. Glutathione, a mitochondrial antioxidant, acts as an important mediator of reactive oxygen species, which cause cell damage when unregulated. Parkinson's patients exhibit reduced glutathione levels in the substantia nigra with increasing evidence suggesting oxidative stress plays a role in both idiopathic and genetic cases leading to cellular dysfunction. We aimed to address the detrimental effects of parkin loss of function by increasing cysteine, the limiting component of glutathione synthesis, by supplementing the diet with folic acid (FA). Control (*park*^{+/+}) and *park* loss-of-function flies (*park*^{-/-}) were raised on food supplemented with 50 μM FA or vehicle. FA-mediated increase in lifespan was detected after placing newly eclosed flies on treated food and counting them until all expired. Climbing behavior was performed at day 10 where individual flies were placed into a *Drosophila* Activity Monitor and climbing attempts representing motivated behavior and average height climbed were recorded. Both metrics were increased by FA supplementation. Finally, brains were dissected from *park*^{+/+} and *park*^{-/-} flies expressing mitochondrially targeted redox-sensitive probes that detect glutathione equilibrium (mitoGrx1roGFP2) or hydrogen peroxide levels (mitoOrp1roGFP2) on days 5 and 20. When oxidized, roGFP maximal excitation shifts from 488 to 405 nm. Z-stacks of roGFPs in oxidized and non-oxidized confirmations within one PPL1 region per brain were captured and analyzed by taking a ratio of the total volume of oxidized to non-oxidized reporter with higher ratios indicating an imbalance in glutathione equilibrium and increased hydrogen peroxide levels respectively. While FA supplementation increased the median lifespan and climbing behavior and initially decreased hydrogen peroxide levels and glutathione equilibrium in PPL1 mitochondria of *park*-null flies, by day 20 the improvement was lost, suggesting FA treatment may delay pathology.

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Poster

449. Parkinson's Disease Animal Models

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Program #/Poster #: 449.04

Topic: C.03. Parkinson's Disease

Support: Georgetown University funding to Charbel Moussa

Title: A novel Ubiquitin Specific Protease-13 inhibitor reduces alpha-synuclein in models of Parkinson's disease

Authors: *X. LIU¹, K. BALARAMAN², C. C. LYNCH², M. HEBRON¹, M. STEVENSON¹, C. WOLF², C. MOUSSA¹;

¹Dept. of Neurology, Translational Neurotherapeutics Program, Lab. for Dementia and Parkins,

²Dept. of Chemistry, Georgetown Univ. & Medicinal Chem. Shared Resource, Georgetown Univ. Med. Ctr., Washington DC, DC

Abstract: Ubiquitin Specific Protease-13 (USP13) promotes protein de-ubiquitination. A novel small molecule inhibitor of USP13, named BK50118-C, increases proteasome activity and clears alpha-synuclein *in vitro*. BK50118-C displayed a favorable therapeutic dose range *in vivo*. Expression of lentiviral human alpha-synuclein in USP13 deficient mice showed motor and behavioral defects in wild type (USP13^{+/+}) but not in partially (USP13^{+/-}) or completely (USP13^{-/-}) deficient USP13 mice. Treatment with BK50118-C once daily for 4 consecutive weeks resulted in a significant reduction of ubiquitinated alpha-synuclein, an increase in dopamine levels and an improvement of motor and behavioral symptoms. These data suggest that USP13 is critical to the neuropathology of alpha-synuclein, whereas a novel small molecule inhibitor of USP13 is a potential therapeutic agent of alpha-synucleinopathies.

Disclosures: X. Liu: None. K. Balaraman: Other; Inventor on issued US Georgetown University patent to use USP13 inhibitors as a treatment for neurodegenerative diseases. C.C. Lynch: None. M. Hebron: None. M. Stevenson: None. C. Wolf: Other; Inventor on issued US Georgetown University patent to use USP13 inhibitors as a treatment for neurodegenerative diseases. C. Moussa: Other; Inventor on issued US Georgetown University patent to use USP13 inhibitors as a treatment for neurodegenerative diseases.

Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 449.05

Topic: C.03. Parkinson's Disease

Support: This study was funded by Contera Pharma A/S.

Title: Synergistic effect of serotonin 1A and serotonin 1B/D receptor agonists in the treatment of L-DOPA-induced dyskinesia in 6-hydroxydopamine-lesioned rats

Authors: M. THOMSEN¹, *A. STOICA², K. CHRISTENSEN², T. FRYLAND², J. D. MIKKELSEN³, J. HANSEN⁴;

¹Mikael Soendergaard Thomsen Aps, Hvidovre, Denmark; ²Contera Pharma A/S, Hørsholm, Denmark; ³Inst. of Neurosci., Univ. of Copenhagen, Copenhagen, Denmark; ⁴Preo Pharma ApS, Copenhagen, Denmark

Abstract: Treatment with L-DOPA, currently the gold standard for symptomatic relief of Parkinson's disease (PD) in late-stage patients, often causes motor complications such as L-DOPA-induced dyskinesia (LID). Extended-release amantadine (Gocovri™) is the only approved therapy for dyskinesia in Parkinson's patients on the American market but is associated with neurological adverse effects and limited efficacy. Therefore, a high unmet need for addressing LID in Parkinson's disease patients worldwide still remains. The present study aimed to compare the serotonin receptor 1A (5-HT1A) and 5-HT1B/D agonists buspirone and zolmitriptan to amantadine in the 6-hydroxydopamine unilaterally lesioned rat model for PD. The hemi-parkinsonian 6-OHDA lesioned rats were induced dyskinesia by chronic treatment with L-DOPA and were subsequently used for efficacy testing of multiple drugs, measured as abnormal involuntary movement (AIM) scores after L-DOPA challenge. Safety pharmacology studies consisted of the rotarod, open field and forelimb adjusting tests, performed in naïve and model rats. The results revealed a significant reduction of AIM scores by the 5-HT1A and 5-HT1B/D agonists buspirone and zolmitriptan, and indirectly pointed to a putative mechanism of action. The anti-dyskinetic efficacy of buspirone and zolmitriptan was synergistic in nature and sub-chronic administration did not lead to tolerance development. The drug combination was safe and did not interfere with the effectiveness of L-DOPA treatment in hemi-parkinsonian rats. Head-to-head comparison with amantadine showed superior performance of buspirone and zolmitriptan in this rat model. The robust anti-dyskinetic effect resulting from combined 5-HT1A and 5-HT1B/D agonism qualifies buspirone and zolmitriptan as a promising treatment for LID in Parkinson's disease.

Disclosures: **M. Thomsen:** F. Consulting Fees (e.g., advisory boards); Contera Pharma A/S. **A. Stoica:** A. Employment/Salary (full or part-time); Contera Pharma A/S. **K. Christensen:** A. Employment/Salary (full or part-time); Contera Pharma A/S. **T. Fryland:** A. Employment/Salary (full or part-time); Contera Pharma A/S. **J.D. Mikkelsen:** F. Consulting Fees (e.g., advisory boards); Contera Pharma A/S. **J. Hansen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Contera Pharma A/S.

Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 449.06

Topic: C.03. Parkinson's Disease

Title: Pharmacologic inhibition of NLRP3 by OLT1177 reduces the levels of α -synuclein and protects dopaminergic neurons from degeneration in a neurotoxic model of Parkinson's disease

Authors: *J. AMO-APARICIO¹, J. DALY¹, D. B. SKOURAS², C. A. DINARELLO¹;
¹Univ. of Colorado, Denver, CO; ²OLATEC Therapeut. LLC, New York, NY

Abstract: Parkinson's disease is characterized by a progressive degeneration of dopaminergic neurons that leads to irreversible loss of peripheral motor functions. It is well established that the death of dopaminergic neurons induces an inflammatory response in microglial cells that further exacerbates neuronal loss. Modulating inflammation is expected to ameliorate tissue degeneration and, consequently, arrest motor defects. Because of the contribution of the NLRP3 inflammasome to the inflammatory response in the central nervous system, we targeted NLRP3 using OLT1177[®] in a neurotoxic model of Parkinson's disease. We show that the treatment with OLT1177[®] 1) prevented the loss of motor function, 2) suppressed the levels of pro-inflammatory cytokines in the nigrostriatal areas of the brain, 3) reduced the amount of α -synuclein, and 4) protected dopaminergic neurons from degeneration. These data suggest that targeting the NLRP3 inflammasome by OLT1177[®] should be considered a novel therapeutic approach to arrest neuroinflammation and protects against neurological deficits of Parkinson's disease in humans.

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Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 449.07

Topic: C.03. Parkinson's Disease

Title: The Effects of the multimodal serotonin compound Vortioxetine on L-DOPA induced dyskinesia in a rat model of Parkinson's disease

Authors: *K. ELDER¹, C. BUDROW¹, M. COYLE¹, J. GLINSKI¹, S. COHEN², N. LIPARI¹, A. CENTNER¹, N. KINZONZI¹, E. WHEELIS¹, C. BISHOP¹;
¹Binghamton Univ., Binghamton, NY; ²Drexel Univ., Philadelphia, PA

Abstract: Parkinson's Disease (PD) is a debilitating, neurodegenerative motor disorder characterized by bradykinesia, tremor, stiffness, and postural instability, that result from progressive nigrostriatal dopamine (DA) loss. The current treatment for PD is the DA precursor L-3,4-dihydroxyphenylalanine (L-DOPA); however, 90% of patients eventually experience choreic and dystonic side effects termed L-DOPA induced dyskinesia (LID) that affect their quality of life. One possible cause of LID is neuroplasticity within the raphe-striatal serotonin (5-

HT) system which can release of L-DOPA-derived DA from 5-HT terminals, suggesting engaging autoregulatory targets like 5-HT_{1A} receptors and 5-HT transporter (SERT) may be effective in attenuating LID. The present study examined: 1) the chronic efficacy of Vortioxetine, a multimodal drug that acts as a 5-HT_{1A} agonist and SERT blocker, on LID expression and 2) acute Vortioxetine on striatal DA efflux. All adult female and female Sprague-Dawley rats received unilateral 6-hydroxydopamine into the medial forebrain bundle to lesion nigrostriatal DA neurons. In the second experiment, cannulae were implanted into striata ipsilateral to lesion. Thereafter rats received 2 weeks of daily L-DOPA treatment (6 mg/kg) until they developed stable abnormal involuntary movement (AIMs) akin to LID. In experiment 1, rats were also assessed for motor performance with the forepaw adjusting steps (FAS) test at baseline and on L-DOPA with or without Vortioxetine treatment. In experiment 2, *in vivo* microdialysis was performed to collect extracellular striatal DA and 5-HT release during acute Vortioxetine and L-DOPA treatment. Results revealed Vortioxetine significantly reduced AIMs during chronic and acute administration with maintenance of L-DOPA's beneficial prokinetic effects. Neurochemical analyses are currently underway. Overall, these findings support Vortioxetine, an FDA-approved compound, as a potential adjunct therapeutic to optimize L-DOPA treatment for PD patients.

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Poster

449. Parkinson's Disease Animal Models

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Program #/Poster #: 449.08

Title: WITHDRAWN

Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 449.09

Topic: C.03. Parkinson's Disease

Title: Gt-02287, a brain-penetrant, structurally targeted allosteric regulator of glucocerebrosidase, shows promising activity in an α -synuclein seed and spread mouse model of parkinson's disease

Authors: B. CALVO-FLORES GUZMAN¹, N. PEREZ², A. GARCIA-COLLAZO², E. CUBERO², X. BARRIL², M. BELLOTTO¹, R. MAJ¹, *J. TAYLOR³;
¹Gain Therapeut., Lugano, Switzerland; ²Gain Therapeut., Barcelona, Spain; ³Gain Therapeut., Gain Therapeut., Bethesda, MD

Abstract: Glucocerebrosidase (GCase) deficiency due to cellular stress, aging, and mutations in the *GBA1* gene leads to toxic accumulation of sphingolipid substrates in the lysosome. GCase is attracting increasing attention given its potential role in Parkinson's disease (PD) pathophysiology: deficiency can lead to elevated α -synuclein (α -syn) that reciprocally inhibits GCase activity, leading to a vicious cycle that magnifies the pathogenic process. GCase deficiency can also increase the spread of α -syn pathology, which may contribute to earlier onset, faster progression and worse cognitive decline seen in PD patients carrying *GBA* mutations. Enhancing GCase activity may therefore represent a potential therapeutic strategy in PD. Gain Therapeutics has applied its innovative proprietary drug discovery platform, Site-directed Enzyme Enhancement Therapy (SEE-TxTM), to the discovery and development of GT-02287, a small-molecule structurally targeted allosteric regulator (STAR) that stabilizes GCase by binding to an allosteric site, facilitating its maturation and trafficking to the lysosome, where it exerts its catalytic activity. In this study we have tested the effect of GT-02287 on α -syn spreading in a mouse model of PD. 8 weeks old C57BL/6J mice received a single unilateral intrastriatal stereotaxic injection of synthetic mouse α -syn preformed fibrils (mPFFs) and were orally treated with GT-02287 for 9 weeks. We report *in vivo* evidence that GT-02287 reduces the spread of pathological α -synuclein in this model. We have demonstrated that our safe, orally bioavailable and brain penetrant PD lead, GT-02287, is protective against the spread of α -syn pathology. Therefore, STAR therapy continues to represent a potential novel pharmacological tool for the treatment of PD warranting further development towards the clinic.

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Poster

449. Parkinson's Disease Animal Models

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Program #/Poster #: 449.10

Topic: C.03. Parkinson's Disease

Support: R15 NS121784

Title: Senp-1 inhibition ameliorates the pff induced toxicity and alleviates Parkinson's disease-related signs in mice

Authors: *D. VERMA¹, A. GHOSH¹, K. OFORI¹, B. SEO², G. CABRERA¹, D. B. WHEELER¹, H. KO², Y.-H. KIM¹;

¹Dept. of Biol. Sci., Delaware State Univ., Dover, DE; ²Inst. for Cell Engin., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Small Ubiquitin-like modifier (SUMO) conjugation is a dynamic post-translational modification on lysine residues, catalyzed by SUMO-specific ligases and removed by SUMO-specific proteases (SENPs). The physiological consequences of (de)SUMOylation in Parkinson's disease (PD) pathology are not well understood. In this study, we characterized which isoform of SENPs is involved in detaching SUMOs from α -synuclein when 1-methyl-4-phenylpyridinium (MPP⁺) or pre-formed fibrils (PFF) of α -synuclein induces toxicity, in the scope of understanding the idiopathic mechanisms of PD pathology. After PFF or MPP⁺ exposure to N27 rat dopaminergic cells, we found that SENP-1 expression was particularly elevated among other SENP family proteins, such as SENP-1, 3, 5, 6, 7 and 8. An increase in SENP-1 expression and a decline in SUMO-1 level were detected in PFF-treated primary cortical neuron culture, midbrain and striatum of PFF injected C57Bl/6 mice and the SNpc of human PD patient brains. The knock-down of SENP-1 by siRNA resulted in an increase of SUMO-1 level in PFF-treated N27 cells. Next, we assessed various commercially available SENP-1 inhibitors targeting SENP-1 suppression and found significant decreases in the levels of ROS and protein aggregates, derived from PFF toxicity. Since a SENP-1 inhibitor, Momordin Ic was more efficacious than others based on our *in vitro* assays, we have orally treated Momordin to PFF-injected >12-month-old mice at two doses (10 or 50 mg/kg) for 6-7 weeks. The SENP-1 inhibition alleviated motor deficits induced by PFF injection in behavioral tests, such as rotarod, nesting, grooming, hindlimb clasping and pole test. In the striatum and midbrain region of PFF injected mice, immunohistochemical analyses revealed the increased levels of protein aggregates in Thioflavin-T staining and phosphorylated alpha-synuclein, a pathological marker, were significantly reduced by Momordin treatment. These results were further verified by an increase in the number of TH⁺ neurons in the SNc and enhanced TH intensity in the striatum in a blinded analysis. Taken together, our results strongly suggest that SENP-1 inhibition can be applied to halt and further reverse the pathology of PD, due to the reduction of protein aggregation and oxidative stress.

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Poster

449. Parkinson's Disease Animal Models

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Program #/Poster #: 449.11

Topic: C.03. Parkinson's Disease

Support: # 1ZIAES103310 to G.C.
NES22001-001-00000
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Title: The impact of dietary nicotinamide riboside on Parkinson's Disease

Authors: *S. SAHA¹, S. WANG¹, H. CAI², G. CUI¹;

¹Neurobio., Natl. Inst. of Environ. Hlth. Sci. (NIEHS), Durham, NC; ²Neurogenetics, Natl. Inst. on Aging, Bethesda, MD

Abstract: Parkinson's Disease (PD), resulting from the progressive degeneration of nigrostriatal dopaminergic (nDA) neurons, affects more than 60,000 Americans each year. PD is characterized as a complex disorder owing to its etiology being both genetic and environmental cues, with pesticides as well as fungicides being among the main causalities. Aldehyde dehydrogenases (ALDHs) protect against the neuronal loss through catabolizing the cytotoxic dopamine intermediate 3,4-dihydroxyphenylacetaldehyde (DOPAL), whereas benomyl being a potent ALDH inhibitor, is associated with increased PD risk. The overarching goal of this study is to identify the cumulative role of mutated α -synuclein(A53T) and benomyl in PD etiopathogenesis and to explore whether activating ALDH pathway can be an effective therapeutic strategy to protect nDA neurons in PD. Here, by bilateral stereotaxic injection of A53T in substantia nigra (SNc), we developed a genetic PD mice model and tested the toxicity of benomyl via dietary intervention in PD progression. Behaviorally, we found that the double hit PD model persistently reduced motor performance in a time dependent manner (compared to control mCherry-expressing mice fed on benomyl diet and mice expressing A53T fed on regular diet) accelerating PD, thus revealing the deficiency of basal ganglia dopamine transmission. Histological studies revealed the loss of nDA neurons and increased A53T accumulation in the SNc of the PD mice indicating that benomyl inhibition of ALDH activity can exacerbate the SNCA aggregation-induced nDA neuron damage in PD. To investigate whether enhancing ALDH activity can counteract benomyl-induced toxicity in this PD model, we added NAD⁺ precursor Nicotinamide Riboside (NR) to the drinking water in mice that had received either A53T AAV or mCherry AAV injections and subsequently exposed to benomyl. We found that NR supplementation rescued the motor deficits in A53T AAV expressing benomyl administered PD mice in rotarod and open field tests. However, histological analysis revealed that there was no significant protective effect of NR supplementation on the loss of nDA and ALDH1a1 neurons. This study demonstrates the notorious contribution of A53T and benomyl in the etiopathogenesis of PD development and the ameliorative efficacy of NR supplementation in this complex PD model.

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Poster

449. Parkinson's Disease Animal Models

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Topic: C.03. Parkinson's Disease

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Title: Axonal transport of CRISPR/Cas9 ribonucleoprotein and edited protein products as a therapeutic neural-network strategy for Parkinson's Disease

Authors: S. NEUMAN¹, J. M. METZGER¹, Y. WANG¹, J. A. FELTON¹, V. BONDARENKO¹, K. SAHA², J. E. LEVINE³, S. GONG¹, *M. EMBORG²;
¹UW Madison, Madison, WI; ²Univ. of Wisconsin-Madison, Madison, WI; ³Wisconsin Natl. Primate Res. Ctr., Madison, WI

Abstract: Targeted DNA editing is proposed as a therapeutic approach for neurological disorders, such as Parkinson's disease (PD). The pathological hallmark of PD is the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) which project into the striatum. Conversely, the striatal neurons project into the substantia nigra pars reticulata (SNpr). As the brain is organized into neural networks, it is critical to understand how anatomically connected structures are affected by the delivery of genome editors and genome editing. **Our research team has developed biodegradable, synthetic, non-viral PEGylated nanocapsules (NCs) that encapsulate preassembled Cas9-gRNA ribonucleoproteins (RNPs) capable of *in vivo* gene editing.** These NCs can be used to deliver RNPs targeting the stop cassette upstream of the tdTomato gene present in Ai14 mice; therefore **successful genome editing is detectable through production of the red fluorescent protein tdTomato.** To assess the effect of gene editing in the nigrostriatal neural network, Ai14 reporter mice received intra-striatal unilateral stereotactic injections of NCs decorated with no ligand (n=3), cell penetrating peptide (CPP) (n=3), or both CPP and rabies virus glycoprotein (RVG) (n=2). Two weeks post-injection, brains were collected, fixed in 4% paraformaldehyde, and processed for immunofluorescence. To sample the entire striatum, every 6th serial brain section (40µm thickness) was immunolabeled with antibodies against tdTomato and NeuN and imaged using confocal microscopy. The total striatal area across brain sections with genome-edited tdTomato+ cells and the nigral tdTomato fluorescence intensity were calculated using NIS Elements. TdTomato expression was observed at the injection site in the NC-treated groups and typically colocalized with NeuN, indicating neuronal genome editing. No significant differences were detected in striatal edited area between NC-injected groups. TdTomato-positive fibers were present in the SNpr when the edited striatal area was greater than 5 mm². **These results demonstrate that *in vivo* axonal transport of protein products to the substantia nigra following neuronal genome editing in the striatum can be exploited as a therapeutic strategy for PD.**

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Poster

449. Parkinson's Disease Animal Models

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Program #/Poster #: 449.13

Topic: C.03. Parkinson's Disease

Title: Synthetic peucedanocoumarin IV prevents α -synuclein neurotoxicity in an animal model of Parkinson's disease

Authors: *H. KIM¹, J. KIM¹, Y. OH², H.-J. KIM³, S.-M. PAEK⁴, Y. LEE¹;

¹Sungkyunkwan Univ., Suwon-si, Korea, Republic of; ²Hanyang Univ., Seoul, Korea, Republic of; ³Gyeongsang Natl. Univ., Jinju, Korea, Republic of; ⁴Gachon Univ., Incheon, Korea, Republic of

Abstract: Pathologic protein inclusion formation and propagation is a main cause of neuronal dysfunction in diverse neurodegenerative disease and as such current disease modifying therapeutic strategies have been targeting this disease protein aggregation processes. Recently, we have reported Peucedanocoumarin III (PCiii) as a promising therapeutic compounds with the ability to disaggregate α -synuclein inclusion and protect dopaminergic neurons in Parkinson's disease (PD). Here we found that Peucedanocoumarin IV (PCiv), a structural isomer of PCiii, with higher synthetic yield presented strong anti-aggregate activity to a similar degree comparable to PCiii. PCiv retained effective inhibitory function against β -sheet aggregate-mimic β 23 cytotoxicity and potently prevented α -synucleinopathy in α -synuclein preformed fibril (PFF)-treated mouse cortical neurons. In detailed pharmacokinetic profiling of PCiv, oral administration of PCiv in rats exhibited about 97 min half life and 10 % bioavailability. Moreover, tissue distribution analysis revealed favorable profiles of brain penetration with 6.4 % brain to plasma concentration ratio. Given these pharmacokinetic properties of synthetic PCiv, its therapeutic efficacy was further evaluated in a sporadic PD mouse model with combinatorial coinjection of PFF and rAAV- α Syn. Motor dysfunctions induced in this combinatorial α -synucleinopathy PD mice were almost completely rescued by PCiv diet administration and this therapeutic effect is consistent with the marked prevention of dopaminergic neuron loss and suppression of α -synuclein aggregation. Taken together, our translational study using synthetic PCiv suggest that PCiv is advantageous as therapeutic agents for neurodegenerative diseases especially with good synthetic yield, high brain distribution and anti-aggregate activity. PCiv could be useful in the management of α -synuclein inclusion formation and propagation in different stages of PD.

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Poster

449. Parkinson's Disease Animal Models

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Topic: C.03. Parkinson's Disease

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Title: The neuroprotective effect of the novel barbiturate derivative in an MPTP-Induced Parkinson's disease mouse model

Authors: *Y. LEE¹, S. LEE², C. LEE¹;

¹Cognitive science research group, ²Neurodegenerative research group, Korea Brain Res. Inst., Deagu, Korea, Republic of

Abstract: Parkinson's disease (PD) is one of the most common neurodegenerative disorders, which leads to dopaminergic neuronal damage and neuroinflammation in the nigrostriatal pathway. Neuroinflammation plays important roles in the central nervous system because it prompts the repair of damaged tissues, supplies nutrients, and maintains homeostasis. In addition, neuroinflammation contributes to neurodegeneration, which has been well documented to be closely associated with glial cell activation. This study examined the neuroprotective effects of the novel barbiturate derivative (BD) in MPTP-induced PD mice model. BD inhibited MPP⁺-induced astrocyte activation and ROS production in primary cultured astrocytes and ameliorated the MPP⁺-induced phosphorylation of MAPK and NF- κ B. The anti-inflammatory effects of BD inhibited MPTP-induced motor dysfunction and prevented dopaminergic neuronal death, and suppressed neuroinflammatory responses in PD lesions. Therefore, BD could offer alternative therapeutic possibilities for targeting neuroinflammation-associated neurodegenerative diseases such as PD.

Disclosures: Y. Lee: None. S. Lee: None. C. Lee: None.

Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 449.15

Topic: C.03. Parkinson's Disease

Support: LB692 Nebraska Biomedical Research Development Fund
Dr. George F. Haddix President's Faculty Research Fund (Creighton)

Title: Analysis of CNS-derived exosomes as biomarkers of Parkinson's Disease

Authors: *K. K. ZAND¹, T. T. NILLES-MELCHERT¹, S. H. WEE¹, H. KIM¹, M. S. BURNETT², J.-Y. HWANG¹;

¹Dept. of Pharmacol. and Neurosci., ²Dept. of Neurol., Creighton Univ. Sch. of Med., Omaha, NE

Abstract: Parkinson's Disease (PD) is the second most common neurodegenerative disease in the world. PD is caused by severe loss of nigrostriatal dopaminergic neurons and is characterized by increases in cytoplasmic inclusions of alpha-synuclein. A timely diagnosis of PD is critical to providing the proper treatment and advice regarding care to patients. However, presently a suitable diagnostic laboratory test for PD does not exist. Therefore, the identification of reliable biomarkers is clinically imperative. Exosomes are nanovesicles found throughout the body that carry various proteins, mRNAs, and microRNAs. Central nervous system (CNS)-derived exosomes can cross the blood-brain barrier and have been suggested as a potential source of neurodegenerative disease biomarkers in some pilot studies. Thus, research related to further validation and a large independent cohort study is needed for relevancy and accuracy. To examine the possibility of using CNS-derived exosomes as biomarkers and identify new candidates, we used an animal model of PD. We isolated CNS-derived exosomes from peripheral blood of rats with rotenone-induced PD, collecting samples at 2-week and 4-week time points. The results from Western blot analysis on the samples using antibodies against L1CAM and MAP2, proteins known to be present in CNS exosomes, showed that CNS-derived exosomes were successfully isolated from peripheral blood. Next, we examined whether PD-related pathogenic proteins were changed. We found that phosphorylated alpha-synuclein and DJ-1 were increased at four weeks post rotenone injection compared to sham, suggesting their potential use as biomarkers for diagnosing PD. To identify new candidates in CNS-derived exosomes, we are currently preparing samples for next-generation sequencing and anticipate our analysis may identify novel mRNA and/or miRNA biomarkers from CNS-derived exosomes.

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Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 449.16

Topic: C.03. Parkinson's Disease

Title: Suvn-n9503012: a selective 5-ht_{1a} receptor agonist for the potential treatment of motor, cognitive and behavioral dysfunction associated with parkinson's disease

Authors: ***V. R. GRANDHI**, N. GANUGA, R. MEDAPATI, R. ABRAHAM, P. JAYARAJAN, D. MALLESHWARI, V. MIDDEKADI, P. AMIDALA, A. SHINDE, A. MOHAMMED, R. SUBRAMANIAN, R. NIROGI;
SUVEN LIFE SCIENCES LIMITED, SUVEN LIFE SCIENCES LIMITED, Hyderabad, India

Abstract: Parkinson's disease (PD) patients experience progressive cognitive decline and behavioral complications as the disease progresses over the years. Progression of the disease further deteriorates the health related quality of life (hQOL) of patients which adds additional burden on caregivers. There is an unmet medical need to find effective yet, safe interventions for

cognitive and behavioral problems associated with PD. Drug-induced PD is indistinguishable from the pathological PD, as the symptoms are similar. Haloperidol is known for its propensity to induce extrapyramidal symptoms, hallmark of PD and the associated behavioral complications, by blocking the striatal dopamine D₂ receptors. Recently, selective 5-HT_{1A} receptor agonists were reported to exhibit beneficial effects in motor and other complications associated with PD. SUVN-N9503012 is a selective 5-HT_{1A} receptor agonist and has been evaluated for potential treatment of cognitive and behavioral complications of PD. Haloperidol produces catalepsy behavior in rodents. Prior treatment with SUVN-N9503012 ameliorated the haloperidol-induced catalepsy in a dose dependent manner. SUVN-N9503012 was also found to reverse haloperidol-induced cognitive impairment associated with PD upon 7 days treatment in a social olfactory memory task. Further, SUVN-N9503012 was evaluated in resident-intruder task, where it showed robust anti-aggressive like effect in male CD1 mice. SUVN-N9503012 ameliorated PD associated motor and cognitive deficits and showed anti-aggressive like effects in preclinical models. This makes SUVN-N9503012, a promising potential therapeutic intervention for motor as well as behavioral complications associated with PD.

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Poster

449. Parkinson's Disease Animal Models

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS109608
NIH Grant NS122805-01
Arizona Biomedical Research Commission (ABRC) Grant ADHS18-198846

Title: Analysis of the role of opioid receptors in the anti-dyskinetic and antiparkinsonian effects of sub-anesthetic ketamine

Authors: *R. PARMAR¹, C. J. STOPERA², M. J. BARTLETT⁴, C. LIU³, A. ESQUEDA¹, M. L. HEIEN³, S. J. SHERMAN³, T. FALK⁵;

¹Univ. of Arizona, Tucson, AZ; ²Neurosci., ³The Univ. of Arizona, Tucson, AZ; ⁴Dept. of Neurol., ⁵Dept. Of Neurol., Col. of Med., Tucson, AZ

Abstract: We have previously demonstrated that treatment with sub-anesthetic ketamine (10-hrs; 5 x 20 mg/kg; *i.p.*; 2-hrs apart) can reduce L-DOPA-induced dyskinesia (LID) in 6-hydroxydopamine (6-OHDA)-lesioned rats and is acutely antiparkinsonian. We expand this work here with pharmacokinetic analysis and further mechanistic studies. The concentrations of ketamine and its metabolites norketamine, hydroxynorketamine and dehydronorketamine in

plasma and CSF were measured with LC-MS/MS in catheterized Sprague-Dawley rats (n=6) with microdialysis probes placed into the striatum after injection of 20 mg/kg ketamine (*i.p.*). Ketamine is a multifunctional ligand with similar binding affinity to both *N*-methyl-D-aspartate (NMDA) and opioid receptors. Through NMDA receptor antagonism ketamine modulates neuroplasticity. Recent data indicates that opioid agonism is required for the anti-depressive activity of ketamine. In addition, the striatum is rich in both μ - and δ -opioid receptors and long-term L-DOPA therapy changes the levels of opioid peptides. This leads to the hypothesis that ketamine may also activate opioid receptors in the basal ganglia, contributing to either anti-dyskinetic or antiparkinsonian action. In a 1st study, we used the pan-opioid receptor antagonist naloxone to investigate if opioid receptor activation is necessary for reducing LID. Unilateral 6-OHDA lesioned male Sprague-Dawley rats were treated with 6 mg/kg of L-DOPA for 21 days to establish a model of LID. Once abnormal involuntary movements (AIMs) were stable over three testing days, the rats (n=10/group) were then treated on Day 0 with either vehicle, ketamine (20 mg/kg), or ketamine (20 mg/kg) + naloxone (3 mg/kg) via the same 10-hour treatment protocol. L-DOPA was administered immediately following the protocol and AIMs were assessed. All animals received a 6 mg/kg dose of L-DOPA every 3-4 days to maintain the LID and AIMs were scored once per week. Similar to our published data, ketamine significantly reduced AIMs by 36% immediately following the treatment ($p < 0.05$). The addition of naloxone did not prevent the effect of ketamine ($p < 0.05$ vs. vehicle) to reduce LID (mean AIMs \pm SEM; ketamine = 24.1 \pm 1.9 vs. ketamine + naloxone = 22.9 \pm 4.4). This data suggests that opioid receptor activation may not be necessary for the anti-dyskinetic effects of ketamine. In a 2nd study, we investigated in unilateral 6-OHDA-lesioned rats if the antiparkinsonian activity of ketamine is dependent on opioid receptor activation, using the forelimb adjusting steps (FAS) and rotarod tests in groups treated with ketamine, or ketamine + naloxone (n=9/group). The analysis is ongoing and results will be presented.

Disclosures: **R. Parmar:** None. **C.J. Stopera:** None. **M.J. Bartlett:** None. **C. Liu:** None. **A. Esqueda:** None. **M.L. Heien:** None. **S.J. Sherman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SJS has a pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc.. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TF has a pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc...

Poster

449. Parkinson's Disease Animal Models

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Program #/Poster #: 449.18

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS109608
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Arizona Biomedical Research Commission (ABRC) Grant ADHS18-198846

Title: Differential effects of two types of statins on the anti-dyskinetic activity of sub-anesthetic ketamine

Authors: ***M. J. BARTLETT**¹, C. J. STOPERA², S. J. SHERMAN¹, T. FALK¹;
¹Neurol., ²GIDP Neurosci., The Univ. of Arizona, Tucson, AZ

Abstract: Previous work published from our lab shows that sub-anesthetic ketamine-treatment (10-hrs; 5 x 20 mg/kg; *i.p.*; 2-hrs apart) attenuates L-DOPA-induced dyskinesia (LID), as measured by abnormal involuntary movements (AIMs), in a 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease (PD). Given that pravastatin has been shown by others to block the long-term anti-depressive activity of ketamine by interfering with the direct binding of ketamine to the TrkB receptor, we set out to test if statins also interfere with ketamine's anti-dyskinetic activity. In this pilot, first we replicated our published data, demonstrating that ketamine attenuates the development of AIMs by ~45% compared to vehicle rats ($p < 0.05$; Mann-Whitney test) treated with L-DOPA for 14 days. We then investigated the differential interactions of two types of statins, lovastatin (non-polar) and pravastatin (polar), on AIMs independently and when combined with ketamine. Unilateral 6-OHDA lesioned male Sprague-Dawley rats were pretreated for 14 days with either vehicle, lovastatin (10 mg/kg; *i.p.*), or pravastatin (10 mg/kg; *s.c.*). The vehicle or statin treatment was maintained for an additional 14 days during which the rats were primed with daily L-DOPA (6 mg/kg) and treated with either ketamine or vehicle on days 0 and 7, as described above. Total limb, axial, and oral AIMs were scored every 3-4 days. When combined together, lovastatin and ketamine, attenuated AIMs by ~65% at day 14, compared to vehicle treated rats ($p < 0.05$). However, this was not significantly different when compared to ketamine alone. Unlike with the combination of lovastatin and ketamine, ketamine's long-term anti-dyskinetic effect was blocked in rats co-treated with pravastatin ($p < 0.05$ vs. ketamine alone). Likewise, pravastatin alone had no anti-dyskinetic effect. Instead, when given alone, pravastatin seemed to accelerate the development of AIMs, reaching its maximum score by day 3 versus day 7 in vehicle treated rats. Moreover, on the first exposure to L-DOPA, pravastatin-treated animals had an ~239% increase in AIMs, as compared to vehicle, while ketamine blocked this effect in pravastatin co-treated animals. Our data suggests that the non-polar statin drug, lovastatin, does not interfere with ketamine's anti-dyskinetic effects, unlike pravastatin. Moreover, despite its polarity, pravastatin has significant central effects as seen by the sensitization in rats on first exposure to L-DOPA and the rapid increase in AIMs within the first days of L-DOPA priming. Combined, this pilot provides further support for the preferential use of non-polar statins, like lovastatin, when needed in individuals with PD or LID.

Disclosures: **M.J. Bartlett:** None. **C.J. Stopera:** None. **S.J. Sherman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SJS has a pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc.. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TF has a pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc...

Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 449.19

Topic: C.03. Parkinson's Disease

Support: Arizona Biomedical Research Commission (ABRC) Grant ADHS18-198846
NIH Grant NS122805-01
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Title: Neuroprotective activity of sub-anesthetic ketamine-treatment in the progressive unilateral 6-OHDA-lesion rat Parkinson's disease model

Authors: *C. J. STOPERA¹, M. J. BARTLETT³, M. R. SEXAUER¹, K. BERNARD¹, J. A. STANCATI⁵, S. J. SHERMAN², H. W. MORRISON¹, K. STEECE-COLLIER⁶, T. FALK⁴; ²Dept. of Neurol., ¹The Univ. of Arizona, Tucson, AZ; ³Dept. of Neurol., ⁴Dept. Of Neurol., Col. of Med., Tucson, AZ; ⁶Translational Neurosci., ⁵Michigan State Univ., Grand Rapids, MI

Abstract: Sub-anesthetic ketamine is neuroprotective in rodent models of cerebral ischemia and traumatic brain injury. Our lab has shown that sub-anesthetic ketamine reduces L-DOPA-induced dyskinesia in rat 6-hydroxydopamine (6-OHDA) Parkinson's disease (PD) models, is acutely antiparkinsonian, and ketamine's long-term anti-dyskinetic effects were brain-derived neurotrophic factor (BDNF)-dependent. Here, we tested if sub-anesthetic ketamine exhibits neuroprotective effects in a progressive 6-OHDA rat PD model. Male Sprague-Dawley rats were treated with either ketamine or vehicle (6-hr treatment; 3 x 20 mg/kg; *i.p.*; 2-hrs apart) beginning 6-hrs prior to a unilateral intrastriatal 6-OHDA lesion (13.75 µg / site), and then treated daily with the 6-hr protocol for 7 days post-lesion. Using the amphetamine-induced rotation test to estimate lesion severity rotational asymmetry was assessed by counting the net ipsilateral rotations (mean ± SEM) over 90-min. On Day 14 post-lesion, rats treated with ketamine (168 ± 90) showed a decreased number of net ipsilateral rotations compared to vehicle (497 ± 170), and on Day 28, this effect of ketamine (211 ± 116) vs vehicle (666 ± 187; two-tailed t test; p < 0.05; n=15) reached significance, suggesting a neuroprotective effect. Forelimb adjusting steps (FAS) tests were also conducted, and on Days 14 and 28 post-ketamine akinesia was improved by >200% (p' = 0.001; two-tailed t tests, Holm-Bonferroni Correction). Semi-quantitative western analysis showed that relative striatal tyrosine hydroxylase (TH) content (Lx/Intact) was increased by 85% in ketamine-treated rats compared to vehicle (n=7). We stained nigral dopaminergic neurons for TH, unbiased stereology (n=8) is ongoing. The role of BDNF was evaluated via dual labeling immunohistochemistry of TH and DAPI with *in situ* hybridization for RNA of BDNF and its receptor, TrkB, in substantia nigra (SN) and striatum (STR); and a group treated with ketamine and the TrkB blocker ANA-12 (0.5 mg/kg) was added. To investigate anti-inflammatory action via activation of microglia, we have stained the SN and STR with the microglia marker, IBA1, and analyzed branch length/cell and end points/cell. We found no effects in the SN, but a significant reduction of branch length/cell by 6-OHDA, compared to the

intact STR (Two-way ANOVA; $p < 0.001$; $n=8$). Ketamine further significantly reduced branch length/cell in the intact STR ($p < 0.05$). The number of end points/cell was also significantly reduced by 6-OHDA in the vehicle ($p < 0.001$), but not the ketamine group. In conclusion, an acute neuroprotective activity of ketamine is further supporting the ongoing clinical evaluation in individuals with PD.

Disclosures: **C.J. Stopera:** None. **M.J. Bartlett:** None. **M.R. Sexauer:** None. **K. Bernard:** None. **J.A. Stancati:** None. **S.J. Sherman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SJS has a pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc... **H.W. Morrison:** None. **K. Steece-Collier:** None. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TF has a pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc...

Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 449.20

Topic: C.03. Parkinson's Disease

Title: Behavioral and electrophysiological characterization of antipsychotic treatments in a rodent model of Parkinson's disease psychosis

Authors: T. LOREDAN STAN¹, A. RONAGHI², S. BARRIENTOS¹, P. HALJE¹, *L. CENSONI², E. GARRO MARTÍNEZ², E. MALININA², K. SAHLHOLM², P. PETERSSON^{1,2};
¹Lund Univ., Lund, Sweden; ²Umeå Univ., Umeå, Sweden

Abstract: Parkinson's disease psychosis (PD-P) affects more than a quarter of all Parkinson patients and can severely reduce the quality of life for patients and cause increased distress among caregivers. Clinically useful treatment options for PD-P include the atypical antipsychotic clozapine and the selective serotonin-2A antagonist pimavanserin. However, clozapine must be used with great care, due to adverse side effects, and pimavanserin, while better tolerated, is not always an effective treatment in the long-term, making improved and complementary treatments for PD-P an urgent clinical need.

To this aim, we have here developed a new method to characterize brain states associated with PD-P. Hemiparkinsonian rats were implanted with multi-electrode arrays and their spontaneous motor behavior and associated brain activity patterns were characterized following administration of a low dose of MK-801 (0.05-0.07 mg/kg), in conjunction with one of the clinically used antipsychotic substances pimavanserin and clozapine, or the novel compound mesdopetam, which has been suggested to have both anti-dyskinetic and anti-psychotic

properties in PD. Our results suggest that high-frequency oscillations (HFOs) in cognitive-limbic cortico-basal ganglia circuits may be a useful biomarker for the psychotic state and that mesdopetam can reverse HFOs in a similar way as clozapine and pimavanserin but probably via a different mechanism of action, involving antagonism of dopamine type-3-receptors. In conclusion, our results indicate that the treatment effects of the compounds tested share a mechanism related to suppression of HFOs in cognitive-limbic structures, while a more detailed characterization of their physiological profiles could provide insights into their respective treatment and side effect profiles.

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Poster

449. Parkinson's Disease Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 449.21

Topic: C.03. Parkinson's Disease

Title: Behavioral and electrophysiological characterization of the antidyskinetic treatments in a rodent model of PD-LID

Authors: *A. RONAGHI¹, T. LOREDAN STAN², S. BARRIENTOS BAEZA², S. SULIS SATO¹, E. MALININA¹, L. CENSONI¹, P. HALJE², P. PETERSSON¹;

¹Integrative Med. Biol. (IMB), Umeå Univ., Per Petersson lab, Umeå, Sweden; ²Exptl. Med. Sci., Integrative Neurophysiol. and Neurotechnology, Lund, Sweden

Abstract: In Parkinson's disease (PD), pharmacological treatment with the dopamine precursor levodopa, in the long-term, often leads to troublesome side effects in the form of levodopa-induced dyskinesia (LID). To mitigate these problems, an anti-dyskinetic drug, such as amantadine, may be used together with levodopa treatment. However, the use of amantadine is sometimes limited by other side effects, particularly in the psychiatric domain. We have here investigated how the novel drug candidate, mesdopetam, compares to amantadine and pimavanserin with respect to reduction of LID in a rat model of PD-LID. In order to clarify the mechanistic effects of the different drugs, the severity of dyskinesia and body rotations were quantified in eight hemiparkinsonian rats while neurophysiological local field potentials in motor related and limbic brain structures were recorded (in total 128 electrodes distributed in 17 structures in each hemisphere). On a behavioral level, both amantadine and mesdopetam lowered the scores of abnormal involuntary movements and reduced the number of contralateral turns. Preliminary analyses of the electrophysiological recordings demonstrated that narrow band gamma oscillations in the local field potential recorded in motor cortical structures, known to be associated with LID (Halje et al 2012), followed a similar pattern with a comparable suppression by amantadine and mesdopetam of aberrant gamma-oscillations. By further characterizing the

pattern of physiological changes in motor and to cognitive-limbic brain circuits, more detailed treatment profiles of the different compounds will be provided. Taken together, our data indicate that mesdopetam and amantadine have comparable anti-dyskinetic effects in behavioral assessments of LID and of cortical narrow-band gamma, whereas pimavanserin showed very modest effects. We also suggest that characterizations of differences in the induced activity patterns in non-motor circuits is of importance for a deeper mechanistic understanding of treatment effects as well as potential side effects.

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Poster

449. Parkinson's Disease Animal Models

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Topic: C.03. Parkinson's Disease

Support: CSIR Grant 09/263(1219)/2019-EMR-I

Title: Oral treatment with the NADPH oxidase antagonist Apocynin ameliorates pathological features of Parkinsonism in the Paraquat-induced rat model.

Authors: *S. XXXX, A. C. MONDAL;
Sch. of Life Sci., Jawaharlal Nehru Univ., NEW DELHI, India

Abstract: Abstract

Background: Parkinson's disease (PD) is the second most common progressive neurodegenerative disease characterized by the presence of intra- cytoplasmic inclusion bodies known as Lewy bodies containing α - synuclein aggregate and the loss of dopaminergic neurons in the substantia nigra regions of the brain. The exact cause of dopaminergic neuronal loss in PD remains unknown for a long time, however, recent studies report that oxidative stress plays a key role in the pathogenesis of PD. Paraquat (PQ), a widely used herbicide is an oxidative stress inducer that has been implicated as a potential risk factor for the development of PD. Pharmacological approaches targeting antioxidant machinery may have therapeutic value against PD. Flavonoids are naturally occurring polyphenolic compounds that display a variety of therapeutic properties against oxidative stress. Apocynin (4-hydroxy-3- methoxyacetophenone) is a natural flavonoid obtained from medicinal plant *Picrorhiza kurroa* that exhibits neuroprotection against PD-related pathology. However, studies on its neuroprotective role and the underlying mechanisms are scarce.

Aim: The proposed study will explore the potential beneficial effect of Apocynin in the Paraquat-induced PD model.

Methods: As a part of the preliminary study, we have developed PQ-induced *Parkinsonism* model in adult Wistar rats. We performed motor coordination-related behavioral experiments and

histopathological studies in order to validate the establishment of PQ-induced *Parkinsonism*. Then we determined the effect of apocynin on motor function in PQ-induced rat model of *Parkinsonism*.

Results: Paraquat-induced nigrostriatal dopaminergic neurodegeneration in the rat model of *Parkinsonism*. Apocynin improved motor deficits in PQ-induced rat model of *Parkinsonism*.

Conclusion: Apocynin treatment alleviates PQ-induced *Parkinsonism*. In the future, we will be assessing the neuroprotective effect of Apocynin in the developed model of *Parkinsonism*.

Keywords: Parkinson's disease, paraquat, neuroprotection, apocynin, neurodegeneration.

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Poster

449. Parkinson's Disease Animal Models

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Topic: C.03. Parkinson's Disease

Support: NPLC, NIPER SAS Nagar
CSIR, GOVT of India

Title: Hc070, a potent trpc5 inhibitor, attenuates increased trpc5 channel expression and associated downstream signalling in parkinson's disease models

Authors: *B. VAIDYA¹, I. ROY², S. S. SHARMA¹;

¹Dept. of Pharmacol. and Toxicology, ²Dept. of Biotech., NIPER SAS NAGAR, Mohali, India

Abstract: Transient receptor potential canonical 5 (TRPC5) channels are cation permeable channels activated in response to oxidative stress. Physiologically, TRPC5 channels are involved in neuronal development as well as temperature and mechanical sensation. Additionally, the involvement of these channels has also been postulated in different neurological disorders, such as cerebral ischemia, depression, anxiety and Huntington's disease. However, its role has not yet been explored in the context of Parkinson's disease (PD). Thus, in the present study, potential the role of TRPC5 channels and associated downstream signalling was explored in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and MPP⁺ (1-methyl-4-phenylpyridinium) model of PD. MPTP was infused intranigrally into the substantia nigra while MPP⁺ exposure was carried out in SH-SY5Y cells to induce the PD *in vivo* and *in vitro*, respectively. PD rats exhibited increased TRPC5 channel levels in the striatum and mid-brain, accompanied by reduced expression of tyrosine-hydroxylase (TH) in comparison to sham animals. Moreover, MPTP/MPP⁺ treatment produced mitochondrial dysfunctions, which were studied using rt-pcr and immunoblotting for Peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1 α) and transcription factor A, mitochondrial (TFAM). We also observed reduced

tetramethylrhodamine (TMRE) intensity and increased mitoxox levels in the SH-SY5Y cells treated with MPP⁺. These changes were reversed after co-treatment with a selective TRPC5 inhibitor, HC070. HC070 further reduced calcium influx and attenuated the expression of calcium buffering proteins such as parvalbumin and calmodulin. We also investigated the effect of HC070 on apoptotic signalling using terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling (TUNEL) assay and expression of apoptosis-inducing factor (AIF) in the striatum as well as mid-brain. Overall, our results provide novel insights into the potential of TRPC5 channels as a therapeutic target for the development of pharmacological interventions for the treatment of PD.

Disclosures: B. Vaidya: None. I. Roy: None. S.S. Sharma: None.

Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Kurt B. Seydow Dystonia Foundation

Title: Methods for Recording Local Field Potentials during Movement and Stimulation from Externalized Deep Brain Stimulation Leads

Authors: *S. L. ALBERICO¹, J. E. AMAN², M. C. PARK¹, L. A. JOHNSON³, D. ESCOBAR SANABRIA⁶, J. WANG⁴, R. PATRIAT⁵, S. E. COOPER⁷, L. E. SCHROCK¹, C. D. MACKINNON⁴, N. HAREL⁹, J. L. VITEK⁸;

²Dept. of Neurol., ³Univ. of Minnesota Dept of Neurol., ⁴Neurol., ⁵CMRR / Radiology, ¹Univ. of Minnesota, Minneapolis, MN; ⁶Biomed. Engin. (Lerner Res. Institute), Cleveland Clin., Bay Village, OH; ⁷Neurol., ⁸Dept. of Neurol., Univ. of Minnesota Dept. of Neurol., Minneapolis, MN; ⁹Radiology, Univ. of Minnesota Syst., Minneapolis, MN

Abstract: Background: Previous studies have successfully recorded local field potentials (LFPs) and/or provided stimulation via deep brain stimulation (DBS) leads externalized to the body, often at the scalp. Here, we describe a method for externalizing DBS leads for the purpose of recording network physiological activity from Parkinson's disease (PD) patients, simultaneously recording LFPs from DBS leads, electroencephalography (EEG), electromyography (EMG) and biomechanical measures of limb movement during rest and motor tasks with high temporal resolution. Methods: Following DBS lead placement, a lead extension was connected to the

proximal end of the DBS lead and tunneled to the upper chest wall, per standard of care. Rather than connecting to an implantable pulse generator (IPG), a second lead extension was connected and tunneled to the upper abdomen region where a stab incision was made to externalize the end of the second lead extension, which was wrapped and covered. Approximately 5 days later, subjects returned and were admitted to our Clinical Research Unit where they performed a battery of motor tasks in combinations of ON/OFF medication and traditional stimulation as well as closed-loop stimulation. Following completion of the recordings, patients returned to the surgical suite for removal of the externalized hardware and placement of the IPG. **Results:** To date, we have externalized 11 leads from 10 PD patients, 4 subthalamic nuclei (STN) and 7 internal segment of the globus pallidus (GPi), using leads from Abbott Laboratories, Boston Scientific Corp., and Medtronic plc. There have been no reported adverse events related to the externalization surgical procedure or data collection. Synchronized oscillations and associated movement-related modulation of oscillatory activity recorded from DBS leads have demonstrated stable and consistent neural activity across testing conditions, amenable to testing closed-loop stimulation algorithms. **Conclusion:** These methods demonstrate an effective method for recording LFPs from permanently implanted DBS leads during motor tasks and for testing closed-loop algorithms while offering a new location (upper abdomen) for externalization, potentially reducing infection risk at critical incision sites. Importantly, our results show stable physiological recordings from STN or GPi at rest and during motor tasks. Our methods utilize a high sampling rate (44 kHz) of LFPs synchronized to EEG, EMG, and biomechanical measures, allowing us to study physiological features of PD across the cortex (EEG) and GPi/STN (DBS lead) with high temporal resolution and apply closed-loop stimulation in real-time.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Program #/Poster #: 450.02

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Title: Cell type and synapse specific adaptations in the motor cortical circuits following the loss of midbrain dopamine neurons

Authors: *L. CHEN, S. DANIELS, H.-Y. CHU;
Van Andel Res. Inst., Van Andel Res. Inst., Grand Rapids, MI

Abstract: The hypokinetic motor symptoms of Parkinson's disease (PD) have been long thought to be caused by an impaired motor cortical output associated with pathological basal ganglia activity. However, whether and how the loss of dopamine (DA) alters the cellular and synaptic properties of motor cortical neurons remains undefined. We induced parkinsonism in adult C57BL/6 mice of both sexes by injecting neurotoxin, 6-hydroxydopamine (6-OHDA), into the medial forebrain bundle. By using ex vivo electrophysiology, optogenetics, and retrograde tracing approaches, we found that the intrinsic excitability of pyramidal tract neurons (PTNs) in the primary motor cortical layer 5 was greatly decreased following the loss of DA, but the intratelencephalic neurons (ITNs) were not affected. Further, we showed that the thalamocortical excitation to the PTNs, but not that to the ITNs, was selectively decreased following the loss of DA. In contrast, cortico-cortical excitation to the PTNs in M1 is largely intact in mice with 6-OHDA lesions. We are using a combination of molecular, genetic, and behavioral approaches to study the molecular mechanisms that underlie the cell type- and synapse-specific circuit adaptations in the motor cortex in parkinsonism. These results provide novel insight into our understanding of the pathophysiology of motor deficits in PD.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Title: Characterization of Beta and High-Frequency Oscillations in the Pallidum Across the Sleep-Wake Cycle in Parkinson's Disease

Authors: *A. K. VERMA¹, S. ACOSTA LENIS¹, J. E. AMAN¹, D. ESCOBAR SANABRIA¹, J. WANG¹, A. PEARSON¹, M. HILL¹, R. PATRIAT², L. SCHROCK¹, S. E. COOPER¹, M. PARK¹, N. HAREL³, M. HOWELL¹, C. D. MACKINNON¹, J. L. VITEK¹, L. A. JOHNSON¹;
¹Dept. of Neurol., ²CMRR / Radiology, ³Radiology, Univ. of Minnesota, Minneapolis, MN

Abstract: People with Parkinson's disease (PD) show excessive beta (β , 13-35 Hz) and high-frequency (HF, 150-350 Hz) oscillations in the internal segment of the globus pallidus (GPi) in the off-medication awake state. These oscillations are thought to play a role in the development of PD motor signs since they are typically suppressed with dopaminergic medication or deep brain stimulation (DBS) that improves motor signs. Recent preclinical studies also implicate pallidal β oscillations in contributing to sleep-wake disturbances, which are commonly reported in people with PD. The dynamics of β and HF oscillations in the GPi during sleep and their potential relationship to disordered sleep in patients with PD, however, is not well understood. As a first step, the primary aim of this study was to improve our understanding of how β and HF oscillations in the GPi modulate across the sleep-wake cycle in people with PD. We simultaneously collected video-polysomnography and GPi local field potentials from four PD patients with externalized DBS leads. Video-polysomnography included surface electroencephalogram (EEG), chin electromyography (EMG), and left and right electrooculogram (EOG). Sleep recordings were scored as WAKE, REM, and NREM 1 (N1), NREM 2 (N2), and NREM 3 (N3) by an expert sleep technician. β and HF spectral power from GPi local field potentials were extracted during WAKE, NREM, and REM sleep, and a within-subject comparison was performed to quantify the modulation of β and HF oscillations across sleep-wake states. Compared to wake, β and HF power decreased in all subjects during NREM sleep. During REM sleep compared to NREM sleep β and HF power increased in all subjects. Compared to the wake state, β power decreased during REM sleep in 3 subjects but increased in one. Interestingly, this subject (unlike the others) was documented to have REM sleep behavior disorder (RBD). HF power during REM sleep compared to wake was variable across subjects (increasing in 2 subjects, decreasing in 1 subject, and no change in 1 subject). Our results show that β and HF oscillations are modulated differently by REM and NREM sleep. Moreover, we observed subject-specific variability in the polarity of modulation of β and HF oscillations during REM sleep compared to wake. Further investigation is required to understand the extent to which β and HF GPi power during REM sleep are associated with sleep disturbances exhibited by people with PD. These findings can inform the development of GPi-DBS strategies tailored to patients' sleep-wake cycle, with the long term goal of developing biomarker-based sleep-specific DBS strategies to improve sleep and quality of life in PD patients.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Title: Parkinsonism alters neuronal activity in the primary motor cortex during active movement

Authors: Z. GUO¹, *N. HJELLE⁴, L. A. JOHNSON², J. WANG³, J. L. VITEK⁵;
²Univ. of Minnesota Dept of Neurol., ³Neurol., ¹Univ. of Minnesota, Minneapolis, MN; ⁴Univ. of Minnesota, Twin Cities, Minneapolis, MN; ⁵Dept. of Neurol., Univ. of Minnesota Dept. of Neurol., Minneapolis, MN

Abstract: Background: Parkinson's disease (PD) has been associated with alterations in neuronal activity in the basal ganglia thalamocortical network. Although the primary motor cortex (M1) is a critical node in this circuit and previous studies have suggested that cortical disinhibition is a feature of PD there has been little direct evidence of the changes that occur in M1 to support this hypothesis. Objective: The goal of this study is to investigate the effects of parkinsonism on movement-related neuronal activity in M1. Method: Two nonhuman primates (NHPs) were each implanted with a high-density Utah array over M1, trained to perform a reaching task and rendered moderately parkinsonian by administering 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Kinematic and M1 neuronal data were collected in the naïve and parkinsonian states when the animals were performing the reaching task. M1 single unit activities were sorted in Offline Sorter (Plexon) and then analyzed in Matlab. Each neuron's response during active movement was compared to a pre-movement period and categorized as activated, inhibited or mixed, i.e., sequence of activation ↔ inhibition. The rate of change of the neuronal response to reach onset was also calculated. Results: We found in both animals that the number of M1 units responding to active movement was significantly reduced in the parkinsonian state compared to the naïve state. This resulted from a significant reduction in the number of neurons that were inhibited during active movement (28.5% to 6.5% in one animal and 24.7% to 7.2% in the other). The proportion of neurons excited during movement did not change significantly. As a result, the ratio of the number of neurons inhibited versus excited, i.e., I/A, during movement decreased significantly in both animals (0.91 to 0.16 in one animal and 0.65 to 0.23 in the other). Moreover, the rate of change of neuronal activity in M1 was also reduced in the parkinsonian state. Conclusion: The balance between inhibition and excitation in M1 was significantly altered in the parkinsonian condition and is consistent with the hypothesis that a reduction in cortical inhibition is an underlying feature of the motor dysfunction in PD. These results provide some of the first direct evidence of the loss of cortical inhibition in M1, and are consistent with previous studies demonstrating similar changes during passive movement in the globus pallidus internus (GPi). Together, these data support the concept that dopaminergic

loss in parkinsonism promotes a loss of inhibition and disruption in spatial-temporal processing of information within the BGTC circuit contributing to the motor dysfunction observed in PD.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Title: Effect of parkinsonism on basal ganglia-thalamocortical neuronal dynamics during sleep-wake behavior

Authors: ***B. NANDAKUMAR**¹, A. VERMA², K. ACEDILLO³, A. L. DENICOLA⁵, H. YAO³, Y. YU⁷, T. HAVEL⁴, C. D. MACKINNON⁵, M. HOWELL⁴, J. L. VITEK⁷, L. A. JOHNSON⁶;

¹Univ. Of Minnesota Twin cities, Med. Sch., Minneapolis, MN; ²Dept. of Neurol., Univ. of Minnesota twin cities, Minneapolis, MN; ³Neurol., ⁴Univ. of Minnesota, Twin Cities medical school, Minneapolis, MN; ⁵Neurol., ⁶Univ. of Minnesota Dept of Neurol., Univ. of Minnesota, Minneapolis, MN; ⁷Dept. of Neurol., Univ. of Minnesota Dept. of Neurol., Minneapolis, MN

Abstract: Sleep disturbances are present in 74-98% of Parkinson's disease (PD) patients and can adversely affect their quality of life. Sleep fragmentation, the extent to which sleep is disrupted by microarousals, is one of the common sleep dysfunctions in PD patients. A recent study also suggested that pathological increase in beta oscillations in the basal ganglia might be involved in driving insomnia in the MPTP non-human primate model of PD. The neurophysiological changes that occur across the basal ganglia-thalamocortical (BGTC) network throughout the sleep-wake cycle remain poorly understood, however. In order to address this, we characterized neuronal activity (e.g., slow oscillations, fast spiking activity) in the BGTC across different stages of sleep in healthy and mild parkinsonian conditions. Neuronal activity (local field potential (LFP), spiking activity) was collected wirelessly during naturalistic sleep a nonhuman primate (NHP) using a 96-channel microelectrode drive targeting sensorimotor and premotor cortex as well as motor thalamus. LFPs were also collected from deep brain stimulation (DBS)

leads targeting the subthalamic nucleus (STN) and globus pallidus (GP). Data were collected across the sleep-wake cycle in the normal and mild PD states, induced by systemic injections of MPTP (0.2-0.5mg/Kg). Preliminary data suggest that both slow wave and beta oscillations were modulated across the sleep-wake cycle in cortical areas, motor thalamus and basal ganglia. Oscillatory activity in the high beta band (20-35 Hz) was reduced in cortical areas while slow wave oscillations increased in all regions as the animal transitioned from wake to slow wave sleep. Low beta (8-20 Hz) activity was differentially modulated across the cortical and thalamic areas as animals transitioned to sleep. Spontaneous firing rates of neurons in the cortical and thalamic areas decreased with changes in firing patterns from a more tonic to irregular bursting state. Mild parkinsonism induced sleep fragmentation accompanied by reduced amplitude of slow wave oscillations in the cortex. We also observed increased oscillatory activity in the low beta band in the basal ganglia during both wakefulness and slow wave sleep in the PD state. Understanding the temporal evolution of BGTC neuronal dynamics across the sleep wake cycle will serve as a framework from which sleep disturbances associated with Parkinson's disease can be understood and set the stage for the development of new DBS approaches that also focus on improving sleep dysfunction in PD.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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Title: A heuristic study of simulated spike-train entropy characteristics during neuromodulation: myths and praxis

Authors: ***E. M. BELLO**, M. D. JOHNSON;
Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Calculation of spike-train entropy based on the distribution of inter-spike intervals can be useful, particularly in quantifying neuronal spike-pattern changes during neuromodulatory stimuli such as deep brain stimulation. Our goal was to heuristically ascertain what biases or variability in entropy calculation may be expected in realistic experimental conditions, both with and without any added effect of regular stimulus events. Furthermore, we aimed to outline appropriate bias-mitigating techniques to ensure rigorous and scientifically meaningful conclusions based on spike-train entropy. To that end, we simulated neuronal activity with

biologically-relevant spike-pattern and timescale qualities. Trains of spike-time events were simulated as simple Poisson processes, with lambda parameters set to simulate realistic mean firing rates. As a secondary goal, temporally-regular DBS-events were included with the spike-train data. As the timing of DBS-events and spike trains were uncorrelated with each other by definition, this simulation allowed for the exploration of whether data processing around DBS events incurs any intrinsic “structure” or “information” to entropy analysis, thus causing external stimulus-induced bias in entropy calculation. This simulation study allowed for an intuitive understanding of the following information regarding entropy-analysis of spike-train data: 1) entropy-estimation is biased downwards by spike-sparsity, but can often be adequately estimated with neural recordings at most experimentally attainable recording durations; 2) comparison of entropy between two spike-trains can be normalized by a sample-matching step, with a small but predictable bias; 3) the co-occurrence of external time-event stimuli does not intrinsically bias spike train entropy estimation via some artifactual by-product of data processing. It is recommended that, for any given study of spike trains using entropy, baseline spiking conditions be similarly simulated prior to any final confirmatory analyses as was done here to better inform the expectations of effect sizes and the design of appropriate statistical tests. The principles observed in the current study may be generalized to many different timescales of neuronal spike activity as well as many modalities of neuromodulation.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

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Title: Single Unit Activity in the Supplementary Motor Area is Disrupted during the Baseline [Pre-Movement] State in Parkinsonism

Authors: ***C. HENDRIX**, H. E. BAKER, D. L. BAUER, Y. YU, J. WANG, L. A. JOHNSON, J. L. VITEK;
Dept. of Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: Multiple studies have reported that Parkinson’s disease (PD) is associated with changes in neuronal activity patterns throughout the basal ganglia-thalamocortical (BGTC) motor circuit. There is limited electrophysiological data, however, describing how parkinsonism

impacts the supplementary motor area (SMA), an area that is known to be involved in movement planning and motor control. In this study, SMA single unit activity (SUA) was recorded contralateral to the working arm in two non-human primates during a visually cued reaching task. Recordings were made in the same subjects in both the naive and parkinsonian state using the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of parkinsonism. The fixed baseline (0-3 sec) period of interest is a non-movement period which occurs well before a go cue (i.e., instruction of reach onset and direction). During this period the subjects were alert and waiting for instruction. Cell activity was categorized as either tonic (i.e., sustained, non-modulating firing rates) or as modulating/changing within the trial baseline period. For cells classified as modulating, the depth of modulation was calculated as the difference between the peak and lowest mean firing rate during the fixed baseline period.

In the Naive state, more than half of SMA cells were classified as tonic as compared to modulating (percentage of total cells). In the PD state, the ratio of tonic over modulating cells increased significantly in both animals. Firing rates in the tonic cell population increased with onset of parkinsonism in one animal but did not change in the second. In contrast, the ratio of cells classified as modulating decreased in both animals with the onset of parkinsonism. The magnitude of the depth of modulation also decreased in one animal but remained the same in the second animal.

We also explored the relationship between changes in SMA SUA during this precue period to behavior. The mean firing rate during the fixed baseline period was compared to the reaction time within each trial for all cells and recording sessions. In the Naive condition, a subset of cells showed that SMA SUA correlated linearly with reaction time (RT) on a trial-to-trial basis, suggesting cell activity may predictively encode for RT. In PD, however, the percentage of cells that changed with RT was significantly diminished in both animals ($\chi^2=12.89$, $p=0.0003$). The results suggest that SMA activity although overactive is largely disengaged from motor planning in the Parkinsonian condition. The significant reduction of predictive encoding of RT in the SMA in PD may contribute to errors in motor planning leading to prolonged RT's.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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MnDRIVE Brain Conditions Program

Title: The Selection of Cycle Rate Impacts the Effect of Coordinated Reset Deep Brain Stimulation on Parkinsonian Gait

Authors: ***K. M. BOSLEY**, Z. LUO, C. M. HENDRIX, A. L. DENICOLA, L. A. JOHNSON, J. L. VITEK, J. WANG;
Neurol., Univ. of Minnesota Neuromodulation Res. Ctr., Minneapolis, MN

Abstract: Background: Impaired gait is a disabling symptom in Parkinson's Disease (PD). While traditional deep brain stimulation (DBS) has shown significant benefit on parkinsonian motor signs, its efficacy on gait impairment has been limited. Coordinated Reset DBS (CR DBS) is a novel DBS approach that uses lower levels of burst stimulation through multiple contacts of the DBS lead. Though CR DBS has been demonstrated to have sustained therapeutic effect on rigidity, tremor, bradykinesia, and akinesia, following cessation of stimulation, i.e., carryover effect, its effect on parkinsonian gait has not been well studied.

Objective: The goal of this study was to explore the carryover effect of subthalamic nucleus (STN) CR DBS using different cycle rates on parkinsonian gait.

Method: An adult non-human primate (NHP) was rendered moderately parkinsonian by administering 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The NHP was implanted with an 8-contact DBS lead in the STN and received two sessions of CR DBS. The only difference between the two sessions was the CR cycle rate, i.e., 6.95 and 8.88Hz. For each session, CR DBS was delivered for two hours per day for five consecutive days. A clinical rating scale modified for NHP use (mUPDRS) was assessed daily on stimulation days and at least five consecutive days after CR DBS to monitor its effect on rigidity, bradykinesia, and akinesia. Gait was assessed as the animal ambulated across a gait mat before CR DBS, immediately following five days of CR DBS, and the third and sixth day after five days of CR DBS. Spatial and temporal gait mat data were analyzed to assess the changes in gait parameters, e.g., stride length, swing time, stance time, stride speed, and temporal overlap between limbs.

Results: While CR DBS using the 8.88Hz cycle rate produced greater acute and carryover improvements in rigidity, bradykinesia, and akinesia indicated by reduced mUPDRS, gait was only improved using the 6.95Hz cycle rate. Specifically, increased stride length and speed, decreased swing time, and decreased temporal overlap between right and left hind limbs were observed with CR DBS at 6.95Hz, but not at 8.88Hz.

Discussion: Although these findings are preliminary, they indicate that the selection of cycle rate can impact the effect of CR DBS on parkinsonian motor signs and can differentially affect gait and other motor symptoms. Additional experiments in more subjects will be needed to confirm these findings and define the changes in neuronal activity in the basal ganglia thalamocortical network associated with the effects on motor signs during CR DBS in future studies.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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MnDRIVE Brain Conditions

Title: Investigation of dorsal premotor cortex neuronal activity during reaching in a rhesus macaque using neuropixels.

Authors: *D. L. BAUER, B. T. POBIEL, K. M. HILBER, J. L. VITEK, L. A. JOHNSON;
Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: Recently developed Neuropixels (NP) probes are a major advancement in recording technology that enable electrophysiological recording of hundreds of neurons simultaneously. Application of this technology has been predominantly in rodents and use in non-human primates (NHP) has been limited. Primary factors that have limited widespread use of Neuropixels in NHPs include: lack of implantation methodology, probe size and fragility, and thicker dura mater compared to rodents. In this project we developed a robust, reproducible approach to implant a Neuropixel probe within a standard Crist cranial recording chamber to perform repeated acute recordings in an awake, behaving rhesus macaque. Using CAD modeling and 3D printing, a sliding guide tube assembly was designed to hold and lower a Neuropixel probe through a miniature guide tube into cortical tissue with a two stage Alpha Omega microdrive. In five experimental sessions we successfully implanted Neuropixel probes through thick dura mater and stabilized the probes for neural recordings in the dorsal premotor cortex (PMd) during reach and retrieval behavioral tasks. Successful reimplantation of probes from one session to the next was feasible. During the reaching task, spike activity was simultaneously collected across the depth of the Neuropixel probe from populations of cells in the PMd cortex. 1187 single- and multi-unit clusters were identified using Kilosort 2.5 and manual curation with PhyGUI. A change point analysis was used to classify neuronal responses to movement onset. Analysis showed 47% of units responded to movement onset and 53% showed no response. Of the responders, 24% were excited, 50% were suppressed, and 26% showed multiple responses to reach onset. Median (IQR) response times relative to reach onset for excited and suppressed units were -0.003 (-0.107-0.113) seconds and -0.063 (-0.193-0.067) seconds, respectively. The response times of suppressed units were significantly earlier than excited units and typically occurred prior to reach onset. Distributions of these measures across the array were explored to further investigate movement-related information processing across cortical layers. Given this experience, future work will (1) aim to further refine the approach to

improve implant repeatability, recording stability, and targeting accuracy and (2) expand the analysis to further characterize neuronal population dynamics and information processing in PMd. In conclusion, this implant method provides a good platform to acutely implant Neuropixel probes in NHPs which allows for investigation of laminar information processing in NHPs during reaching behaviors.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Title: Evolution of beta band activity across the BGTC network in a progressive model of Parkinson's disease

Authors: ***E. BHARTI**, Y. YU, A. DENICOLA, T. HAVEL, H. YAO, A. VERMA, J. WANG, L. A. JOHNSON, J. L. VITEK;
Dept. of Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: Exaggerated oscillatory activity in the beta band (8-35 Hz) has been observed in the basal ganglia-thalamocortical (BGTC) circuit in Parkinson's disease (PD). Recent studies have reported changes in synchronized oscillatory activity both within and across nodal points of the BGTC network. Where these changes begin and how they evolve within the network is unclear. The goal of this study is to investigate the relationship between beta-band activity and motor signs as motor signs progressively worsen using a progressive 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) non-human primate (NHP) model of PD. Two rhesus macaques were instrumented with chronically implanted recording electrodes in the subthalamic nucleus (STN), globus pallidus internus (GPi), globus pallidus externus (GPe), motor thalamus, primary motor cortex (M1), and supplementary motor area (SMA). Baseline local field potential (LFP) activity and behavioral data were collected in the normal state. Animals were then given weekly or biweekly systemic intramuscular injections to induce a gradual change in PD motor signs, with additional neuronal and behavioral data collected over 3-5 days after each injection during an awake, resting state. Power spectral densities (PSDs) and magnitude squared coherence were

used to quantify changes in neural activity across brain structures as motor signs developed and increased in severity.

The results demonstrate that changes in the beta band activity occur early as parkinsonian motor signs first begin to develop and are present across both subcortical and cortical structures. Increases in low-beta band activity (8-20 Hz) in the STN occurred with the onset of mild motor signs. We observed a progressive increase in low beta band power in the STN and GPi coincident with the progressive increase in severity of motor signs. In the high beta-band, we saw a decrease in power in the SMA as motor signs progressed. There were no consistent changes in beta power observed in M1 with increased motor signs. Analysis of neuronal recordings from the motor thalamus is currently underway. We also observed a direct correlation between increasing coherence of subcortical and cortical structures across the 8-35 Hz power spectrum and severity of motor signs. These data provide compelling evidence in support of the relationship between changes in beta-band activity to the development and increasing severity of motor signs in PD and further inform the use of biomarker-based stimulation strategies.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 450.11

Topic: C.03. Parkinson's Disease

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MnDRIVE (Minnesota's Discovery, Research and Innovation Economy) Brain Conditions Program
Engdahl Family Foundation

Title: Effect of Parkinsonism on Basal Ganglia-Motor Cortex Synchrony During Slow-Wave Sleep

Authors: *K. ACEDILLO, A. K. VERMA, B. NANDAKUMAR, A. DENICOLA, H. YAO, Y. YU, T. HAVEL, M. HOWELL, C. D. MACKINNON, J. L. VITEK, L. A. JOHNSON;
Dept. of Neurol., Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: People with Parkinson's disease (PD) exhibit less slow-wave sleep (SWS). Studies have reported that reduced SWS is associated with poor motor and cognitive functions in people with PD. Despite the recognition that reduced SWS negatively impacts quality of life in PD

patients, the neural mechanisms underlying disordered SWS in PD remain poorly understood. Excessive synchrony between the basal ganglia and motor cortex in the low beta band (8-20 Hz) has been associated with PD motor signs and is also hypothesized to play a role in sleep dysfunction in PD. The primary aim of this study was to test whether the synchrony between the basal ganglia and motor cortex during SWS is altered in parkinsonian compared to the normal state. To address this question, we implanted a nonhuman primate (NHP, rhesus macaque) with deep brain stimulation (DBS) leads targeting the subthalamic nucleus (STN) and globus pallidus (GP). We used a microelectrode drive to target the primary motor cortex (M1). STN, GP, and M1 local field potentials were acquired wirelessly using TDT (Tucker and Davis Technology) and TBSI (Triangle BioSystems International) systems overnight during natural sleep in the NHP's home environment. At the conclusion of normal sleep state recordings, the NHP was rendered parkinsonian by administering four low-dose (0.2 mg/Kg) intramuscular injections of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Epochs of SWS were identified as high-amplitude low-frequency (1-5 Hz) oscillations in the M1 local field potential. STN↔M1 and GP↔M1 synchrony was assessed by calculating the magnitude-squared coherence (range, 0-1) during normal and parkinsonian SWS in MATLAB. We observed that the STN↔M1 and GP↔M1 coherence in the beta band during SWS was higher in the parkinsonian state compared to the normal state. Our findings demonstrate the presence of pathological basal ganglia and motor cortex synchrony which may contribute to the dysfunction of SWS. Future studies will 1) extend the analysis to more subjects to test whether this observation is consistent across subjects and 2) correlate basal ganglia↔motor cortex synchrony with behavioral changes in SWS (e.g., fragmentation of SWS) to further our understanding of how pathological basal ganglia↔cortical synchrony may disrupt SWS in parkinsonian state. A better understanding of SWS pathophysiology will provide a rationale for the development of future therapeutic strategies to disrupt the pathological beta band synchrony between the basal ganglia and motor cortex and improve SWS in people with PD using DBS.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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P50 NS123109

Engdahl Family Foundation
MnDRIVE Brain Conditions Program

Title: Phase desynchronization of subthalamic beta oscillatory activities associated with therapeutic coordinated reset deep brain stimulation

Authors: *Z. LUO, S. FERGUS, L. A. JOHNSON, J. L. VITEK, J. WANG;
Dept. of Neurol., Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Coordinated reset deep brain stimulation (CR DBS) is a promising treatment for Parkinson's disease. It delivers short pulse trains through multiple stimulation contacts of the DBS lead at a lower intensity. It is hypothesized that CR DBS desynchronizes the target neuronal population by activating subpopulations of neurons in a phase shifted manner. However, this hypothesis hasn't been tested in vivo. In this study, we investigated the changes in neural activity in the subthalamic nucleus (STN) associated with the therapeutic effect of STN CR DBS in a non-human primate (NHP) model of Parkinson's disease. A NHP was implanted with an 8-contact DBS lead (NuMed) in the STN and acclimated to the assessment of the modified Unified Parkinson Disease Rating Scale (mUPDRS). CR DBS was delivered through contacts within/close to the STN (C0, C1, C2) over five consecutive days for 2 hours per day. The mUPDRS was assessed and STN local field potentials (LFPs) recorded before and immediately after each stimulation block, as well as for a minimum of five days following stimulation cessation. Power spectral density (PSD) and phase locking index (PLI) analyses were performed on the bipolar LFP signals from contact pairs C0-C1 and C1-C2. STN CR DBS was associated with a reduction in the mUPDRS during and after stimulation which persisted for more than 1 week after stimulation cessation. The high (21-35Hz) and low (10-20Hz) beta oscillatory power at C0-C1 and C1-C2 didn't change with CR DBS. However, the PLI between two LFP signals in the low and high beta frequency band were significantly decreased after each stimulation block and this reduction persisted for one day after stimulation cessation. Although these results are preliminary, they further our understanding of the mechanism of CR DBS and support the hypothesis that CR DBS produces its therapeutic effect by desynchronizing neuronal subpopulations within the target brain structure.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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Title: Establishing the Impact of Aerobic Exercise on Biomarkers, Mobility, and Cognitive Functioning of Parkinson's Disease: A Translational Study

Authors: *I. SOTO¹, V. NEJTEK², G. BOHEM⁵, K. BRICE⁵, P. BRADEN⁶, E. A. KASANGA³, W. NAVARRETE BARAHONA⁷, R. MCMANUS⁷, M. F. SALVATORE⁴;
¹UNTHSC, Fort Worth, TX; ²Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; ³Dept. of Neurodegenerative Sci., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; ⁴Univ. of North Texas Hlth. Sci. Ctr., Ft Worth, TX; ⁵Texas Christian Univ., Fort Worth, TX; ⁶Texas Christian Univ., Fort Worth, TX; ⁷UNT Hlth. Sci. Ctr., FT WORTH, TX

Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by a substantial loss of mobility. A key premotor symptom of PD is cognitive decline, which often goes undetected in the prodromal stage. However, verifying subtle cognitive loss in prodromal PD would benefit the patient if this loss was easier to recognize for treatment purposes. As an angle for detecting prodromal signs of PD, along with cognitive decline, we investigated whether the PD-associated serum biomarkers UCH-L1, GFAP, and s100b could provide a signature bio-cognitive profile. If so, these peripherally obtained markers combined with cognitive testing would be highly valuable for enhancing diagnostic tools and therapeutic outcomes. Early and accurate disease detection would inform the clinician as to whether available non-pharmacological treatments (i.e., aerobic exercise) could help slow disease progression prior to initiating dopamine replacement therapy. While aerobic exercise may be a viable treatment for arresting PD progression, the CNS mechanisms associated with treatment efficacy are not well understood. To increase translation of CNS benefits of exercise, we implemented a reverse cross-species translational paradigm between humans and a genetic PD rat model, the Pink1 knock-out (PKO) rat. Using a cross-sectional study design, we compared motor, cognitive and biomarker data from exercising and non-exercising early-stage PD subjects along with matched controls. We found that PD subjects participating in moderate intensity aerobic exercise showed significantly better cognitive flexibility and mobility than non-exercising PD subjects. Moreover, exercise also showed significantly higher serum concentrations of UCH-L1 ($p < 0.05$) and significantly lower concentrations of neuronal injury markers GFAP ($p < 0.0001$) and s100b ($p < 0.05$). To examine the translatability of the PKO rat, we longitudinally collected motor, cognitive data, and serum from PKO and wild-type (WT) rats and discovered premotor cognitive decline at 4 mo old, with a decline in distance traveled at 6mo in PKO rats. Additionally, we found s100b in PKO rats was significantly higher in substantia nigra ($p=0.02$) and in serum ($p=0.02$) compared to WT rats. This is the first translational PD study to find that cognitive flexibility, mobility, UCH-LI, GFAP, and s100b are highly responsive to moderate intensity exercise in early-stage PD subjects. Our data also support that PKO rats may be a reliable model for cross-species translational research for early-stage PD.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant F31NS124269

Title: Striatal projection neuron hyperactivity in MPTP-treated macaques correlates with severity of parkinsonism

Authors: *B. KOCHOIAN, C. SINON, T. YABUMOTO, C. BURE, K. I. PENDERGRAST, Y. LI, R. HALVERSON, J. DOOYEMA, S. M. PAPA;
Emory Primate Ctr., Emory Univ., Atlanta, GA

Abstract: Introduction: Degeneration of nigrostriatal dopaminergic neurons is a hallmark of Parkinson's Disease (PD) pathology. Striatal dopamine (DA) depletion can lead to profound changes in the baseline activity of striatal projection neurons (SPNs). Previous work has shown that severe parkinsonism in MPTP-treated non-human primates (NHPs) leads to hyperactivity of SPNs predominantly in the motor territory of the striatum. We now applied a retrospective analysis of data from several previous studies to assess the degree to which SPN hyperactivity predicted the level of motor impairment in MPTP-treated NHPs. Methods: Data collected from 16 macaques (12 *macaca mulatta*, 4 *macaca fascicularis*) with chronic parkinsonism and DA replacement therapy were used for this analysis. Subjects' eligibility for inclusion was determined based on two factors: availability of video recordings showing OFF-state motor performance and availability of OFF-state SPN recordings from postcommissural putamen. A motor disability scale for NHP was used for scoring the animals by two blinded observers. OFF state recordings of SPN activity included at least 3 minutes for all neurons. Results: Motor disability scores (MDS) ranged from mild symptoms (MDS = 13) to severe parkinsonism (MDS = 23). OFF state recordings of 900 individual units were included from all NHPs (minimal number per NHP = 16). Firing rates for all SPNs were pooled and divided into 4 quartiles: normal, increased, high, or very high. Linear regression analysis was applied to compare the percentage of total units from each quartile with the NHP's MDS. The percentage of units with normal SPN firing rates for each NHP (Quartile 1) was negatively correlated with the severity of the MDS. Alternatively, NHPs with more severe parkinsonism were found to have a high percentage of high SPN firing rates (Quartile 3). Discussion: Motor symptoms in PD have been noted to develop only after the degeneration of a substantial number (>60%) of nigral dopaminergic neurons. Previous analyses have shown that the degree of nigral cell loss following MPTP-treatment in NHPs is only loosely correlated with the severity of parkinsonism. Our data indicate that SPN dysfunctional state as shown by basal hyperactivity plays a role in the progression of PD motor symptoms. Future experiments assessing the mechanisms underlying SPN hyperactivity may provide insights into PD pathophysiology and potential new treatment strategies.

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Poster

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Title: Functional and structural plasticity of gabaergic pallidosubthalamic terminals in mptp-treated parkinsonian monkeys

Authors: *A.-C. MARTEL^{1,2}, R. M. VILLALBA^{1,2}, T. WICHMANN^{1,2,3}, A. GALVAN^{1,2,3}, Y. SMITH^{1,2,3};

¹Emory Natl. Primate Res. Ctr., Atlanta, GA; ²UDALL Ctr. of Excellence for Parkinson's Dis., Atlanta, GA; ³Dept of Neurol., Emory Univ., Atlanta, GA

Abstract: The subthalamic nucleus (STN) and the external globus pallidus (GPe) are part of the “indirect” pathway of the basal ganglia. Alterations of STN firing rates and patterns following nigrostriatal dopaminergic denervation are linked to the severity of parkinsonism. Mice with severe nigrostriatal degeneration display increased strength of GPe-STN GABAergic synapses along with an increase in the number of synapses per GPe terminals (Fan et al., 2012), an adaptation that may contribute to abnormal firing of STN neurons and the development of parkinsonism. The functional and structural plasticity of the pallidosubthalamic system in nonhuman primate models of parkinsonism remains poorly understood. To address this issue, we undertook in healthy and MPTP-treated parkinsonian monkeys: (1) a quantitative ultrastructural 3D electron microscopic analysis of the morphometry of GPe terminals and their synapses in the STN and (2) an *in vivo* electrophysiological analysis of STN neuron responses to electrical stimulation of the GPe. We fully reconstructed 45 and 52 putative GABAergic pallidal terminals in the STN of 2 control and 2 parkinsonian monkeys, respectively. For each terminal we measured: the volume, the flat area of synapses formed by the terminal, and the number and volume of mitochondria within the terminal. GPe terminals were easily recognized in the STN neuropil by their large size and their complex fenestrated synaptic architecture with multiple short active zones. We found that the volume of GPe terminals is significantly smaller in parkinsonian monkeys ($3.69 \pm 0.22 \mu\text{m}^3$) compared with controls ($4.70 \pm 2.01 \mu\text{m}^3$). However, the average flat area of synapses was larger in parkinsonian monkeys ($0.40 \pm 0.04 \mu\text{m}^2$ vs $0.24 \pm 0.2 \mu\text{m}^2$ in controls). Significant differences were also found in the total number and volume of mitochondria between the control and parkinsonian animals. This 3D-ultrastructural analysis indicate that GPe-STN terminals are endowed with neuroplastic properties that could contribute

to changes in their synaptic strength, energy supply, and physiological properties in the state of parkinsonism. To complement these ultrastructural observations, in vivo electrophysiological studies are in progress to determine the responses of STN neurons to electrical stimulation of GPe in the normal and parkinsonian state. Data obtained so far (one normal monkey) showed that GPe stimulation evoked a short inhibition in the firing activity of 51% (14/27) of recorded STN neurons with 13ms mean latency onset. Studies of this monkey in the parkinsonian state are in progress.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: IK2RX003253-01A2

Title: Repetitive mild TBI causes pTau aggregation in nigra dopaminergic neurons without altering preexisting fibril induced Parkinson-like pathology

Authors: *V. DELIC^{1,3,4}, J. H. KARP^{1,4}, M. GUZMAN¹, G. R. ARISMENDI^{1,2,5}, K. J. STALNAKER^{1,4}, J. BURTON¹, K. E. MURRAY^{1,4}, J. P. STAMOS¹, K. D. BECK^{1,3,4}, A. SOKRATIAN^{6,7}, A. B. WEST^{6,7}, B. A. CITRON^{1,3,4};

¹Res. and Develop., ²Dept. of Neurol., VA New Jersey Hlth. Care Syst., East Orange, NJ; ³Dept. of Pharmacology, Physiology, and Neurosci., ⁴Sch. of Grad. Studies, ⁵Dept. of Neurol., Rutgers New Jersey Med. Sch., Newark, NJ; ⁶Neurobio. department, ⁷Dept. of Pharmacol. and Cancer Biology, Duke Ctr. for Neurodegeneration Res., Duke Univ. Sch. of Med., Durham, NC

Abstract: Prospective and retrospective studies have shown that traumatic brain injury (TBI) is associated with an increased risk for Parkinson's disease (PD). The most common type of TBI is mild (mTBI) and often occurs repeatedly among certain groups including athletes, military personnel, and victims of domestic violence. PD is characterized by deficits in fine motor movement control resulting from progressive loss of dopamine producing neurons in the substantia nigra pars compacta (SNpc). This neurodegeneration is preceded by the spread of synuclein (aSyn) protein inclusions with unique architecture. Whether repetitive mTBI (r-mTBI) can initiate PD pathology or accelerate existing PD pathology remains unknown. To answer whether r-mTBI can cause or accelerate preexisting PD-like pathology, a device was constructed to deliver a surgery-free r-mTBI to rats and human-like PD pathology was induced by a unilateral injection of recombinant aSyn preformed fibrils into the SNpc. At the 3-month endpoint, the r-mTBI caused encephalomalacia throughout the brain, similar to neuroimaging findings in patients with a history of mTBI, accompanied by astrocyte expansion and microglial

activation. The pathology associated with PD, which includes dopaminergic neurodegeneration in the SNpc and Lewy body-like aSyn inclusion formation in the surviving neurons, was not produced *de novo* by r-mTBI nor was the preexisting PD-like pathology accelerated. r-mTBI did cause aggregation of phosphorylated Tau (pTau) protein in the SNpc of rats with and without preexisting PD-like pathology. These findings suggest that r-mTBI causes pTau pathology in dopaminergic neurons without directly affecting preexisting aSyn pathology at early chronic 3 month timepoint post fibril injection.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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Title: Efficient cell-type specific mutagenesis in adult neurons using single, conditional, lentiviral CRISPR/Cas9 vectors - new strategy to study early effects of noradrenergic cells degeneration in Parkinson's disease.

Authors: *J. BARUT¹, K. RAFA-ZABLOCKA¹, C. PIOTR¹, M. WILCZKOWSKI¹, M. BAGINSKA¹, A. DOMANSKYI³, M. HERIAN², G. KRYSZYNA², G. KREINER¹;

¹Brain Biochem., ²Pharmacol., Maj Inst. of Pharmacology, Polish Acad. of Sci., Krakow, Poland;

³Helsinki Univ., Helsinki, Finland

Abstract: A common feature of neurodegenerative diseases is the progressive death of neurons in particular regions of the brain associated with specific symptoms. However, a disturbance in the functioning of other neurotransmission systems are commonly observed. Clinical data show that degeneration of noradrenergic neurons of locus coeruleus (LC) is also associated with Parkinson's Disease (PD) and may precede the loss of dopaminergic cells. Here we developed Cre-controlled, lentiviral CRISPR/Cas9-based transgenic tool for targeting Cre-expressing noradrenergic neurons in DBHCre mice. The aim of this study was to trigger progressive degeneration of LC neurons and exploit this new model in the contexts of early, pre-symptomatic phase of PD. Our unique vector is contained in a single 12-kbp plasmid molecule. It contains the RNA guide under hU6 promoter as well Cas9 flagged with loxP sites under synapsin promoter. In vitro efficiency of CRISPR/Cas9-DiO vector first was tested on primary culture of mouse dopaminergic neurons, with gRNA targeted GFP to visualize the effect of the mutation. Co-transduction with lenti-hSYN-GFP-Cre vector was performed to obtain GFP and Cre expressed neurons. Lentivirus transduction lowered GFP expression by 44% after 14 days. Next, gRNA

targeted *Rrn3* (gene encoding transcription factor TIF-IA, controlling polymerase I activity) resulted in neurons impairment and death after 7 days. Vector silencing *Rrn3* was administered by stereotactic surgery to the LC of DBHCre mice. We achieved a progressive degeneration of LC neurons (5-50% cell loss, depending on the age of the mice) along with behavioral phenotype. HPLC analysis performed in striatum, a brain structure with strong projection of noradrenergic and dopamine neurons, showed no changes in dopamine metabolites, but revealed lowered level of noradrenaline in male mutant mice. Also, decreased gene expression of NET (noradrenaline transporter) in hippocampus proved the efficiency of mutagenesis by 61%. Proteomic analysis in SN/VTA revealed mitochondrial impairment which is an important determinant of processes at the forefront of PD. We found this approach to be more efficient than conventional gene knockout allowing targeting cells that would be difficult to differentiate. Cre-controlled CRISPR/Cas9 mutagenesis revealed to be an effective approach in targeting TIF-IA protein as a tool to trigger progressive neurodegeneration associated with human neurodegenerative diseases.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Program #/Poster #: 450.18

Topic: C.03. Parkinson's Disease

Title: Pff induced striatal neurodegeneration model for preclinical proof of concept studies targeting alpha-synuclein pathology

Authors: *W. DEJONCKHEERE¹, S. CARMANS², T. CORNELISSEN³;
¹reMYND, ²reMYND nv, Leuven, Belgium; ³reMYND, reMYND, Leuven, Belgium

Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder and is characterized by the accumulation of alpha-synuclein protein aggregates in the PD brain. These inclusions, accompanied with the degeneration of dopaminergic neurons are the neuropathological hallmark of this incurable disease. Currently a lot of effort is put in studying the involvement of different forms of alpha-synuclein, i.e. monomeric, oligomeric, fibrillar and aggregated forms or different post translational modifications of the protein, and their contribution to disease progression. Targeting alpha-synuclein might thus be of potential therapeutic value. Since the research field lacks good and consistent animal models for preclinical research, we've set up a mouse model based on the striatal administration of sonicated preformed fibrils (PFF). In brief, we performed unilateral stereotactical injections in the dorsal striatum of young wild type mice with sonicated PFF's (n = 8/group). PFF injected mice were sacrificed after 5, 9 and 13 weeks, a control group of vehicle injected mice (n = 7)

was sacrificed after 9 weeks. Phosphorylated alpha-synuclein (pSer129) seed and spread and dopaminergic neurodegeneration was visualized by IHC in different brain regions both ipsilateral and contralateral. pSer129 positive inclusions were shown to be present in striatum, substantia nigra (SN) and amygdala in the ipsilateral hemisphere already after 5 weeks, indicating clear spreading of pathology. Interestingly, in the contralateral hemisphere we were able to detect clear pSer129 in the amygdala and striatum 5 and 9 weeks after injection respectively, demonstrating the progressive nature of this model. To assess whether the observed pathology also affected the dopaminergic circuitry we quantified the loss of synaptic tyrosine hydroxylase positive terminals in the striatum and found a significant decrease already 5 weeks after PFF administration. Since a grid hanging test showed no differences between vehicle or PFF injected mice we will also assess their performance on the beamwalk as a functional readout. This alpha-synuclein PFF based model can be of great importance to the research field to assess in vivo therapeutic interventions based on targeting alpha-synuclein pathology.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: National Institute of Neurological Disorders and Stroke under Award Number R15 NS115032-01A1

Title: Aerobic exercise improves depressive symptoms in a unilateral 6-OHDA-lesioned rat model of Parkinson's disease

Authors: ***H. LOUGHLIN**, J. JACKSON, C. LOOMAN, A. STARLL, J. GOLDMAN, C. YU; Michigan Technological Univ., Houghton, MI

Abstract: Parkinson's disease (PD) is a progressive neurological disorder that causes many debilitating motor impairments including muscle rigidity, resting tremor, bradykinesia, and akinesia. Most existing treatment options are aimed at alleviating motor symptoms, despite many PD patients experiencing clinical non-motor symptoms, such as depression. Aerobic exercise has established benefits in motor function and neuroplasticity capabilities in PD. However, the effects of exercise on depressive symptoms are still not fully understood. The objective of this study is to determine whether regular running wheel exercise ameliorates the prevalence of depression while improving motor performance in a unilateral 6-OHDA-lesioned rat model of PD. Animals were split into three groups: PD exercise, PD control, and naïve control. The PD exercise group performed wheel running exercises 5 days per week for 11 weeks. The behavioral

effects of excises on motor deficits and depressive symptoms were quantified using the rotarod test (RT), forelimb adjusting step test (FAST), sucrose consumption (SCT), and novelty sucrose splash test (NSST). We found that PD animals displayed obvious depressive symptoms indicated by decreased sucrose consumption in the SCT and reduced exploratory activity in the NSST compared to the naïve control animals. After 11 weeks of exercise, the PD exercise group presented the most improvement among the three groups in the SCT, indicated by the highest sucrose preference indexes (Two-way ANOVA, $p < 0.001$). Further, the PD exercise group exhibited decreased immobility and increased time spent of exploring compared to the PD control ($P = 0.008$) in the NSST. In the meanwhile, the PD exercise group demonstrated the greatest improvement in correcting forelimb stepping bias ($p < 0.012$). Interestingly, we did not find an improvement in the RT among all three groups. Our results suggested that a regimen of running wheel exercise expedited recovery in motor abilities while reducing the occurrence of depressive behaviors caused by 6-OHDA dopamine depletion.

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Poster

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Program #/Poster #: 450.20

Topic: C.03. Parkinson's Disease

Title: Effects of L-DOPA on gait, forelimb stepping, and dyskinesia induction in the unilateral 6-hydroxydopamine rat model of Parkinson's disease

Authors: *H. HOLDEN, S. NEZARIA, S. VENKATESH, C. BUDROW, C. BISHOP; Binghamton Univ., Binghamton Univ., Vestal, NY

Abstract: Parkinson's Disease (PD) is a neurodegenerative movement disorder that is characterized by dopamine (DA) loss in the substantia nigra (SN). Patients often experience worsening gait impairment as the disease progresses, resulting in disrupted walking patterns. The aim of this study is to evaluate the association between motor performance, gait deficits and their potential reversal by measuring forelimb stepping and spatiotemporal gait patterns in a hemi-parkinsonian rat model before and after DA replacement treatment with L-DOPA. To do so, 2 cohorts of male and female Sprague-Dawley rats ($N = 19$) were rendered hemi-parkinsonian by unilateral injections of 6-hydroxydopamine (6-OHDA) into the left medial forebrain bundle. An automated, quantitative gait analysis (CatWalk) system was used to assess gait pre and post 6-OHDA lesion, as well as pre and post various doses of L-DOPA treatment (0, 3, 6 mg/kg). In addition to gait assays, the forehand adjusting steps (FAS) test was used to evaluate lesion success and monitor motor performance, while the abnormal involuntary movements (AIMs) test was employed to monitor the development of L-DOPA induced dyskinesia (LID), a side effect of L-DOPA therapy. Gait analyses using the CatWalk demonstrated lesion-induced decreases in

velocity and stride length and an increase in max contact area. Currently under investigation, data suggests that the low dose (3 mg/kg) of L-DOPA appears to improve gait and motor performance, while the higher dose (6 mg/kg) seems to lead to more LID and less gait improvement. This work will lead to increased knowledge regarding the relationship between gait parameters, motor performance and L-DOPA treatment, implicating shared and distinct mechanisms for motor symptoms in PD and more effective strategies for their treatment.

Disclosures: **H. Holden:** None. **S. Nezaria:** None. **S. Venkatesh:** None. **C. Budrow:** None. **C. Bishop:** None.

Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 450.21

Topic: C.03. Parkinson's Disease

Title: Long-term dopaminergic pharmacotherapy is associated with psychosis-like behaviors and aberrant high-frequency oscillations in a marmoset model of parkinson's disease

Authors: M. F. P. DE ARAUJO¹, ***L. ZACHRISSON**², P. HALJE³, L. CENSONI², M. S. L. NASCIMENTO⁴, J. F. R. NETO^{5,4}, R. FUENTES^{6,7,8}, P. PETERSSON^{3,2};

¹Federal Univ. of Espírito Santo, Vitória, Brazil; ²Integrative Med. Biology, Umeå Univ., Umeå, Sweden; ³Integrative Neurophysiol. and Neurotechnology, Dept. of Exptl. Med. Sciences, Lund Univ., Lund, Sweden; ⁴Federal Univ. of Rio Grande do Norte, Natal, Brazil; ⁵Santos Dumont Inst., Natal, Brazil; ⁶Biomed. Neurosci. Institute, Univ. of Chile, Santiago, Chile; ⁷Lab. de Control Motor y Neuromodulación, Facultad de Medicina, Univ. de Chile, Santiago, Chile; ⁸Dept. de Neurociencia, Facultad de Medicina, Univ. de Chile, Santiago, Chile

Abstract: Dopamine replacement therapy remains the standard treatment for Parkinson's disease (PD), but after long-term treatment, motor fluctuations and dyskinesia are frequently experienced problems. In addition, late-stage PD patients often suffer from non-motor symptoms exacerbated by dopamine replacement therapy, such as PD-psychosis characterized by delusions and visual hallucination. Over a one-year period, we have characterized the gradual development of signs of PD-psychosis and motor signs in two 6-OHDA lesioned marmosets in association with dopaminergic pharmacotherapy. In parallel, neuronal activity was recorded in distributed neuronal circuits in different parts of the cortex, basal ganglia and thalamus. Our results indicate that both motor and non-motor symptoms were differentially displayed by the two monkeys but showed a relatively consistent pattern for each individual throughout the one-year recording period. Brain recordings revealed a broad increase in firing rates in both sensory and motor circuits in the pharmacological on-state and a relative suppression of beta-band activity in motor structures. Intriguingly, towards the second half of the year, a distinct high-frequency oscillation (at approximately 130 Hz) in the local field potentials of the subthalamic nucleus developed, which became even more prominent following dopaminergic stimulation. Our observations

demonstrate that primates like the common marmoset, through their human-like behaviors, offer a special opportunity to study symptoms of psychosis. Based on the obtained brain recordings, we propose that oscillatory activity in the high gamma band should be further investigated as a potential pathophysiological factor associated with both hyperkinetic and psychotic symptoms in PD.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 450.22

Topic: C.03. Parkinson's Disease

Title: In vivo extracellular recordings of dopamine substantia nigra neurons in a human Lewy Body-based Parkinson Disease mouse model

Authors: *J. BOEHM¹, J. ZALDIVAR-DIEZ^{1,2}, J. A. OBESO^{2,3,4}, J. ROEPER¹;

¹Johann Wolfgang Goethe-University Frankfurt, Frankfurt a.M., Germany; ²HM CINAC (Centro Integral de Neurociencias Abarca Campal), Hosp. Universitario HM Puerta del Sur, HM Hospitales, Madrid, Spain; ³Univ. San Pablo-CEU, Madrid, Spain; ⁴CIBERNED, Inst. de Salud Carlos III, Madrid, Spain

Abstract: Parkinson Disease (PD) is the second most common neurodegenerative disease exhibiting the characteristic neuropathological hallmarks of dopaminergic neurodegeneration in the Substantia nigra as well as the occurrence of cytoplasmic inclusions called Lewy bodies (LB). LB contain several different proteins such as hyperphosphorylated alpha-synuclein, ubiquitin and p62. Our aim was to functionally characterize a PD mouse model previously introduced by Recasens et al. (2014) where human Lewy bodies (LB) extracted from postmortem human PD brains were infused into the substantia nigra (SN) of adult mice. In contrast to vehicle-infused controls, LB-infused mice showed a progressive loss of TH-immunoreactivity as well as neurodegeneration in the SN starting at 4 months post-injection. Furthermore, it was shown that misfolded human alpha-synuclein recruited endogenous murine alpha-synuclein to form Proteinase-K-resistant misfolded aggregates within the SN. To identify potential electrophysiological differences between dopamine SN neurons in male LB-infused and vehicle-infused control mice, we chronically implanted multiple stereotrode bundles into the medial SN 4-5 months after infusion (N=8). After implantation and one week of recovery, we recorded from identified DA SN neurons (>50% inhibition of mean firing rate after Quinpirole i.p.) in awake mice during open-field exploration. In total, we collected data from n=35 DA SN neurons in vehicle infused control mice (N=4) and n=35 DA SN neurons LB-infused mice (N=4). The median firing rate of DA SN neurons in LB-infused animals was 4.5Hz in comparison to a lower median firing rate of 3.3 Hz in the vehicle infused mice. Other spike train parameters like

variability (CV), mean burst frequency or spikes fired in bursts were very similar between the two groups. We are currently implanting additional mice (male and female) and will also record at several time points to capture the development and progression of a potentially hyperexcitable DA SN phenotype in the human LB-based PD model.

Disclosures: **J. Boehm:** None. **J. Zaldivar-Diez:** None. **J.A. Obeso:** None. **J. Roeper:** None.

Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 450.23

Topic: C.03. Parkinson's Disease

Title: Clinical and histological evaluation of a plant species (Montanoa) in the process of healing superficial wounds in murine model applicate in Parkinson's disease.

Authors: ***G. REYNOSO GÁLVEZ**¹, **P. VERGARA ARAGÓN**², **E. A. RODRÍGUEZ PÉREZ**², **S. GALAVIZ HERNÁNDEZ**², **V. GALLEGOS HERNÁNDEZ**², **F. GARCÍA VALDÉS**², **B. HERNÁNDEZ TÉLLEZ**³, **R. BUSTAMANTE GARCÍA**⁴;
¹UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, ³BIOLOGIA CELULAR Y TISULAR, ⁴FACULTAD DE QUIMICA, ²UNAM, MEXICO CITY, Mexico

Abstract: Healing and regeneration processes take place in all parts of the human body, while regeneration describe the specific substitution of the tissue, i.e. the superficial epidermis, mucosa, skin repair displays an unspecific form of healing in which the wound heals by fibrosis and scar formation. In the long life expectancy patient with Parkinson can occurred wounds for diverse causes that interfering with quality life, aggravate condition and reduce life expectancy. Rats and mice represent an ideal preclinical model to study new products. However, wound healing in a mouse is fundamentally different to that of humans where the repair process is then dependent on epithelialization, cellular proliferation and angiogenesis, which closely mirror the biological processes of human wound healing, allows for testing of promising agents that may promote rapid healing like Montanoa Grandiflora, which have been considered as promising systems of phytopharmaceutical administration by the pharmaceutical industry, mainly because they are biocompatible, available in nature, nontoxic and economical in its elaboration in order to satisfy the demand presented today. Healing of skin wounds is a highly complex process aimed at recovering the integrity of the tissue, allowing its regeneration and restoring its functions.

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Poster

450. Clinical and Preclinical Strategies in Parkinson's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 450.24

Topic: C.03. Parkinson's Disease

Support: ANR-16-CE37-0007-03

Title: Structural STN-SMA connectivity accounts for the modulation of automatic inhibition and movement initiation by subthalamic stimulation

Authors: *P. BOULINGUEZ¹, G. M. MEYER¹, G. LIO³, A. BELIAKOVA⁴, M. ALBARES², G. POLO⁶, B. LAU⁷, S. THOBOIS⁶, B. BALLANGER⁵;

¹Ctr. de Recherches en Neurosciences de Lyon, Univ. Claude Bernard Lyon 1, Bron, France;

²Ctr. de Recherches en Neurosciences de Lyon, Univ. Claude Bernard Lyon 1, Lyon, France;

³Inst. des Sci. Cognitives, Bron, France; ⁴Ctr. de Recherches en Neurosciences de Lyon, Lyon, France; ⁵Ctr. de Recherches en Neurosciences de Lyon, Bron, France; ⁶Hop. Neurologique P. Wertheimer, Lyon, France; ⁷Inst. du cerveau et de la moelle épinière, Paris, France

Abstract: As an input station of the basal ganglia, the subthalamic nucleus is a key component of the different cortico-basal networks involved in movement control. Subthalamic deep brain stimulation (STN-DBS) in Parkinson's disease (PD) is an efficient therapy for the management of refractory motor symptoms including movement initiation, but it can have adverse effects like impulsivity since it also modulates executive networks supporting inhibitory control. To date, the impaired ability to suppress ongoing actions has not been found to correlate with the beneficial effects of stimulation on movement initiation (e.g., Lofredi et al., 2021), and impulsivity is viewed as an independent side effect. However, response inhibition mechanisms other than stopping have been mostly ignored. Here, we test automatic non-selective inhibition, a function intended to suppress any kind of response when the context is uncertain, whose modulation might account for both the improvement of movement initiation and the greater difficulties in refraining from reacting. We assessed the effects of STN-DBS in 19 Parkinson's disease patients with regard to I) behavioral changes in a motor inhibitory task (Go/NoGo), II) electroencephalographic changes analyzed at the source level, and III) structural connectivity between the stimulated area of the subthalamic nucleus and the supplementary motor area (SMA). STN-DBS decreased reaction times and increased commission errors. These behavioral changes were associated with a modulation of an electrophysiological marker of automatic response inhibition in the supplementary motor complex, and were correlated with the number of fibers connecting the volume of tissue activated in the stimulated area of the STN and the upstream SMA. Our study shows that STN-DBS, by modulating automatic inhibition within a functional network that does not fully overlap with the stopping network, facilitates movement initiation by reducing action restraint. Only the markers of impulsive behavior under DBS were found to correlate with the connectivity strength between the stimulated volume of the STN and the SMA, but not the clinical markers of motor improvement (UPDRS-III total or bradykinesia subscore). Such dissociation might offer opportunities to better balance between the therapeutic benefits and the adverse effects of stimulation as a function of fiber-specific neuromodulatory effects.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 451.01

Title: WITHDRAWN

Poster

451. Tau: Cellular and Molecular Mechanisms I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 451.02

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: The Swedish Research Council
Knut and Alice Wallenberg Foundation
Västerbotten county council

Title: A novel RT-QuIC method for prion-like SOD1 aggregation associated with ALS pathogenesis

Authors: *L. LEYKAM¹, T. BRÄNNSTRÖM¹, P. M. ANDERSEN², P. ZETTERSTRÖM¹;
¹Med. Biosci., ²Clin. Sci., Umeå Univ., Umeå, Sweden

Abstract: During ALS disease propagation the ubiquitous enzyme superoxide dismutase 1 (SOD1) can misfold and aggregate. Strong evidence supports the theory, that SOD1 aggregation can drive ALS pathogenesis in a prion-like template assisted manner. This leads to accumulation of differently structured aggregates, referred to as strains. These neurotoxic aggregates have a defined conformation composed of a tightly packed core and loose ends extruding on the outside. This allows us to define anti-peptide antibody binding patterns, using an in-house developed method, called binary-epitope mapping (BEM). We have identified two distinctive pathological strains, A and B in transgenic (tg) mouse models expressing mutant human SOD1 (hSOD1). Data obtained from inoculations of these strains into the ventral horn of tg mice, suggests a seeding-nucleation mechanism behind SOD1 aggregation *in vivo*. The collection of pathological strains from patient post-mortem tissue and tg mice in high quantities remains to be a great challenge due to the degeneration of motor neurons during disease progression. Previous efforts to produce these aggregates *in vitro* resulted in structural differences when compared to *in vivo*

aggregates obtained from tg mice. This is also observed for other neurodegenerative conditions, which are associated with protein inclusions, such as Parkinson or Alzheimer's disease. Thus, we developed a novel Real-time quaking induced conversation protocol (RT-QuIC) to produce disease relevant strain A and B aggregates *in vitro*. Aggregates isolated from tg mice are used as seeds binding unfolded, recombinant hSOD1 and induce the proteins to adopt pathological conformations. This leads to accumulation of misfolded SOD1 and to the exponential growth of fibrils. These fibrils fragment and serve as templates, which initiate further aggregation. We found that seeding with strain A or B aggregates results in the formation of aggregates with the same structure as the *in vivo* template, which confirms that we are able to mimic the aggregation process. By combining *in vitro* SOD1 aggregation with our unique BEM method for structural characterization, we are able to produce pure, strain specific aggregates in larger quantities. These aggregates can be used for inoculations studies to prove neurotoxicity or detailed structural analysis. Additionally, reseeded of *in vitro* produced strain A aggregates leads to formation of aggregates with a new structural profile after the 3rd generation, which we call strain C. This is a strong indicator that SOD1 aggregates can adapt additional pathological conformations distinct from the ones we know today.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 451.03

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: Sarm1 depletion elongates lifespan in a mouse model of tauopathy

Authors: *E. MILIOTOU¹, M. SHEETZ¹, Y. KOMURO¹, J. T. WANG², J. D. HINMAN¹;
¹Neurol., UCLA, Los Angeles, CA; ²Dept. of Neurobio., Stanford Univ., Stanford, CA

Abstract: Axonal pathology represents an early and common pathogenic event among human tauopathies. However, the molecular mechanisms underlying axonal degeneration in neurodegenerative disease are unknown. *Sarm1*, a NAD hydrolase, is required for programmed axonal degeneration and activated in response to specific axonal injuries. Prior work demonstrates that *Sarm1* deficiency enhances axonal survival in ageing, traumatic nerve injury, and traumatic brain injury. In this study, we hypothesized that genetic deletion of *Sarm1* could reduce the phenotypic effects of mutant human tau by reducing axonal degeneration and blunting neurodegeneration. To address this hypothesis, the effects of *Sarm1* deletion on neuropathological changes associated with tauopathy were evaluated in *Sarm1*-null:hemizygous P301S mutant mice. This novel strain combines the PS19 mouse model expressing P301S mutant human microtubule-associated protein tau with *Sarm1*-het or *Sarm1*-null mice. The genetic background of this mixed strain retains the 97.8% C57Bl/6 background of the donor strains.

Notably, median survival in wild-type P301S+ mice (255 days, $n = 8$) is significantly and progressively extended by genetic deletion of *Sarm1* alleles: *Sarm1*-het: P301S+ (335 days, $n = 29$); *Sarm1*-null:P301S+ (368 days, $n = 54$); $p=0.002$ by log-rank test). No apparent sex differences in survival were noted. Immunohistochemistry using antibodies against phospho-tau species suggests that *Sarm1* deletion reduces phosphorylated tau proteins in the hippocampus and cortex at 14 months of age. In contrast to findings in other mouse models of neurodegenerative disease, these findings support the hypothesis that *Sarm1* deletion enhances survival expectancy and neuronal survival by reducing the presence of aggregated, phosphorylated tau. The molecular mechanisms of axonal degeneration remain understudied in neurodegeneration. Inhibition of degenerative pathways in axons may identify novel pathways associated with tau-mediated neurodegeneration.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 451.04

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: R01AG073133 to GT.

Title: Investigating the role of LRP1 in modulating the interaction between A β and tau oligomers at the synaptic interface

Authors: *S. KADAMANGUDI¹, A. FRACASSI¹, M. MARCATTI², G. TAGLIALATELA³; ¹Univ. of Texas Med. Br., Galveston, TX; ²Neurol., UTMB, Galveston, TX; ³Neurol., Univ. of Texas Med. Br. Dept. of Neurol., Galveston, TX

Abstract: Background: The aggregation and propagation of amyloid- β (A β) and pathological tau in the brain remain hallmark features of AD neuropathology, with the extent of tau spreading correlating significantly with cognitive decline. The molecular interplay between A β and tau has been long postulated, however the precise effect of A β on pathological tau spreading remains unresolved. Recent studies elucidate the role of Low-density Lipoprotein Related Receptor 1 (LRP1), a post-synaptic endocytic receptor, in mediating tau uptake and propagation. The role of LRP1 in AD has been characterized extensively in the context of APP and A β metabolism, however, there exist no investigations on whether LRP1 modulates synaptic A β -tau interplay. To this end, we investigated the influence of A β oligomers (A β O) on synaptic binding and uptake of toxic tau oligomer (TauO), as well as the role of LRP1 in this interplay, in human synaptosomes. Methods: We employed synaptosomes isolated from autopsy frontal cortex specimens of 3 non-demented individuals with no evidence of AD neuropathology. First, synaptosomes were incubated with TauO (2.5 μ m) alongside various concentrations of fluorescent labeled preformed

A β O (0, 2.5uM, 5.0uM, and 10.0uM). The same design was then repeated in the presence of the LRP1 inhibitor RAP (0, 0.5uM, 1.0uM, 2.5uM, and 5.0uM). After extensive washing, the resulting oligomer binding and uptake (measured as residual oligomer presence after treatment with PK) was determined using flow-cytometry and EM.

Results: First, we found significantly increased synaptic TauO binding and uptake in the presence of 5.0uM (~12x bound, ~3x uptake; p<0.05) and 10.0uM (~14x bound, ~2x uptake; p<0.05) A β O. Second, we observed a modest decrease in TauO binding in the presence of 0.5uM RAP (~0.5x; p=0.08), but no significant differences in TauO binding or uptake between any of the other concentrations of RAP tested. A qualitative assessment of EM negative stain and immunogold images validated the quality of our synaptosome preparations as well as the possibility of synaptic TauO uptake.

Conclusion: Collectively, our results show that synaptic binding and uptake of TauO was increased by A β O. The mirrored gradient of synaptic TauO binding and uptake suggest shared molecular pathways moderated by A β O, however, this phenomenon does not appear to require tau uptake via LRP1 in these conditions. Nevertheless, these results corroborate the idea that in AD progression, high levels of A β O promote engagement of TauO at synapses, which may underscore increased TauO toxicity at late disease stages.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 451.05

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Leon Levy Postdoctoral Fellowship (HTE)
RainWater Foundation Tau Leadership Fellow Award (HTE)
NIH grant NS121786 (EK)

Title: Dysregulated translation in tauopathies

Authors: ***H. T. EVANS**, S. VENKATESAN KALAVAI, E. KLANN;
Ctr. of Neural Sci., New York Univ., New York, NY

Abstract: Protein synthesis is a vital biological process, important for many neuronal and cognitive processes such as synaptic plasticity and the formation, updating and extinction of long-term memories. Recent studies have identified dysregulated translation as a hallmark of many neurodegenerative diseases, including tauopathies such as Alzheimer's disease (AD), frontotemporal dementia (FTD), and corticobasal degeneration (CBD) (Evans *et al.*, *EMBO J*, 2019; Evans *et al.*, *Acta Neuropathoc Comms*, 2021; Elder *et al.*, *Commun Biol*. 2021). This dysregulation is thought to be driven in part by pathogenic alterations to tau, a neuronally

enriched microtubule binding protein. Here we utilize a series of *de novo* proteomic analyses, including non-canonical amino acid (NCAA) tagging, surface sensing of translation-based ribosome speed of elongation (SunRiSE), and polysome profiling, to explore how mutant human tau alters translation in FTD-patient derived inducible pluripotent stem cells (iPSCs) and induced neurons, as well as in transfected HEK293 cells. Using these techniques, we demonstrate that both P301S and V337M mutant human tau can severely impair protein synthesis, even when protein degradation is inhibited. Our data also suggests that the dysregulated translation is caused, at least in part, by impaired elongation, with SunRiSE assays showing that P301S and V337M mutant human tau both slow elongation rates. In addition to this, we also demonstrate that mutant human tau can impair ribosomal complex formation. Together, our results demonstrate that FTD-mutant tau can severely impact several important components of the cellular translational machinery. Furthermore our results also contribute to the growing evidence that impairments in protein synthesis are a hallmark of neurodegenerative diseases. This work was supported by the Leon Levy foundation (H.T.E.), the Rainwater foundation (H.T.E.) and NIH grant NS121786 (E.K.).

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Poster

451. Tau: Cellular and Molecular Mechanisms I

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Program #/Poster #: 451.06

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

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T32 AG058522

Title: Lysine 63-linked ubiquitination of tau oligomers contribute to the pathogenesis of Alzheimer's disease

Authors: *N. PUANGMALAI¹, U. SENGUPTA¹, N. BHATT¹, S. GAIKWAD¹, M. MONTALBANO¹, A. BHUYAN², S. GARCIA⁴, S. MCALLEN⁵, M. SONAWANE¹, C. JEREZ¹, Y. ZHAO³, R. KAYED¹;

¹Neurol., ²Sch. of Med., ³Dept. of Intrnl. Med., Univ. of Texas Med. Br., Galveston, TX; ⁴Univ. of Texas Hlth. Sci. Ctr., Houston, TX; ⁵Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Ubiquitin-modified tau aggregates are abundantly found in human brains diagnosed with Alzheimer's disease (AD) and other tauopathies. Soluble tau oligomers (TauO) are the most

neurotoxic tau species that propagate pathology and elicit cognitive deficits, but whether ubiquitination contributes to tau formation and spreading is not fully understood. Here, we observe that K63-linked, but not K48-linked, ubiquitinated TauO accumulate at higher levels in AD brains compared to age-matched controls. Using mass spectrometry analyses, we identified 11 ubiquitinated sites on AD brain-derived TauO (AD TauO). We found that K63-linked TauO are associated with enhanced seeding activity and propagation in human tau-expressing primary neuronal and tau biosensor cells. Additionally, exposure of tau-inducible HEK cells to AD TauO with different ubiquitin linkages (wild type, K48, and K63) resulted in enhanced formation and secretion of K63-linked TauO, which was associated with impaired proteasome and lysosome functions. Multi-pathway analysis also revealed the involvement of K63-linked TauO in cell survival pathways, which are impaired in AD. Collectively, our study highlights the significance of selective TauO ubiquitination, which could influence tau aggregation, accumulation, and subsequent pathological propagation. The insights gained from this study hold great promise for targeted therapeutic intervention in AD and related tauopathies.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 451.07

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH UL1 TR002535
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the Boettcher Foundation

Title: Hiv and fiv glycoproteins increase cellular tau pathology via cgmp-dependent kinase ii activation

Authors: *M. DOOLITTLE¹, M. F. SATHLER¹, J. A. COCKRELL¹, I. R. NADALIN¹, F. HOFMANN², S. VANDEWOUDE¹, S. KIM¹;

¹Colorado State Univ., Colorado State Univ. Molecular, Cell. & Integrative Neurosciences, Fort Collins, CO; ²Tech. Univ. of Munich, Munich, Germany

Abstract: As the development of combination antiretroviral therapy (cART) against human immunodeficiency virus (HIV) drastically improves the lifespan of individuals with HIV, many are now entering the prime age when Alzheimer's disease (AD)-like symptoms begin to manifest. It has been shown that hyperphosphorylated tau, a known AD pathological characteristic, is prematurely increased in the brains of HIV-infected individuals as early as in their 30s and that its levels increase with age. This suggests that HIV infection might lead to

accelerated AD phenotypes. However, whether HIV infection causes AD to develop more quickly in the brain is not yet fully determined. Interestingly, we have previously revealed that the viral glycoproteins HIV gp120 and feline immunodeficiency virus (FIV) gp95 induce neuronal hyperexcitation via cGMP-dependent kinase II (cGKII; also known as PRKG2) hippocampal neurons. Here, we use cultured mouse cortical neurons to demonstrate that the presence of HIV gp120 and FIV gp95 are sufficient to increase cellular tau pathology, including intracellular tau hyperphosphorylation and tau release to the extracellular space. We further reveal that viral glycoprotein-induced cellular tau pathology requires cGKII activation. Taken together, HIV infection likely accelerates AD-related tau pathology via cGKII activation.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

Location: SDCC Halls B-H

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Program #/Poster #: 451.08

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH Grant 9R01NS096785-06
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University of Michigan Protein Folding Disease Initiative

Title: Rtl8 facilitates stress-induced nuclear localization of the ubiquitin-adaptor protein, ubqln2

Authors: H. MILAGANUR MOHAN¹, H. TRZECIAKIEWICZ², A. PITHADIA¹, E. CROWLEY¹, N. SAFREN³, H. L. PAULSON¹, ***L. M. SHARKEY**¹;
¹Neurol., Univ. of Michigan Med. Sch., Ann Arbor, MI; ²Michigan State Univ., Grand Rapids, MI; ³Neurol., Northwestern Univ., Evanston, IL

Abstract: The brain-expressed ubiquilin protein, UBQLN2 is one member of a family of ubiquitin adaptor proteins that participate broadly in protein quality control (PQC) pathways, including the ubiquitin proteasome system (UPS). UBQLN2, has been implicated in numerous neurodegenerative diseases including ALS/FTD. UBQLN2 typically resides in the cytoplasm but can translocate to the nucleus under heat shock, proteotoxic stress and disease. UBQLN2 translocation to the nucleus in a mouse model of Huntington's disease promotes the clearance of nuclear aggregates of mutant huntingtin, suggesting that UBQLN2 plays an important role in nuclear PQC. How UBQLN2 translocates to the nucleus and clears aberrant nuclear proteins, however, is not well understood. In a mass spectrometry screen to discover UBQLN2 interactors, we identified a family of small (13 kD), highly homologous, uncharacterized proteins, retrotransposon Gag-like 8 (RTL8). We confirmed the interaction between UBQLN2 and mRTL8A, an RTL8 family member, both *in vitro* using recombinant protein and *in vivo* using mouse brain tissue. When co-expressed with UBQLN2, mRTL8A promotes nuclear translocation

of UBQLN2. UBQLN2 and mRTL8A colocalize within ubiquitin-enriched subnuclear structures containing protein quality control components. Fluorescence recovery after photobleaching (FRAP) experiments show that the colocalized UBQLN2/RTL8 puncta are highly dynamic, phase separated condensates. The robust effect of mRTL8A on the nuclear translocation of UBQLN2 does not extend to the other brain-expressed ubiquilins, UBQLN1 and UBQLN4. Moreover, compared to UBQLN1 and UBQLN4, UBQLN2 preferentially stabilizes RTL8 levels in human cell lines and in mouse brain, supporting functional heterogeneity among UBQLNs. As a novel UBQLN2 interactor that recruits UBQLN2 to specific nuclear compartments, RTL8 may regulate UBQLN2 function in nuclear protein quality control.

Disclosures: H. Milaganur Mohan: None. H. Trzeciakiewicz: None. A. Pithadia: None. E. Crowley: None. N. Safren: None. H.L. Paulson: None. L.M. Sharkey: None.

Poster

451. Tau: Cellular and Molecular Mechanisms I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 451.09

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: 1R21AG069475-01A1
AARFD-22-926379

Title: In vivo neuronal deficits and microglial structural dynamics in the course of tau pathology and during antibody treatment

Authors: *A. MARTIN-AVILA;
Physiol. and Neurosci., NYU Grossman Sch. of Med., New York, NY

Abstract: In vivo neuronal deficits and microglial structural dynamics in the course of tau pathology and during antibody treatment

Authors

Alejandro Martin-Avila and Einar M. Sigurdsson, New York University Grossman School of Medicine, New York, NY.

Disclosures

Alejandro Martin-Avila: None. **Einar M. Sigurdsson:** None

Abstract

A β and tau aggregates are hallmarks of Alzheimer's disease (AD) and tau pathology correlates better with the degree of dementia than A β plaques. Several tau immunotherapies are in clinical trials but their mechanism of action is unclear. Microglia are the professional phagocytes of the brain that help remove dead cells and protein aggregates but not much is known about how microglia interact with cells accumulating tau or with tau-antibody complexes. While reactive microglia are found in close proximity to tau aggregates, microglia away from tau lesions do not display such an activated phenotype. Microglia are highly dynamic and their motility has been

shown to be modulated by neuronal activity. However, both the functional neuronal deficits as well as the structural dynamics of microglia have not been well explored in the course of tau pathology or during tau immunotherapy. In this study, by using two-photon *in vivo* imaging in head-restrained mice attached to a custom-made, free-floating treadmill, we visualize activity in L2/3 pyramidal neurons and microglia dynamics at structural level in the motor cortex during tau pathology progression and plan to monitor microglia-mediated phagocytic clearance of tau-antibody complexes. To achieve that, we crossed the *Thy-1^{GCaMP6}* mice and *Cx3cr1^{GFP}* mice with the transgenic (Tg) PS19 tauopathy P301S mouse model. We have found that while L2/3 pyramidal neurons from young *Thy-1^{GCaMP6}:nonTg* mice (1 to 4 months old) increased their somatic activity during running periods as expected, their littermates *Thy-1^{GCaMP6}:PS19* mice failed to do so. In addition, we found that although tau pathology is evident in the cortex and hippocampus in 2-3 months old *Cx3cr1^{GFP/+}:PS19* mice, their microglia morphology did not differ from control littermates *Cx3cr1^{GFP/+}:non-tg* mice. We are examining these neuronal and microglial parameters in older mice and following tau antibody treatment. Our current findings indicate that functional deficits in cortical neurons associated with tau pathology start early in PS19 mice without structural changes in microglia, which suggests that neuronal deficits linked to tau accumulation precede microglial activation.

Disclosures: A. Martin-Avila: None.

Poster

451. Tau: Cellular and Molecular Mechanisms I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 451.10

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH R01NS088485
VA Merit I01RX002340
American Federation for Aging Research

Title: Damaged PERK affects Tau protein aggregation in Tauopathy brain diseases

Authors: *K. XU¹, G. PARK^{1,2}, L. CHEA¹, K. KIM², L. SAFARTA¹, J. H. LIN^{2,1};
¹Pathology, Stanford Univ., Palo Alto, CA; ²VA Palo Alto Healthcare Syst., Palo Alto, CA

Abstract: Tauopathies, including Alzheimer's Disease (AD) and Progressive Supranuclear Palsy (PSP), are neurodegenerative disorders with complex symptoms and intricate underlying pathologies characterized by Tau deposits in the central nervous system (CNS). The endoplasmic reticulum (ER) stress and Unfolded Protein Response (UPR) are strongly associated to tauopathies. Accumulation of misfolded tau proteins alters ER homeostasis and triggers UPR as an adaptive response. The UPR involves three regulator proteins, including protein kinase RNA-like endoplasmic reticulum kinase (PERK), which are subsequently induced to restore the ER stability. Interestingly, recent genome-wide association studies (GWAS) show PERK as a

genetic risk factor associated with AD and PSP, raising attention to PERK's role in tauopathies. Despite increasing understanding of the structure and function of pathogenic Tau and PERK protein, the correlation between Tau aggregation and PERK is unclear. Based on our previous finding of Tauopathy-associated PERK variants, which shows impaired function, we further examined the role of PERK in regulating Tau aggregation in AD and PSP. Using computational tools, we analyzed the population distribution of PERK variants, predicted the pathogenicity of PERK mutations, and assessed structural modeling to identify functional changes in PERK variants. Next, we tested an in vitro cell culture model, TauRD(P301S)-YFP biosensor cells, to investigate the effect of PERK signaling on Tau aggregation combined with RNA-seq analysis. We also measured Tau and PERK protein levels and PERK-dependent RNA expression levels in human AD patient brain tissues. Our bioinformatic study identified several disease-associated PERK variants with a striking differential frequency between different racial/ethnic groups. Moreover, the R240H mutation, which is associated to AD, was found to be pathogenic and resulted in the loss of several H-bonds. The biosensor cells infected with pathogenic AD brain lysates induced Tau aggregation. In addition, RNA-seq revealed lower PERK- and IRE1-related gene expression in late-stage AD. The biochemical study of human AD brain tissues reveals a significant reduction of PERK phosphorylation in late-stage AD brains compared to non-AD controls. Pharmacological activation and inhibition of PERK in biosensor cells uncovered that PERK inhibition promotes tau aggregation, while PERK activation limits tau aggregation. Our finding unravels a novel PERK-regulated mechanism related to Tau pathology, offering a potential new therapeutic target in treating AD-related tauopathies.

Disclosures: **K. Xu:** None. **G. Park:** None. **L. Chea:** None. **K. Kim:** None. **L. Safarta:** None. **J.H. Lin:** None.

Poster

451. Tau: Cellular and Molecular Mechanisms I

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Program #/Poster #: 451.11

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH Grant AG054025
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Title: Disease-specific brain derived tau oligomers show differential effects on synaptic functioning

Authors: *N. MORENO^{1,2}, A. BITTAR^{1,2}, U. SENGUPTA^{1,2}, N. BHATT^{1,2}, F. LO CASCIO^{1,2}, M. MONTALBANO^{1,2}, N. PUANGMALAI^{1,2}, A. LIMON^{1,2}, R. KAYED^{1,2};
¹Dept. of Neurol., ²Mitchell Ctr., Univ. Of Texas Med. Br., Galveston, TX

Abstract: Alzheimer's disease (AD) is histopathologically characterized by amyloid β ($A\beta$) and tau accumulation as amyloid plaques and neurofibrillary tangles, respectively. Tau oligomers are thought to be the major neurotoxic species in AD, and recent studies have demonstrated that these oligomers can form conformers (prion-like strains) with varying levels of neurotoxicity. To determine the relative neurotoxicity of tau conformers associated with different diseases, we investigated the effect of brain-derived tau oligomers (BDTOs) from multiple tauopathies on synaptic functioning. Here, we isolated BDTOs from AD, dementia with Lewy bodies (DLB), and progressive supranuclear palsy (PSP) brain tissues and performed electrophysiological recordings to investigate the effect of these BDTOs on neuronal transmission and long-term potentiation (LTP). Our results demonstrate that BDTOs negatively impact neuronal transmission and LTP. Interestingly, the AD, DLB, and PSP BDTOs had differential effects on LTP, likely due to unique protein-protein interactions occurring within each tauopathy. Ultimately, these results suggest that the formation of distinct tau oligomeric strains may contribute to the development of disease-specific phenotypes. Further investigation into the influence of strain type on neurotoxicity is needed.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 451.12

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: Single-nuclei RNA sequencing reveals a unique astrocytic population in sporadic progressive supranuclear palsy

Authors: *H. W. RESSLER, K. WHITNEY, K. FARRELL, A. C. PEREIRA, B. ZHANG, W.-M. SONG, A. SHARMA, E. WOODOFF-LEITH, D. DANGOOR, A. CERVERA, J. F. CRARY;

Icahn Sch. of Med. At Mount Sinai, Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Neurodegenerative diseases represent a monumental public health crisis, with misfolded accumulation of tau protein as a prominent pathological disturbance. Tauopathies comprise many neurodegenerative diseases including Alzheimer disease (AD) and progressive supranuclear palsy (PSP). PSP is distinct from other tauopathies in that it is characterized by essentially pathognomonic tufted astrocytes, which occur distinctively in certain subcortical brain regions, especially the subthalamic nucleus (STN) among others. Understanding the molecular changes in astrocytes in PSP has the potential to elucidate the pathogenesis and show how it overlaps and differs from other tauopathies. To understand the unique astrocytic changes in patients with PSP, we employed single-nuclei RNA sequencing (snRNA-seq) on human post-

mortem brain tissue in a cohort of autopsy-confirmed PSP cases and sex and age-matched controls ($n=3$ each). All subjects were male with a median age of death of 70 years (range 64-73) and median post-mortem interval of 18.1 hours (range 11.7-43.8). Single-nuclei RNA sequencing was performed on fresh frozen tissue from the STN region and analyzed using Seurat in R. Cells with greater than 35% mitochondrial RNA and greater than 3,750 features were excluded. 19 clusters were identified using 40 principal components. 9 clusters were glial, 6 were neuronal, and 4 were classified as other. Across all clusters, 450 genes were differentially expressed, with 226 genes upregulated and 224 genes downregulated in cases compared to controls. From our clustering analysis, 2 major astrocytic populations emerged. One was classified as “healthy” and the other as “stressed” due to its elevated proportion of mitochondrial reads. Intriguingly, these stressed cells had increased expression of known tufted astrocyte markers. Subcluster analysis of the stressed astrocytic cluster revealed a large astrocyte population unique to PSP. This subcluster was highly enriched for known reactive astrocyte markers, and the cluster’s associated genes were significantly elevated in the cases compared to the controls across all clusters. Pending further validation, these results may provide important mechanistic insight into the cellular changes underlying tau aggregation in PSP.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

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Program #/Poster #: 451.13

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH Grant AG067741
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Title: Ssh1-mediated suppression of the nrf2 antioxidant pathway in cellular and in vivo models of tauopathy

Authors: *S. CAZZARO^{1,3}, J. A. WOO¹, T. LIU¹, S. REGO³, X. WANG², D. E. KANG^{1,3,4},
¹Pathology, ²Case Western Reserve Univ., Cleveland, OH; ³Univ. of South Florida, Tampa, FL;
⁴Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH

Abstract: SSH1-mediated suppression of the Nrf2 antioxidant pathway in cellular and in vivo models of tauopathy
Authors* S. CAZZARO^{1,2}, J. A. Woo¹, T. LIU¹, S. REGO², K. MCGILL-PERCY¹, V. ZHAO¹, D. E. KANG^{1,2,3}; ¹Case Western Reserve University, Cleveland, USA; ²University of South Florida, Tampa, USA; ³Louis Stokes Cleveland VA Medical Center,

Cleveland, USA.

Disclosures S. Cazzaro: None. J. A. Woo: None. T. Liu: None. S. Rego: None. K. McGill-Percy: None. V. Zhao: None. D. E. Kang: None.

Abstract Neurodegenerative diseases such as Alzheimer's disease (AD) and tauopathies are characterized by the accumulation of misfolded proteins, neuronal loss, mitochondrial dysfunction, autophagy impairment, and oxidative stress. Nuclear factor erythroid 2-related factor 2 (Nrf2), a redox-sensitive transcription factor that upregulates the expression of antioxidant, anti-inflammatory, and autophagy-related genes in response to oxidative damage, is reduced in the hippocampi of AD brains. While the loss of Nrf2 promotes protein aggregation and neurodegeneration, Nrf2 activation ameliorates pathology and cognitive impairment in neurodegenerative disease models. Sequestosome1/p62 (SQSTM1/p62) is a scaffolding protein mutated in the spectrum of ALS-FTD and best known as a selective autophagy cargo receptor activated by phosphorylation on Ser403, which plays a critical role in the clearance of multiple autophagic cargoes. However, SQSTM1/p62 also plays a vital role in cellular oxidative stress response upon phosphorylation of SQSTM1/p62 on Ser349, which increases its affinity for Keap1, thereby competitively releasing Nrf2 for nuclear translocation. We recently showed that Slingshot homolog 1 (SSH1), a protein phosphatase traditionally known for its role in actin dynamics through cofilin activation, dephosphorylates SQSTM1/p62 at pSer403, thereby inactivating SQSTM1/p62-mediated autophagy. In this study, we show that SSH1 simultaneously inhibits Nrf2 antioxidant signaling through a mechanism involving SQSTM1/p62 dephosphorylation, Keap1-Nrf2 complex formation, and SSH1-Nrf2 interaction, resulting in cytoskeletal sequestration of Nrf2 and inhibition of Nrf2 activation in cellular models, animal models, and human patient brains. In the PS19 mouse model of tauopathy, loss of SSH1 significantly mitigates markers of oxidative stress, increases Nrf2 nuclear to cytosol ratio, mitigates tauopathy, and rescues long-term synaptic plasticity deficits. These results elucidate a new function of SSH1 that plays a significant negative regulatory role in the Nrf2 antioxidant pathway relevant for AD and other tauopathies.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

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Program #/Poster #: 451.14

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH/NIA R01AG067762
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NIH/NIA R01NS082730

Title: Mapping the tau protein interactome using the BioID2 approach

Authors: *A. ATWA^{1,2}, M. ALHADIDY^{1,2}, B. COMBS¹, J. LAMP¹, I. E. VEGA^{1,2}, N. M. KANAAN^{1,2,3},

¹Translational Neurosci., Michigan State Univ., Grand Rapids, MI; ²The Neurosci. Program, Michigan State Univ., East Lansing, MI; ³Hauenstein Neurosci. Ctr., Mercy Hlth. St. Mary's, Grand Rapids, MI

Abstract: Aberrant aggregation of tau protein is a hallmark of neurodegenerative diseases collectively known as tauopathies, of which the most common is Alzheimer's Disease. Although tau protein is most commonly known as a microtubule-associated protein involved in regulating microtubule dynamics, accumulating evidence suggests tau likely plays roles in many other biological functions. Deciphering the tau protein-protein interactome is a critical step toward better understating the physiological and pathological roles of tau. This work aims to identify tau interacting partners using the BioID2 method that allows *in situ* protein labelling and identification of protein-protein interactions by biotin-targeted pulldown and mass spectrometry. We generated lentiviruses expressing; a) fusion proteins between full-length human tau (htau40) with BioID2 on either the N-terminus (Myc-BioID2-ht40) or C-terminus (ht40-BioID2-HA) of tau, and b) the respective controls Myc-BioID2 and BioID2-HA. Embryonic day 18 tau knockout (TKO) primary cortical neurons were plated at a density of 3.6E+06 cells per plate. TKO primary cortical neurons were transduced on the 4th day in vitro (DIV4), and lysates were collected on DIV12 (n=3 biological replicates for each lentiviral transduction). Protein lysates were used for biotin-targeted pulldown and mass spectrometry analysis. The following criteria were used to define proteins as ht40-BioID2 or BioID2-ht40 interactors: 1) being identified in at least two of the independent replicates, and 2) being detected at ≥ 1.5 -fold increase compared to the respective BioID2 control. Utilizing this approach, we identified 269 potential interactors with ht40-BioID2 and 169 potential interactors with BioID2-ht40, of which 66 proteins were identified in both ht40-BioID2 and BioID2-ht40. Gene Ontology (GO) enrichment analysis mapped protein interactions in the cytoskeleton, mitochondria, cytosol, dendrites, synaptic vesicles, and RNA-binding proteins. These results suggest that this approach can be applied to identify novel protein-protein interactions via an *in situ* labeling method that could facilitate detection of transient and/or weak interactors. Moreover, the identified proteins could help shed light on tau's growing functional roles in neurons under both physiological and pathological states helping to identify potential tau-targeted therapeutic strategies for neurodegenerative diseases.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

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Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: R01AG061800
U01AG061357

Title: Integrated proteomics to map the tau interacting partners in Alzheimer's Disease

Authors: *S. M. SHAPLEY¹, P. BAGCHI¹, C. BOWEN¹, E. B. DAMMER¹, S. RANGARAJU², N. T. SEYFRIED¹;
¹Biochem., ²Neurol., Emory Univ., Atlanta, GA

Abstract: Neurofibrillary tangles (NFTs) comprised of the phosphorylated Tau protein are a core pathological feature of Alzheimer's disease (AD) and several other neurodegenerative diseases collectively termed tauopathies that include progressive supranuclear palsy, corticobasal degeneration, and subtypes of frontotemporal dementia. Physiological tau is involved in a diverse number of processes important for cell function via protein-protein interactions. These include microtubule-stabilization, proteostasis, translation, energy metabolism, and nuclear import. However, under pathological conditions, the gain or loss of Tau protein interaction partners is an important consequence of disease pathophysiology and may result in the 'rewiring' of Tau protein-protein interaction (PPI) networks. Therefore, modeling and identifying Tau interacting partners could reveal signaling mechanisms and therapeutic targets in tauopathies. To gain an unbiased understanding of the aggregated Tau interactors, we generated a split Turbo Tau (sTurbo Tau) proximity labeling system. We chose to express the isolated Tau repeat domain (RD), containing a pro-aggregation P301L tauopathy substitution since the RD is the core of initial Tau fibrils in disease. To assess the model, immunocytochemistry (ICC) was performed in cells expressing the sTurbo Tau construct following biotin labeling. ICC displayed speckle-like Tau positive aggregates in both the cytoplasm and nucleus that colocalized with biotin. Western blot with streptavidin dye also revealed high and low molecular weight biotinylated proteins in cells expressing the sTurbo Tau. To identify these Tau interacting partners, biotinylated proteins were affinity purified and analyzed in triplicate via mass spectrometry (MS), which identified nearly 1,900 proteins enriched in sTurbo Tau lysates compared to controls. Gene Ontology (GO) analysis of these putative Tau interacting proteins confirmed a wide variety of pathways, including cellular structural components, RNA binding, and translation. Overlap of the sTurbo Tau interactors and Tau interacting partners in AD brain lysates revealed 176 shared partners with roles in translation, synaptic processing, RNA-binding, and proteasome function. This overlap included spliceosome proteins, U1-70K, U1A, and SNRPD2 previously shown to aggregate and colocalize with Tau in AD. Collectively we have established a cell-based Tau proximity labeling approach to identify Tau co-aggregation partners relevant to human AD, which provides insight toward novel pathways and therapeutic targets in disease.

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Poster

451. Tau: Cellular and Molecular Mechanisms I

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Program #/Poster #: 451.16

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: Age-dependent mislocalization of Tau in human neurons

Authors: *S. PELUCCHI¹, L. BÖHNKE¹, S. EICHHORNER¹, O. BORGOGNO¹, L. ZHOU-YANG¹, L. TRAXLER¹, J. MERTENS^{2,1};

¹Univ. of Innsbruck, Innsbruck, Austria; ²LOG-G, The Salk Inst., La Jolla, CA

Abstract: Mislocalization of Tau from axons to neuronal cell bodies has been described in several Tauopathies, such as frontotemporal dementia (FTD), as well as during normal aging. Tau has prominent axonal and synaptic functions, but it has also been shown to exhibit specific functions in the nucleus, where is involved in the stress-mediated DNA damage protection. Recent evidence suggests that Tau, under stress conditions, converges at the soma, and further translocates to the nucleus through the nuclear pore complex (NPC). Furthermore, in Tau-overexpressing transgenic mice and in human AD brain tissue, Tau can be hyperphosphorylated and accumulate into the soma where it impairs NCT function and induces nuclear envelope defects. Moreover, it is well known that nuclear pore is a target for cellular aging. Indeed, age-related protein compartmentalization, nuclear transport dysfunction, and transcriptional changes are major regulators of cellular aging. Taking advantage of the induced neurons (iNs) cell reprogramming method, that preserves donor-specific epigenetic features and aging hallmarks of the starting cell type, we analyzed the subcellular distribution of Tau protein. The study of iNs phenotype is allowing us to develop a platform for exploring how changes in nuclear Tau might contribute to neuronal aging and neurodegeneration, and to assess to what extent changes in nuclear-associated Tau might act as a convergence platform for aging and disease. We set up a cohort of iNs deriving from old human donors and from patients carrying familial mutations that lead to tauopathy and compared those to iNs deriving from young human donors. We show here that glutamate excitotoxicity drives Tau into the nuclear compartment to mediate stress-related response in iNs derived from young donors. On the other hand, old iNs showed higher levels of Tau in basal conditions and no difference has been detected after stress stimuli, as in neuronal cells carrying Tau mutations. On the contrary, iNs with Tau mutations showed a lower basal level of Tau in the nucleus coupled with higher Gamma-H2AX spots detection, suggesting a Tau nuclear misfunction.

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Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

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Program #/Poster #: 452.01

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH Grant R21AG065914
NIH Grant U01NS123658

Title: A novel method to detect large-scale cortical activity patterns in freely-moving mice

Authors: *A. DAS¹, S. HOLDEN², J. BOROVIKA¹, J. ICARDI¹, D. PATEL¹, R. PATEL¹, J. RABER², H. DANA¹;

¹Neurosciences, Lerner Res. Inst., Cleveland, OH; ²Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: A central objective of modern neuroscience research is to understand the causal relationship between neuronal activity and cognitive performance in healthy and disease conditions. There are several existing techniques that allow recording of neuronal activity from the mouse brain with single-cell resolution. However, these techniques have several limitations. For example, they require fixing the mouse head under a microscope or the attachment of a recording device to the animal's skull, both of which significantly affect its behavior and brain activity. Here we introduce a novel recording method to monitor brain activity with cellular resolution from freely-moving mice using a new calcium sensor called CaMPARI. We present a new recording method, which does not require head fixation or the attachment of a miniaturized device to the mouse's head, thereby allowing non-restricted movement during recording. We use CaMPARI, a fluorescent calcium sensor that undergoes an irreversible change from green to red emission when increased intracellular calcium concentration coincides with light illumination at 400nm. We used this unique property of CaMPARI to detect large-scale activity from multiple cortical regions when the animal was performing behavioral and cognitive tests. We identified differential activity patterns across motor and somatosensory cortices that were dependent upon the task being performed. For example, somatosensory areas were more active than motor areas in the same mouse for fear conditioning, but not for novel object recognition tasks. When comparing across young and older mice, we found increased firing rates in the older mice. We also found that aged mice expressing the human tau gene showed different brain activity patterns than wild-type aged mice. The new CaMPARI-based recording method expands the capabilities of recording neuronal activity from freely-moving and behaving mice under minimally-restrictive experimental conditions.

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Poster

452. Animal Models and Tau

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Program #/Poster #: 452.02

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH Grant R61NS115102 P51OD011106
T32 GM081061
TL1 TR0023

Title: 3r-tau and 4r-tau isoforms and pT231-tau are expressed throughout the rhesus macaque brain

Authors: ***J. GAMBARDELLA**, W. SCHOEPHOERSTER, V. BONDARENKO, M. EMBORG;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: Tau is a neuronal protein involved in microtubule stabilization and intracellular vesicle transport. Six isoforms are expressed in the adult human brain, which contain either three (3R) or four (4R) microtubule binding regions in a 1:1 ratio. In neurodegenerative disorders termed ‘tauopathies’, hyperphosphorylated tau protein forms pathological aggregates with altered expression of 3R-tau and 4R-tau isoforms. Rhesus macaques are widely used for studying a variety of neurodegenerative disorders, yet little is known about tau expression in their brains. Here we present the neuroanatomical expression of 3R-tau, 4R-tau, and tau phosphorylated at Thr231 (pT231-tau) in healthy rhesus macaques (n=2; 1 female, 1 male; 5.2-6yrs; 6.7-7.45kg). Animals were necropsied by transcardiac perfusion with heparinized PBS followed by 4% PFA. The brains were extracted and processed for double-label immunofluorescence against 3R-tau (#2A1-1F4; 1:50) and 4R-tau (#ab218314; 1:100) or immunohistochemistry against pT231-tau (#ab151559; 1:200). Protein expression was assessed across 16 brain structures: prefrontal cortex, anterior cingulate cortex, primary motor cortex, corpus callosum, internal capsule, anterior commissure, caudate, putamen, substantia nigra, globus pallidi external and internal, thalamus, subthalamic nucleus, amygdala, hippocampus, and entorhinal cortex. 3R-tau and 4R-tau were ubiquitously expressed in the rhesus brain. Both isoforms were observed in neuronal soma and axons in all grey matter brain regions. Generally, 3R-tau expression was robust in neuronal soma while 4R-tau was intense in the surrounding neuropil. 3R-tau and 4R-tau were also observed in oligodendrocytes of the white matter tracts. pT231-tau was also observed throughout the rhesus brain. In grey matter brain areas, pT231-tau was present in neuronal soma and axons. In white matter brain areas, pT231-tau was weakly expressed in glia-like cells. Overall, this study is the first to demonstrate regional and intracellular tau isoform expression in the rhesus macaque brain. The presence of neuronal pT231-tau throughout the brain suggests this expression is endogenous in rhesus macaques. We hope these data will facilitate future studies for understanding and modeling tau and tauopathies in rhesus macaques.

Disclosures: **J. Gambardella:** None. **W. Schoephoerster:** None. **V. Bondarenko:** None. **M. Emborg:** None.

Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 452.03

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: JSPS KAKENHI K1807618
Mitsui Sumitomo Insurance Welfare Foundation Research Grant 2016

Title: A novel mouse model that recapitulates the isoform-specific, pathological propagation of tau

Authors: *M. HOSOKAWA^{1,2}, M. MASUDA-SUZUKAKE², H. SHITARA², A. SHIMOZAWA², G. SUZUKI², H. KONDO², T. NONAKA², T. ARAI³, M. HASEGAWA²; ¹Fukuoka Univ., Fukuoka Univ., Fukuoka, Japan; ²Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; ³Univ. of Tsukuba, Ibaraki, Japan

Abstract: The phenomenon of "prion-like propagation" in which aggregates of abnormal amyloid-fibrilized protein propagate between neurons and spread pathology, is attracting attention as a new mechanism in neurodegenerative diseases. There is a strong correlation between the accumulation or spread of abnormal tau aggregates and the clinical symptoms of tauopathies. Microtubule-associated protein of tau contains a microtubule-binding domain which consists of 3-repeats or 4-repeats due to alternative mRNA splicing of transcripts for the *Microtubule-associated protein of tau* gene. Although a number of models for tau propagation have been reported, most utilize 4-repeat (4R) human tau transgenic mice or adult wild-type mice expressing only endogenous 4R tau and these models have not been able to reproduce the pathology of Alzheimer's disease in which 3-repeat (3R) and 4R tau accumulate simultaneously, or that of Pick's disease in which only 3R tau is aggregated. These deficiencies may reflect differences between human and rodent tau isoforms in the brain. To overcome this problem, we used genome editing techniques to generate mice that express an equal ratio of endogenous 3R and 4R tau, even after they become adults. We injected these mice with sarkosyl-insoluble fractions derived from the brains of human tauopathy patients such as those afflicted with Alzheimer's disease (3R + 4R tauopathy), corticobasal degeneration (4R tauopathy) or Pick's disease (3R tauopathy). At 8-9 months following intracerebral injection of mice, histopathological and biochemical analyses revealed that the abnormal accumulation of tau was seed-dependent, with 3R and 4R tau in Alzheimer's disease-injected brains, 4R tau only in corticobasal degeneration-injected brains, and 3R tau only in Pick disease-injected brains, all of which contained isoforms related to those found in the injected seeds. The injected abnormal tau was seeded, and accumulated at the site of injection and at neural connections, predominantly within the same site. The abnormal tau newly accumulated was found to be endogenous in these mice and to have crossed the species barrier. Of particular importance, Pick's body-like inclusions were observed in Pick's disease-injected mice, and accumulations characteristic of Pick's disease were reproduced, suggesting that we have developed the first model that recapitulates the pathology of Pick's disease. This model recapitulates characteristics of isoform-specific tau pathologies which were seen in Alzheimer's disease, corticobasal degeneration and Pick's disease.

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Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 452.04

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NHMRC Grant - Ideas 1187156

Title: Spatial reference memory deficits in a SARM1 knockout mouse model of aging tau pathology

Authors: *J. F. MCMANUS, N. H. DANIELS, M. V. KURUVILLA, J. M. COLLINS, R. A. K. ATKINSON, M. T. K. KIRKCALDIE, A. E. KING;
Wicking Dementia Res. and Educ. Ctr., Univ. of Tasmania, Hobart, Australia

Abstract: Cognitive decline is accompanied by a breakdown in neuronal connectivity in neurodegenerative disease, often involving axonal dysregulation. Although the cause may vary, common pro-degenerative pathways are often involved in axon loss. One such pathway is mediated by sterile alpha and TIR motif-containing 1 (SARM1) protein, a potent hydrolase for the metabolic cofactor NAD⁺. NAD⁺ depletion puts the axon into a state of metabolic crisis and triggers pro-apoptotic cytokine and chemokine responses - a process called Wallerian degeneration. In the absence of SARM1, this degeneration is delayed or absent. We sought to determine whether this SARM1-dependent degeneration contributes to *in vivo* neuronal pathology by crossing rTg4510 mutant tau (pTau) mice with a SARM1 knockout (KO) line, to determine whether SARM1 deletion ameliorates the cognitive decline observed in the rTg4510 model, assessed by novel object studies and tests of spatial-reference memory. 10 mice (5 male, 5 female) each from four genotypes were assessed: pTau transgenic, SARM1-KO, a group expressing both (pTau/SARM1-KO), and controls expressing neither. Cognitive tests of spatial reference built around novel object recognition were conducted at 6 months and evaluated via two-way ANOVA with multiple comparisons, showed differences in cognitive scores attributable to SARM1-KO. Control mice outperformed pTau in novel object discrimination scoring ($p = .0033$). Unexpectedly, control mice also outperformed SARM1-KO ($p = .0211$). While pTau/SARM1-KO mice showed an improvement in discrimination scoring compared to control relative to pTau animals, no significant effect was observed ($p = .0659$). pTau expressing animals consistently underperform relative to control, supporting extant literature on this rTg4510 cognitive phenotype. A significant interactive effect ($p = .0049$) of the SARM1-KO genotype was observed in control and pTau expressing mice, wherein the absence of SARM1 improved object discrimination in pTau mice, while proving detrimental to control performance. No attributable difference in total exploration time was observed between groups. These results may indicate an ameliorative effect of the SARM1-KO genotype in cognitive decline associated with tau pathology, but also imply a phenotypic cognitive impairment attributable to an absence of SARM1. A thorough understanding of the potential impacts on inhibiting SARM1 activity will be essential before considering it a direct therapeutic target. Further exploration of functional impact of the SARM1-KO genotype will focus on brain pathology and total tauopathy in regions known to be impaired in the rTg4510 model.

Disclosures: J.F. McManus: None. N.H. Daniels: None. M.V. Kuruvilla: None. J.M. Collins: None. R.A.K. Atkinson: None. M.T.K. Kirkcaldie: None. A.E. King: None.

Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 452.05

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH grant R01AG0066211
VA PSHCS ACOS/R

Title: Photoconvertible fluorescent protein-tagged tau exhibits exceptional stability in a *C. elegans* model of proteostasis

Authors: *M. HAN¹, A. SAXTON², N. LIACHKO², S. WALDHERR², B. KRAEMER²;
¹Univ. of Washington Grad. Program In Neurosci., Univ. of Washington, Seattle, WA;
²Geriatrics Res. Educ. and Clin. Center, Veterans Affairs Puget Sound Hlth. Care Syst., Seattle, WA

Abstract: Tauopathies are a group of neurodegenerative diseases pathologically characterized by aggregation of the microtubule-associated protein tau. *Caenorhabditis elegans* is a powerful tool to study tauopathy due to its easily manipulated genetics, short lifespan, ease of imaging, thoroughly documented connectome, and well established functional assays. Currently, many *C. elegans* neurodegenerative disease models utilize multicopy integration of the disease protein to elicit a phenotype, but controlling the level of transgenic expression is difficult. To address this issue, we used conventional transgenic array and recombinase-mediated cassette exchange to generate multicopy and single-copy genomically integrated strains pan-neuronally expressing the photoconvertible protein Dendra2 fused to wild-type human tau as a system for monitoring tau proteostasis. This fluorescently tagged tau model allows immediate tau visualization and enables optical pulse-chase experiments to measure tau turnover *in vivo*. Preliminary pulse-chase experiments in multicopy strains reveal a neuronally expressed tau half-life that is longer than the median lifespan of *C. elegans* (~12 days). Multicopy Dendra2-tau strains display a wide range of tau burden, and thus fluorescence intensity, that correlates with the degree of locomotion deficit in each strain and differs between strains. In addition, known genetic suppressors of tauopathy ameliorate their disease phenotype. While single-copy Dendra2-tau strains lack distinguishable locomotion deficits due to low tau levels, they can be utilized to identify new enhancers of tau, as evidenced by phenotype exacerbation with human wild-type TDP-43 overexpression. In summary, we present single- and multicopy Dendra2-tau models as a novel tool to study tau proteostasis and modifiers of tauopathy.

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Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 452.06

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Forschungskredit UZH (K-86301-21-01)
Swiss National Foundation (310030_207872)

Title: Transcriptional and 3-dimensional analysis of prion diseases pathophysiology

Authors: *G. MIRACCA¹, D. CAREDIO¹, M. CERISOLI¹, P. SCHWARZ², A. AGUZZI¹;
¹Inst. of Neuropathology, Univ. of Zurich, Zurich, Switzerland; ²Inst. of Neuropathology, Univ. Hosp. of Zürich, Zurich, Switzerland

Abstract: Prion diseases (PrDs) are fatal neurodegenerative conditions caused by the deposition of the scrapie prion protein (PrP^{Sc}), a misfolded isoform of the host-encoded protein. After a long incubation period, PrDs show fast and irreversible neurodegeneration, spongiosis and gliosis, causing death in humans within weeks. Based on the prion strain, PrDs have a wide range of pathological manifestations, resulting in a complex umbrella of phenotypes. To understand strain-related differences in PrDs, I will combine 3D imaging on cleared brain tissues with spatial transcriptomic (ST). I will use wild-type mice of both sexes inoculated with 3 different prion strains (RML6, mNS and ME7) and analyse histological and transcriptomic data at 3 stages of the disease: pre-symptomatic, symptomatic and terminal stage. With 3D imaging, I will obtain whole brain maps of prions deposition patterns and gliosis and information on preferential sites where the PrP^{Sc} strains accumulate to exert their pathological effects over time. Areas with major plaques deposition and glial reactivity will then be analysed by ST. Hypothalamus and cerebellum resulted to be the most affected brain areas when looking at prion deposition and gliosis for all prions strains at terminal stage of the disease (n= 5 for each group). We therefore selected these areas for ST and we are now in the process of analysing the sequencing data. From the transcriptional database, we investigate region-specific markers that might function as cofactors to PrP^{Sc} deposition, correlating the expression levels of markers with the vicinity to prion deposits and/or reactive glia. We interrogate glia's pro-inflammatory phenotypes and molecular changes triggered by PrDs over time. We obtain a detailed overview of regional transcriptional changes occurring during prion diseases in both neurons and glia, to reveal pathways and functions altered by and contributing to the disease. As no therapy is yet available for PrDs, the identification of cofactors is essential to advance prion research. Pharmacological targeting of region-specific markers could be a new approach to target prion accumulation. Information on transcriptional changes in neurons and glial cells will elucidate pathological changes in the brain that might precede clinical symptoms, offering further lines of investigation on the disease's early signs. As molecular alterations are potentially reversible, this project offers an anatomical and functional analysis of TSEs pathological progression and a potential new approach for clinical and diagnostic intervention.

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Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 452.07

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: EU/EFPIA/Innovative Medicines Initiative 2 Joint Undertaking (IMPRiND grant No 116060)
France PSP

Title: Brain injection of patient-derived tau extracts induces progressive supranuclear palsy-like pathology and phenotype in primates

Authors: *G. PORRAS¹, M. DARRICAU², T. KATSINELOS³, F. RASCHELLA⁴, T. MILEKOVIC⁴, G. COURTINE⁴, W. MCEWAN³, Q. LI¹, B. DEHAY², E. BEZARD², V. PLANCHE²;

¹Motac Neurosci. LTD, Motac, Cheshire, United Kingdom; ²Inst. of Neurodegenerative Dis., Bordeaux, France; ³Univ. of Cambridge, Cambridge, United Kingdom; ⁴EPFL, EPFL, Geneva, Switzerland

Abstract: Progressive supranuclear palsy (PSP) is a primary tauopathy affecting both neurons and glia and responsible of motor and cognitive symptoms. Recently, it has been suggested that PSP tauopathy may spread in the brain from cell-to-cell, in a “prion-like” manner. However, direct experimental evidence of this phenomenon, and its consequences on brain function, is still lacking in primates. In this study, we derived sarkosyl-insoluble tau aggregates from human PSP brains. We also isolate the same fraction from healthy age-matched control brains. The *in vitro* characterization of PSP-tau extracts demonstrated high seeding activity in P301S-tau expressing cells compared to control extracts. Furthermore, tau aggregates induced *in vitro* by PSP-tau seeds where abnormally phosphorylated (AT8 and AT100 positive), misfolded, filamentous (pFTAA positive) and sarkosyl-insoluble. Then, we bilaterally injected two male rhesus monkeys in the supranigral area with this solution of PSP-tau extract, and two other animals with a control extract. The quantitative analysis of monkeys’ kinematic features revealed that PSP-tau injected macaques exhibited symptoms suggestive of parkinsonism as early as 6 months after injection compared to controls, which then remained relatively stable over an 18-months follow-up period. Object retrieval task showed a tendency for the progressive appearance of a dysexecutive syndrome over time in PSP-tau injected monkeys compared to controls. Macaques were euthanized 18 months after injection and neuropathological analyses were performed. AT8-positive tau inclusions were found only in PSP-tau injected macaques. Characteristic pathological hallmarks of PSP, including neurofibrillary tangles, globose tangles, tufted astrocytes and coiled bodies were found close to the injection sites but also in connected brain

regions usually affected in human PSP. Our results demonstrate for the first time that patient-derived PSP-tau aggregates can induce typical pathological PSP lesions that can in turn, trigger motor and behavior impairments in a primate host. On top of pathophysiological information regarding the “prion-like” nature of the disease, our results provide support for PSP-tau inoculated macaques as relevant animal models to accelerate drug development in this rare neurodegenerative disease.

Disclosures: **G. Porras:** A. Employment/Salary (full or part-time);; Motac neuroscience. **M. Darricau:** None. **T. Katsinelos:** None. **F. Raschella:** None. **T. Milekovic:** None. **G. Courtine:** None. **W. McEwan:** None. **Q. Li:** A. Employment/Salary (full or part-time);; Motac neuroscience. **B. Dehay:** None. **E. Bezard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac neuroscience LTD. **V. Planche:** None.

Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 452.08

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Kaufman Foundation

Title: Impact of reduced gut bacteria on neurodegeneration in a *Drosophila* model of human tauopathy

Authors: O. BAT-ERDENE, L. DIGGAN, J. R. FIGURA, L. GRAY, M. J. HIRST, V. R. WILSON, ***K. M. LOHR;**
Biol., Washington and Jefferson Col., Washington, PA

Abstract: Deposition of the microtubule-associated protein tau is a hallmark pathology of the family of neurodegenerative diseases known as tauopathies, including Alzheimer’s disease, frontotemporal dementia, and chronic traumatic encephalopathy. Ongoing work on mechanisms of tau-mediated neurodegeneration suggest that genetic contributions interact with peripheral or environmental factors to contribute to disease onset and severity. Recently, the gut microbiota has emerged as a potential modifier of brain function in human, rodent, and invertebrate models via changes to neurotransmitter levels and systemic inflammation. We have previously shown that *Drosophila* expressing human tau in neurons show reduced gut motility and an increased bacterial load compared to control animals. Further, tau transgenic flies show activation of the innate immune system as shown by antimicrobial peptide expression. To expand upon these studies, we have grown control and tau transgenic flies in an environment with limited bacterial exposure and show enhanced neurodegenerative outcomes. Taken together, these data suggest that tau transgenic flies have an innate deficit in gut function and that manipulation of the gut microbiota is capable of altering neuronal health in this *Drosophila* model of human tauopathy.

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Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 452.09

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: College of Arts and Sciences, Quinnipiac University
Psychology Department, Quinnipiac University
Biomedical Sciences, Quinnipiac University

Title: Effects of partial or full deletion of the microtubule-associated protein tau or administration of minocycline on *Drosophila Melanogaster* olfactory-based avoidance learning

Authors: K. CASTELL¹, *J. MIRRA¹, G. BURMAN², J. BLAKE³, G. R. TANNER⁵, A. BETZ⁴;

²Biomed. Sci., ³Computer Sci., ⁴Psychology, ¹Quinnipiac Univ., Hamden, CT; ⁵Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT

Abstract: The microtubule-associated protein tau is an important stabilizing component of the cytoskeleton, but it also has the potential to become dysregulated and contribute to the pathogenesis of neurodegenerative diseases such as Alzheimer's' disease, other tauopathies, and neuroinflammatory disorders. Elucidating the loss of function of tau or neuroprotection of inflammation is critical to understanding the mechanisms of neurodegeneration. *Drosophila Melanogaster* genetic mutants, partial deletion or tau knock-out, were used to assess the function of differential tau deletions on associative olfactory learning and memory. We observed both impairments and gain of function depending on the level of tau expression in our learning assay. Along with changes in learning scores, there were differences in protein expression of CREB, PKA, and synapsin in the central nervous system of non-aged flies. Further, we found sex and age-specific differences in olfactory learning and related protein expression. Together our results indicate that the amount of tau has a functional impact on short-term memory with age and sex differences suggesting additional insights into tau's impact on cognitive function over the lifespan.

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Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 452.10

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: RF1 NS117628
CurePSP

Title: Non-selective expression of human tau in the pedunculopontine tegmentum of the rat mimics progressive supranuclear palsy-like pathology and symptomology

Authors: *S. SURESH, M. P. K. LEIGH, S. D. CLARK;
State Univ. of New York, Univ. of Buffalo, State Univ. of New York, Univ. at Buffalo, Buffalo, NY

Abstract: Non-selective expression of human tau in the pedunculopontine tegmentum of the rat mimics progressive supranuclear palsy-like pathology and symptomology Shrutu Suresh¹, Martin P.K. Leigh¹, Stewart Clark¹ Department of Pharmacology and Toxicology, University at Buffalo, Buffalo, New York, USA

No conflict of interest

Progressive Supranuclear Palsy (PSP) is an atypical Parkinsonism. Symptoms include motor impairment, mood changes, and dysexecutive dementia among others. PSP is a tauopathy, characterized by the aggregation of tau protein into neurofibrillary tangles. The tangles are most commonly observed in neurons and glia. The accumulation of tau is greatest in the pons, globus pallidus, caudate, subthalamic nucleus, and substantia nigra (SN), all of which overlap with the projection sites of the pedunculopontine tegmentum (PPT). PSP patients exhibit loss of dopaminergic neurons in the SN, degeneration of the PPT with an extensive loss of cholinergic neurons, and atrophy of the midbrain. The lack of accurate preclinical animal models is a caveat to drug discovery and effective treatments.

Based on previous work, it was hypothesized that an overexpression of human tau (htau) in the neurons of the PPT will produce tauopathy, resulting in PSP-like symptomology and pathology. Previously, we restricted the overexpression of htau to the cholinergic neurons of the PPT, by using Cre-dependent AAV vectors and Chat-CRE rats. However, it is unclear if abnormal tau is restricted to the cholinergic neurons in PSP patients. Therefore, in the current study we used Cre-independent AAV vectors, to nonspecifically overexpress wildtype htau (PSP relevant isoform-1N4R) in the PPT. When compared to controls, at eight months post-AAV transduction, rats with overexpressed htau displayed several PSP-like behavioral deficits: acoustic startle reflex, hindlimb clasp, and vertical descent. PSP patients also have deficits in working memory and behavioral flexibility. Therefore, the cognitive flexibility of the animals was assessed using a rodent task similar to the Wisconsin Card Sorting Task that is used clinically. In addition to behavior, immunohistochemistry assessment is ongoing to assess the extent of tau pathology and determine whether there is loss of SN and PPT neurons. Our findings suggest that non-specific htau expression with the PPT causes PSP-like symptomology. Through our immunohistochemistry results, it will be determined which PPT neuron subtype is the most vulnerable to htau overexpression. Overall, our model serves as a springboard for a future, more comprehensive pre-clinical animal model of PSP.

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Disclosures: S. Suresh: None. M.P.K. Leigh: None. S.D. Clark: None.

Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 452.11

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: DoD Defense Health Agency 311661-2.00-66323

Title: Behavioral and histological outcomes in human tau transgenic laboratory rats after repeated mild blast traumatic brain injury

Authors: *C. KOSTELNIK¹, A. FAN^{1,2}, J. LIU^{1,2}, G. A. CARLSON^{3,4}, J. AYERS^{3,4}, S. B. PRUSINER^{3,4,5}, J. T. MCCABE¹;

¹Dept. of Anatomy, Physiol. & Genet., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; ²Henry M. Jackson Fndn. for the Advancement of Military Med., Bethesda, MD; ³Inst. for Neurodegenerative Dis., ⁴Dept. of Neurol., Univ. of California San Francisco Weill Inst. for Neurosciences, San Francisco, CA; ⁵Dept. of Biochem. and Biophysics, Univ. of California San Francisco, San Francisco, CA

Abstract: Traumatic Brain Injury (TBI) has been called a signature injury sustained by United States Military Service members in recent conflicts, particularly due to the increased incidence in blast exposure. While most cases are classified as mild, many service members experience repeated mild TBI (rmTBI) which has been associated with a heightened risk for early-onset dementia. This chronic neurodegenerative process after rmTBI has been attributed to the abnormal accumulation of tau, an essential microtubule-associated protein. Since tauopathies can currently only be diagnosed post-mortem, the mechanistic link between the initial injury and the ongoing degenerative process is still not well understood. There is a need to develop better preclinical models that encompass both the progression of tau pathology and wide range of behavioral impairments. Such a model would help us better evaluate treatment options, while also augmenting our understanding of causal relationships between exposure to rmTBI, tau accumulation, and eventual cognitive decline. The goal of this study is to create a valid preclinical model of a rmTBI related progressive tauopathy in male and female rats. To accomplish this, we utilized both wild type (WT) rats and rats that carry the abnormal, mutated human tau gene (htau) seen in familial frontotemporal dementia, which has an increased propensity to develop tau neuropathology. A total of sixty-nine male and female rats were exposed to five total blast rmTBIs using the Advanced Blast Simulator (ABS) or sham procedures at two-four months of age and behavioral and histological outcomes were evaluated at ten months post injury. Behavioral measures include the Open Field Test (OFT), Novel Object Recognition Test (NOR), and Y-Maze spontaneous alteration task. We hypothesized that htau rats exposed to rmTBI will present with greater behavioral abnormalities and tau accumulation compared to injured WT and sham controls. We found that injured female htau rats displayed

hyperactivity on the OFT compared to sham htau rats. Injured male htau rats displayed cognitive deficits on NOR compared to sham htau rats. No differences were seen on the Y maze. Preliminary immunohistological studies indicate greater accumulation of phosphorylated tau in the piriform cortex in htau rats compared to WT rats. Our data suggest exposure to rmTBI early in life in rats with a predisposition to tauopathy may contribute to specific behavioral abnormalities at chronic timepoints which may differ by sex. This is the first report of rmTBI in transgenic tau rats.

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Poster

452. Animal Models and Tau

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 452.12

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: CIHR

Title: Tau pathology in the lateral entorhinal cortex-hippocampal circuit alters memory and morphological plasticity

Authors: *A. ASH¹, S. R. BAEK², S. SINGH¹, J. S. SNYDER³;

¹Psychology, ³Dept. of Psychology, ²Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The lateral entorhinal cortex (LEC) is the site of tau accumulation in early stages of Alzheimer's disease and has projections downstream to the dentate gyrus (DG) of the hippocampus. The pathological form of tau has the ability to propagate across synapses, spreading to connected brain regions and correlating with memory deficits and synaptic loss. Downstream to the LEC, the hippocampal DG is the site of ongoing adult neurogenesis, with new granule neurons added showing enhanced plasticity and increased survival compared to older neurons. We are interested in examining the role of neuron age for vulnerability to tau pathology in the LEC-DG circuit. We used an inducible cre-recombinase transgenic mouse model to label neurons born at different ages (development vs. adulthood) with TdTomato, and an AAV injection either expressing wild-type human Tau or a control vector into the LEC to mimic early, localized tau pathology. Following a 4-month incubation, animals are tested for memory deficits specific to the LEC and hippocampus with Novel Object Recognition (NOR) and Novel Place Recognition (NPR) and tissue is immunohistochemically processed to analyze tau levels and cellular morphology in TdTomato-labelled DG neurons. We assessed synaptic changes in DG neurons via measuring dendritic spines, dendritic complexity and mossy fibre boutons. Our findings demonstrate that tau animals perform worse on NOR (LEC-dependent) compared to healthy controls, while no difference is found for NPR (hippocampal-dependent). Morphological results show that both developmentally- and adult-born neurons have an increase

in thin dendritic spines and decrease in mushroom spines in tau animals relative to controls, as well as reduced mossy fibre bouton filopodia length, indicating structural changes that could disrupt synaptic efficiency and plasticity. Our results demonstrate the effect of tau pathology on structure and function of the LEC-DG circuit, with altered synaptic structures and memory performance.

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Poster

453. Therapeutics: ALS and SMA

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 453.01

Topic: C.06. Neuromuscular Diseases

Support: NIH/NINDS Grant R01NS100835

Title: Effect of cell type specific-RAGE ablation on the progression of the disease in hSOD1^{G93A}ALS mice

Authors: N. A. KINSCHERF¹, L. LIU², M. AKTHER¹, Y. YAMAMOTO³, S. YAN⁴, M. PEHAR^{1,5};

¹Med., Univ. of Wisconsin-Madison, Madison, WI; ²Biomed. Sci. Training Program, Dep. Pharmacol. and Exptl. Therapeut., Med. Univ. of South Carolina, Charleston, SC; ³Dept. of Biochem. and Mol. Vascular Biol., Kanazawa Univ. Grad. Sch. of Med. Sci., Kanazawa, Japan; ⁴Dept. of Surgery and Dept. of Mol. Pharmacol. & Therapeutics, Columbia Univ., New York, NY; ⁵Geriatric Res. Educ. and Clin. Ctr. (GRECC) Madison VA Hosp., William S. Middleton Mem. Veterans Hosp., Madison, WI

Abstract: ALS is characterized by the progressive degeneration of motor neurons in the motor cortex, brainstem, and spinal cord. The molecular mechanisms responsible for motor neuron degeneration in ALS remain uncertain. Astrocytes, key regulators of central nervous system homeostasis, play a major role in the progression of the disease. Accordingly, primary astrocytes expressing different ALS-linked mutant proteins, or astrocytes differentiated from fibroblast-derived induced pluripotent stem cells (iPSCs) from sporadic and familial ALS patients, induce the death of motor neurons in cell culture models. We recently showed that the motor neuron death induced by astrocytes overexpressing hSOD1^{G93A} ALS-astrocytes in co-culture involves the activation of RAGE signaling in motor neurons. Accordingly, motor neurons isolated from RAGE-knockout mice were not sensitive to the neurotoxic factor/s derived from ALS-astrocytes. To evaluate the relevance of this neurotoxic mechanism in ALS pathology, we crossed hSOD1^{G93A} mice with RAGE-knockout mice. RAGE haploinsufficiency [hSOD1^{G93A};RAGE(+/-)] preserved hind-limb grip strength during the progression of the disease. However, this beneficial effect on grip strength was not observed in hSOD1^{G93A} mice with complete RAGE ablation [hSOD1^{G93A};RAGE(-/-)]. In addition, genetic RAGE ablation significantly shortened the

median survival of hSOD1^{G93A} mice. Here, we show that total RAGE ablation increases the expression of fibrosis and inflammatory markers in the lung of hSOD1^{G93A} mice, suggesting that altered lung function in hSOD1^{G93A};RAGE(-/-) mice could contribute to shorten the lifespan of these mice. These results indicate the need to target RAGE signaling in a cell type/tissue-specific manner. We explore here the effect of ablating RAGE expression specifically in neurons, astrocytes or skeletal muscle.

Disclosures: N.A. Kinscherf: None. L. Liu: None. M. Akther: None. Y. Yamamoto: None. S. Yan: None. M. Peihar: None.

Poster

453. Therapeutics: ALS and SMA

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Program #/Poster #: 453.02

Topic: C.06. Neuromuscular Diseases

Title: The clinical stage small molecule NPT520-34 improves disease-relevant endpoints in a preclinical transgenic mouse model of amyotrophic lateral sclerosis

Authors: *A. KHAN¹, R. W. JOHNSON¹, C. WITTMER², D. BONHAUS¹, D. L. PRICE¹;
¹Neuropore Therapies Inc., San Diego, CA; ²Luminex Corp., Austin, TX

Abstract: BACKGROUND: NPT520-34 is a novel small molecule under clinical development as a disease-modifying therapeutic for neurodegenerative disorders. Evaluations in the Line 61 (mThy-ASYN) transgenic mouse model of PD demonstrate benefits of NPT520-34 on dysregulated processes common to most neurodegenerative disorders, including neuroinflammation and impaired protein clearance mechanisms, accompanied by accumulation of alpha-synuclein pathology and impaired motor function. Furthermore, no safety issues were identified in a Phase 1 safety evaluation of NPT520-34 in normal healthy human participants dosed twice daily for 14 days (NCT03954600). **OBJECTIVE & STUDY DESIGN:** The objective of the current studies was to explore the effects of NPT520-34 on mortality, health, SOD1 pathology, neuroinflammation and motor function in an advanced symptomatic SOD1-G93A transgenic mouse model of ALS (*B6SJL-Tg(SOD1*G93A)1Gur/J*), which develops progressive ALS-like muscle wasting, weight loss, motor performance deficits, SOD1 aggregate neuropathology, spinal motor neuron degeneration and decreased life span. Best practices for conducting *in vivo* pharmacology studies (blinding, 2 factor randomization of group assignment by weights and grip strength), rigorous statistical analyses, as well as published SOD1-G93A mouse-specific guidelines were followed for all *in vivo* evaluations. Male SOD1 Tg and non-Tg (non-carrier) littermates received vehicle or NPT520-34 (5 mg/kg, IP; N=11-15/group) once daily for 1 month starting at 13 weeks of age. **RESULTS:** 1 month of NPT520-34 administration resulted in improved ALS-relevant endpoints in SOD-G93A transgenic mice with; i) Confirmed reductions in SOD1 protein pathology in spinal cords of NPT520-34 ii) Demonstrated reduced multiple central & peripheral markers of inflammation (glial markers, plasma cytokines) iii)

Normalized marker of inflammation and mitochondria (TSPO) iv) These reduced disease-relevant endpoints were accompanied by reduced study mortality rates and improvements in clinically-tractable motor endpoints (grip strength and gait and balance). **CONCLUSIONS:** A successful disease-modifying therapeutic will rectify multiple pathogenic mechanisms underlying progressive and complex neurological diseases. Taken altogether, our in vivo evaluations of NPT520-34 in transgenic mouse models have provided support for further clinical development of NPT520-34 as a disease-modifying therapeutic for Parkinson's disease (NCT03954600) and amyotrophic lateral sclerosis (ALS) (granted FDA ODD status).

Disclosures: **A. Khan:** A. Employment/Salary (full or part-time);; Neuropore Therapies Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies Inc. **R.W. Johnson:** A. Employment/Salary (full or part-time);; Neuropore Therapies Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies Inc.. **C. Wittmer:** None. **D. Bonhaus:** A. Employment/Salary (full or part-time);; Neuropore Therapies Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies Inc. **D.L. Price:** A. Employment/Salary (full or part-time);; Neuropore Therapies Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies Inc..

Poster

453. Therapeutics: ALS and SMA

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Program #/Poster #: 453.03

Topic: C.06. Neuromuscular Diseases

Support: ALS Association

Title: NPT1220-478, a small molecule inhibitor of TLR2 and TLR9 improves disease-relevant endpoints in the SOD1-G93A transgenic mouse model of amyotrophic lateral sclerosis

Authors: ***R. W. JOHNSON**, A. KHAN, J. K. BOWDEN-VERHOEK, D. W. BONHAUS, D. L. PRICE;
NeuroPore Therapies, San Diego, CA

Abstract: BACKGROUND: Toll-like receptors (TLRs) are positioned within the CNS to play a role in pathogenic processes driving neurodegeneration. Chronic dysregulation of the immune system and mitochondrial dysfunction in patients with ALS is mediated in part by TLR2 and TLR9. Neuropore Therapies has discovered and is developing novel orally bioavailable small molecule dual Toll-like receptor 2 and 9 (TLR2/9) antagonists for the treatment of ALS and other neurodegenerative disorders. **OBJECTIVE & STUDY DESIGN:** In vivo pharmacology

studies were conducted utilizing early symptomatic SOD1-G93A (*B6SJL-Tg(SOD1*G93A)1Gur/J*) transgenic mice to inform the preclinical development of NPT1220-478 as a disease modifying ALS therapeutic. Best practices were followed while conducting these studies (blinding, 2 factor randomization of group assignment by body weight and grip strength using rigorous statistical analyses, and following published SOD1-G93A mouse-specific guidelines). The key studies and associated parameters are presented in Figure 1. **RESULTS:** **Study #1 (2-month efficacy):** There were multiple disease-relevant improvements in NPT1220-478-treated SOD1 tg mice, including: delayed weight loss, reduced mortality rates, improvements in motor function, reductions in SOD1 pathology and reduced markers of inflammation. **Studies #2 & 3 (Survival):** NPT1220-478 treatments resulted in improved median survival rates of 28% (10 mg/kg) and 14-17% (1 & 10 mg/kg) in male and female cohorts of SOD1 tg mice, respectively. **Study #4 (Early biomarker development):** The in vitro TLR2 agonist-provoked plasma cytokine evaluation in samples collected from a 1-month study arm (10 mg/kg; IP) revealed disease-relevant differences in the basal level and functional responses of specific analytes as well as possible treatment-responsive analytes. **CONCLUSION:** These studies with NPT1220-478 in the SOD1-G93A transgenic mouse line provide further rationale for targeting TLR2 & TLR9 as a disease modifying approach for ALS.

Figure 1. Study listing with key experimental parameters

Study	Subjects	Treatment groups (N = 11-16/group; IP ROA)	Regimen	Endpoints
2-month efficacy	Male, SOD1 tg and non-tg littermates	Non-tg/Vehicle SOD1 tg/Vehicle SOD1 tg/1 mg/kg SOD1 tg/3 mg/kg SOD1 tg/10 mg/kg SOD1 tg/30 mg/kg	1x daily for 2 months starting at 9 weeks of age	<ul style="list-style-type: none"> Health (weights, mortality) Grip strength Catwalk Spinal cord(SC) pathology Plasma Cytokines TLR gene expression (SC)
Survival-specific	Male, SOD1 tg and non-tg littermates		1x daily treatments starting at 9 weeks of age	Survival
Survival-specific	Female, SOD1 tg and non-tg littermates		1x daily treatments starting at 9 weeks of age	Survival
Early biomarker development	Male, SOD1 tg and non-tg littermates	Non-tg/Vehicle Non-tg/10 mg/kg SOD1 tg/Vehicle SOD1 tg/10 mg/kg	1x daily for 1 month starting at 9 weeks of age	Whole blood collected on last day 1 hr post-final injection In vitro TLR2 agonist-provoked plasma cytokine profile

Disclosures: **R.W. Johnson:** A. Employment/Salary (full or part-time); NeuroPore Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPore Therapies. **A. Khan:** A. Employment/Salary (full or part-time); NeuroPore Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPore Therapies. **J.K. Bowden-Verhoek:** A. Employment/Salary (full or part-time); NeuroPore Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPore Therapies. **D.W. Bonhaus:** A. Employment/Salary (full or part-time); NeuroPore Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPore Therapies. **D.L. Price:** A. Employment/Salary (full or part-time); NeuroPore Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPore Therapies.

Poster

453. Therapeutics: ALS and SMA

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

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Topic: C.06. Neuromuscular Diseases

Support: NINDS/NIA R56NS112296
Maxine and Winstone Wallin Neuroscience Discovery Fund
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Title: Fda-approved pde4 inhibitors reduce the dominant toxicity of als-ftd-associated chchd10^{S59L} in drosophila and human cells

Authors: *N. KIM, M. BAEK, Y.-J. CHOE;
Univ. of Minnesota, Univ. of Minnesota, Duluth, MN

Abstract: Mutations in coiled-coil-helix-coiled-coil-helix domain containing 10 (CHCHD10) are a genetic cause of amyotrophic lateral sclerosis and/or frontotemporal dementia (ALS-FTD). Using *in vivo Drosophila* models expressing C2C10H^{S81L}, and human cell models expressing CHCHD10^{S59L}, we have identified that the PINK1/Parkin pathway is activated and causes cellular toxicity. Furthermore, we demonstrated that pseudo-substrate inhibitors for PINK1 and mitofusin2 agonists mitigated the cellular toxicity of CHCHD10^{S59L}. Therefore, we have further evaluated various additional small molecule compounds that can modulate the PINK1/Parkin pathway and reduce CHCHD10^{S59L}-induced cytotoxicity. Among these compounds, FDA-approved PDE4 inhibitors successfully reduced CHCHD10^{S59L}-induced morphological and functional mitochondrial defects in human cells and an *in vivo Drosophila* model expressing C2C10H^{S81L}. Multiple PDE4 inhibitors decreased PINK1 accumulation and downstream mitophagy induced by CHCHD10^{S59L} via the cAMP-PKA pathway. These findings suggest that PDE4 inhibitors currently available in the market can be repositioned to treat CHCHD10^{S59L}-mediated ALS-FTD and possibly other related diseases.

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Poster

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Program #/Poster #: 453.05

Topic: C.06. Neuromuscular Diseases

Support: NYSCF: DP2-NS106664

Title: Phenotypic drug screen in human induced pluripotent stem cell (hiPSC)-derived neurons for modifiers of dipeptide repeat-induced toxicity in C9orf72 ALS/FTD

Authors: *C. SONG¹, C. MARQUES¹, J. SUNG¹, B. J. WAINGER²;
¹Neurol., ²Neurology; Anesthesia, Critical Care & Pain Med., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Title: Phenotypic drug screen in human induced pluripotent stem cell (hiPSC)-derived neurons for modifiers of dipeptide repeat-induced toxicity in C9orf72 ALS/FTD

Authors: Catherine Song, Christine Marques, Joon Sung, Brian J. Wainger

Abstract: Expansion of (G₄C₂)_n in the C9orf72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). These (G₄C₂)_n repeats can cause neurodegeneration through a combination of aggregation of RNA foci, dipeptide repeat proteins (DPRs) via repeat-associated non-ATG (RAN) translation, and loss of function of the C9orf72 gene. Antisense oligonucleotides (ASOs) targeting the repeat expansion have shown promise in reducing RNA foci and DPR expression, but current strategies have not yet been sufficient to reduce disease progression in clinical trials. While several studies have evaluated genetic modifiers of such toxicity, less effort has been directed toward small molecule screens. To identify FDA-approved drugs that can be repurposed for C9orf72 ALS/FTD, our study aims to develop and execute a phenotypic screen for modifiers of DPR toxicity in human induced pluripotent stem cell-derived cortical neurons (hiPSC-CNs).

To model DPR toxicity in neurons, we cultured control hiPSC-CNs expressing a nuclear blue fluorescent protein (BFP) marker for optimized neuron counting over time and treated them with increasing concentrations of hemagglutinin (HA)-tagged poly-PR (PR₂₀), poly-GR (GR₂₀), and control poly-GAPR (GAPR₁₀) peptide for 24 hours. Unbiased automatic quantification of BFP+ nuclei using custom scripts in MATLAB and R revealed a significant dose and time-dependent decrease in cell viability with PR₂₀ and GR₂₀, but not a GAPR₁₀ control, consistent with *in vitro* and animal studies demonstrating arginine DPR-induced toxicity (*Z'* 0.75/0.78 for 5nM PR₂₀/GR₂₀ as negative control and no DPR (0.1% DMSO) as positive control, n = 3 biological replicates). Two validated epigenetic drugs, PFI-1 (5uM) and bromosporine (10uM), significantly reduced DPR toxicity within 24 hours and serve as additional positive controls for the screen (*P* < 0.05). We use a small molecule drug screen in 384-well plates using a Molecular Devices ImageXpress Micro Confocal platform and robotic liquid handler. Secondary analyses include analysis of neurite outgrowth and nuclear integrity (nuclear vs. cytoplasmic BFP), as well as immunostaining for PR₂₀ and GR₂₀ localization. Our experiments help identify candidates and potential mechanisms by which poly-PR and poly-GR toxicity in hiPSC-CNs may be mitigated.

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Poster

453. Therapeutics: ALS and SMA

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Topic: C.06. Neuromuscular Diseases

Support: Scientific Research (C) (17K09751)
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Title: Antisense oligonucleotides that prevent the impairment of TARDBP exon splicing and accumulation of TDP-43 caused by loss of nuclear TDP-43 function

Authors: *A. SUGAI¹, T. YAMAGISHI², S. KOIDE², O. ONODERA²;

¹Dept. of Mol. Neuroscience, Brain Res. Institute, Niigata Univ., Niigata, Japan; ²Dept. of Neurology, Brain Res. Institute, Niigata Univ., Niigata, Japan

Abstract: Intrinsically disordered regions (IDRs) are primarily involved in TAR DNA-binding protein 43 (TDP-43; gene *TARDBP*) aggregation, a major pathological feature of amyotrophic lateral sclerosis (ALS). The IDR is encoded by an exon (an intron within an exon) that is spliced upon TDP-43 autoregulation. Exon splicing can be compromised by several ALS-causing *TARDBP* mutations, aging-associated epigenetic alterations, and a decrease in the nuclear TDP-43 amount. Decreased splicing of the exon increases the expression of the IDR, which can cause TDP-43 accumulation. Therefore, to suppress this pathogenic process, we developed antisense oligonucleotides (ASOs) for promoting exon splicing. First, to determine how exon splicing is regulated, we analyzed RNA-Seq data from the ENCODE project, in which 233 RNA-binding proteins (RBPs) were knocked down. We found that the reduction in several RBPs increased splicing of the *TARDBP* exon. Among these, we focused on RBPs that have a high number of binding motifs in the exon. In particular, the binding motifs of heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1), which is reportedly involved in intron retention along with the splicing factor U2AF, was clustered on the 5' side of the exon, whereupon it was confirmed that HNRNPA1 does indeed bind to this region. Therefore, we tested the splicing-promoting effect of ASOs targeting the binding region of HNRNPA1 and found that those targeting the binding region promoted exon splicing, whereas those not targeting this region did not. Next, we tested the effects of the ASOs on HEK293T cells in which chromosome segregation 1 like (CSE1L), a factor involved in TDP-43 nuclear migration, was knocked down. Consequently, TDP-43 mislocalization due to CSE1L reduction decreased the level of exon splicing, but this effect was suppressed by several ASOs. Furthermore, similar TDP-43 mislocalization in the SH-SY5Y human neuroblastoma cell line increased the level of insoluble TDP-43, but ASO that promoted exon splicing suppressed this abnormality without changing the amount of soluble TDP-43. These results indicate that enhancing the splicing of the exon with ASOs may be an effective treatment for ALS.

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Poster

453. Therapeutics: ALS and SMA

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Program #/Poster #: 453.07

Topic: C.06. Neuromuscular Diseases

Title: Targeting Tau Tubulin kinase 1(TTBK1) for TDP-43 pathology in ALS and FTLD

Authors: ***Y. TIAN**¹, **Y. WANG**¹, **J. SUGAM**¹, **M. KOGLIN**², **S. STACHEL**¹, **H. ZHOU**², **B. VOLETI**¹;

¹Merck Res. Labs., West Point, PA; ²Merck Res. Labs., Rahway, NJ

Abstract: TDP-43 pathology is a hallmark of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal lobar degeneration (FTLD). Namely, both diseases feature aggregated and phosphorylated TDP-43 containing inclusions in the cytoplasm and a loss of nuclear TDP-43 in affected neurons. It has been reported that tau tubulin kinase (TTBK)1/2 phosphorylate TDP-43 and TTBK1/2 overexpression induces neuronal loss and behavioral deficits in a *C. elegans* model of ALS. Here we aimed to elucidate the molecular mechanisms of TTBK1 in TDP-43 pathology. TTBK1 levels were observed to be elevated in ALS patients' postmortem motor cortex. Also, TTBK1 was found to directly phosphorylate TDP-43 at disease-relevant sites in vitro, and this phosphorylation accelerated TDP-43 aggregation. Overexpression of TTBK1 in mammalian cells induced TDP-43 phosphorylation and aggregation, which were concurrent with TDP-43 mislocalization and cytoplasmic inclusions. In addition, when TTBK1 was knocked down or pharmacologically inhibited, TDP-43 phosphorylation and aggregation were significantly alleviated. Functionally, TTBK1 knockdown could rescue TDP-43 overexpression-induced neurite and neuronal loss in iPSC-derived GABAergic neurons. These findings suggest that phosphorylation plays a critical role in the pathogenesis of TDP-43 pathology and that TTBK1 inhibition may have therapeutic potential for the treatment of ALS and FTLD.

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Poster

453. Therapeutics: ALS and SMA

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Topic: C.06. Neuromuscular Diseases

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SERIKA FUND

The Tsubaki Memorial Foundation
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Title: A TDP isoform lacking the intrinsically disordered region alleviates in vitro TDP-43 aggregation and corrects aberrant splicing in TDP-43 overexpression mice

Authors: ***T. YAMAGISHI**¹, **S. KOIDE**¹, **Y. YAMADA**¹, **A. SUGAI**², **O. ONODERA**^{1,2};
¹Dept. of Neurology, Brain Res. Institute, Niigata Univ., Niigata, Japan; ²Dept. of Mol. Neuroscience, Brain Res. Institute, Niigata Univ., Niigata, Japan

Abstract: TDP-43 has been identified as a major component of neuronal intracellular inclusion in amyotrophic lateral sclerosis (ALS) patients. TDP-43 aggregation and aberrant splicing mediated by TDP-43 contribute to the pathogenesis of TDP-43 proteinopathies, such as ALS and frontotemporal dementia. The processes of TDP-43 aggregation and TDP-43-mediated splicing involve liquid-liquid phase separation (LLPS). However, little is known about intracellular molecules that modulate LLPS, correct aberrant splicing, and further inhibit TDP-43 aggregation. TDP-43 forms oligomers via its N-terminal domain (NTD), and the intrinsically disordered region (IDR) at the C-terminus contributes to aggregation and the LLPS phenomenon. The IDR can be excluded by alternative splicing, generating a short TDP isoform (sTDP). Thus, we investigated whether sTDP affects LLPS and inhibits aggregation by forming hetero-oligomers with TDP-43 via the NTD and reducing the proximity among IDRs. We purified and analyzed maltose-binding protein (MBP)-fused TDP-43, sTDP, and NTD-deleted sTDP after the cleavage of MBP by TEV protease. We investigated how sTDP affects TDP-43 LLPS, observed as droplets on differential interference contrast microscopy, and TDP-43 aggregation, indicated by a time-dependent increase in turbidity. The size of TDP-43 droplets was reduced in the presence of sTDP but not NTD-deleted sTDP. Aggregation assay revealed that TDP-43 turbidity was increased over time; however, the turbidity of sTDP and NTD-deleted sTDP did not change considerably. The increase in the turbidity of TDP-43 was markedly suppressed by sTDP but not NTD-deleted sTDP. To investigate whether sTDP corrects aberrant splicing mediated by TDP-43 in vivo, we used C57BL/6-Tg(Prnp-TARDBP)^{3cPtrc}/J mice and an adeno-associated virus (AAV) vector expressing sTDP under the control of the hSyn1 promoter. The vector was administered intracerebroventricularly to P1 hemizygous mice. Untreated wild-type and untreated hemizygous mice were used as the control group. RNA was extracted from the cerebral cortex of the mice, aged eight weeks or older, and analyzed by droplet digital PCR for the retention of exon 18 of Sort1 mRNA, one of the splicing targets of TDP-43. In comparison with untreated wild-type mice, untreated hemizygous mice showed the decreased retention of exon 18 of Sort1 mRNA; however, this abnormality was corrected in hemizygous mice treated with the AAV vector. Therefore, sTDP derived from alternative splicing could inhibit TDP-43 LLPS and aggregation and correct aberrant splicing mediated by TDP-43, suggesting that sTDP generation may be a therapeutic approach for TDP-43 proteinopathies.

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Poster

453. Therapeutics: ALS and SMA

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Topic: C.06. Neuromuscular Diseases

Support: UCSD Academic Senate
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Title: Evaluating the neuroprotective role of CK1-dependent TDP-43 phosphorylation in ALS

Authors: *V. KO¹, K. ONG¹, B. GIANG¹, D. KWON², P. LOOS², M. LULLA², J. HARVEY², G. BHAT², S. PARMENTIER-BATTEUR², D. W. CLEVELAND¹, H. YU³, J. RAVITS¹; ¹UCSD, La Jolla, CA; ²Biogen, Cambridge, MA; ³Univ. of Texas, Southwestern Med. Ctr., Dallas, TX

Abstract: ALS is the most common incurable adult-onset motor neuron disease. Despite heterogeneity in familial versus sporadic ALS, over 90% of all patients exhibit the key pathological hallmark of TDP-43 (TAR DNA-binding protein 43) mislocalization from the nucleus to the cytoplasm where it forms hyperphosphorylated TDP-43 aggregates which are suggested to be toxic in neurons. The role of phosphorylation in this pathological mechanism is unknown, such as whether phosphorylation is a driver or consequence of aggregation, and whether phosphorylation is protective or toxic in neurons. In our published study (Krach, *et al.* 2018), we used laser capture microdissection technique to isolate motor neurons in lumbar spinal cords of sporadic ALS patients for RNA-seq and eCLIP analysis. By comparing the list of genes whose mRNA levels correlated with pTDP43 burden and a list of genes whose mRNA is bound by TDP43 in the human brain, we identified the upregulation of casein kinase 1 epsilon gene (CSNK1E gene encoding CK1ε protein) as being tightly correlated with pTDP-43 pathology. Recent literature has revealed a closely related family member, CSNK1D (encoding CK1δ), to be implicated in TDP-43 phosphorylation and toxic aggregation. Thus, we are evaluating the roles of CK1ε and CK1δ in cellular and mouse models that generate cytoplasmic phosphorylated TDP-43 inclusions. We found that inhibiting CK1δ and/or CK1ε kinase activity results in significant reduction of phosphorylated TDP-43 in cell models. We are now furthering our studies with an ALS mouse model to characterize the PK/PD profile and efficacy of CK1 inhibitors to evaluate the potential neuroprotective effect of the CK1 inhibitor as well as provide insight on the role of phosphorylation in pathologic mechanisms of disease. If proof-of-principle is established, CK1 inhibitors could rapidly translate for therapeutic use.

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Poster

453. Therapeutics: ALS and SMA

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Alcyone Therapeutics

Title: Combination therapy approaches for the treatment of Amyotrophic Lateral Sclerosis

Authors: *M. BAIRD^{1,2}, J. CAPORALE¹, H. GIRARD¹, M. SCHWARTZ^{1,2}, X. ZHANG¹, A. REED¹, F. ROUSSEL¹, A. HOWARD¹, K. FAULCONER¹, S. LIKHITE¹, K. MEYER^{1,2};
¹The Abigail Wexner Res. Inst. at Nationwide Children's Hosp., Columbus, OH; ²Col. of Med., The Ohio State Univ., Columbus, OH

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a severe progressive neurodegenerative disease, leading to muscle weakness and paralysis. As no cure exists, the disease is always fatal, with most patients dying within 3-5 years of symptom onset. Approved therapeutics, such as riluzole and edaravone, manage symptoms but treatments that halt disease progression are lacking. One of the challenges in effectively treating ALS is the disease hallmark of non-cell autonomous toxicity. Specifically, aberrant processes in multiple cell types such as motor neurons, astrocytes, and microglia contribute differentially to disease pathogenesis. We have previously shown that AAV9-mediated SOD1 downregulation in motor neurons and astrocytes significantly improves motor function in ALS mouse models and strongly extends their lifespan. Unfortunately, AAV9 does not efficiently target or correct ALS microglia and, despite neuron and astrocyte correction, the ALS mice still died of the disease. Previous studies from us and others have shown that transgenic targeting of microglial inflammation is beneficial in ALS mice. Thus, the optimal therapeutic strategy needs to simultaneously correct motor neurons, astrocytes, and microglia. In this currently ongoing study, we evaluate a novel cell-specific treatment using our previously developed ALS gene therapy in tandem with techniques to modulate microglia inflammation. Utilizing this combination approach, we indirectly target microglia, thereby dampening the chronic neuroinflammation in ALS mice. The single or combined treatment was administered in neonatal SOD1^{G93A} mice by intracerebroventricular (ICV) delivery. We compare the combination treatment against single treatment of the ALS gene therapy. Motor function is tested by rotarod performance and grip strength twice per week. Disease onset, duration, and survival are monitored and compared to appropriate controls. Combination treatment of SOD1^{G93A} mice utilizing AAV9 mediated downregulation of SOD1 and microglia modulation significantly extended survival and delayed onset. These results confirm that the optimal ALS treatment will target multiple key players in non-cell autonomous toxicity and that targeting of inflammatory microglia will be an important approach in future therapeutic development.

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Poster

453. Therapeutics: ALS and SMA

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 453.11

Topic: C.06. Neuromuscular Diseases

Support: Funded in part by a gift from the Ralph L. Smith Foundation (to DAL).

Title: Therapeutic effects of red dragon fruit betacyanins in the G93A hSOD1 mouse model of ALS.

Authors: *C. PENA¹, L. A. KOZA¹, A. C. SMITH¹, A. N. BAYBAYON-GRANDGEORGE¹, C. SARANGI², T. SAVOLT², D. A. LINSEMAN¹;
¹Biol. Sci., ²Univ. of Denver, Denver, CO

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal, progressive neurodegenerative disorder that affects nerve cells in the brain and spinal cord. It is characterized by the loss of cortical and spinal motor neurons and by the presence of oxidative and nitrosative stress, inflammation, and mitochondrial dysfunction, which are thought to be the underlying causes of disease progression. Research on nutraceuticals known as betacyanins, water-soluble pigments found in red dragon fruit, have demonstrated the powerful antioxidant, anti-inflammatory, and free-radical scavenging properties of these compounds; activities which may be beneficial for ameliorating underlying disease pathology and slowing disease advancement in disorders like ALS. The present study aimed to characterize the therapeutic effects of a betacyanin-rich red dragon fruit extract (DFE) in a transgenic mouse model of ALS harboring a point mutation of glycine to alanine at codon 93 in the human Cu, Zn-superoxide dismutase 1 gene (G93A hSOD1). In this animal model, disease onset typically occurs at 90 days old with the development of skeletal muscle atrophy, hind limb weakness (and eventual paralysis), and weight loss; disease end-stage is reached at approximately 120 days of age. G93A hSOD1 mutant mice were treated orally with 5% (v/v) DFE in drinking water ad libitum, from disease onset until end-stage. Each group of mice (n=16; equal number of males and females) were sex-matched littermates, consisting of two mutants (one treated with DFE, one untreated) and a wildtype (WT) control. Body weight was monitored weekly throughout the treatment, and grip strength and rotarod behavioral tests were conducted to assess muscle strength and endurance. The treated G93A hSOD1 mice had a median lifespan of 135 days while their untreated G93A hSOD1 littermates had a median survival of 124.5 days, as assessed by the Mantel-Cox test; this is roughly equivalent to an

extended survival of ~400 days in human lifespan. One-way ANOVA was used to compare body weight differences and showed a conservation of body weight in the treated mutants vs their untreated littermates. Additionally, paired t-test analyses showed a significant preservation of muscle strength and endurance in the G93A hSOD1 mice treated with DFE, when compared to their untreated littermates. We are currently performing histopathological analyses to evaluate the effects of DFE on spinal cord astrogliosis, microgliosis, motor neuron survival, and neuromuscular junction complexity and innervation. Overall, these findings indicate that DFE, or purified betacyanin compounds, could potentially be used as a therapeutic intervention for patients with ALS.

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Poster

453. Therapeutics: ALS and SMA

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Program #/Poster #: 453.12

Topic: C.06. Neuromuscular Diseases

Title: Mir-126 5p as a therapeutic target in als pathology

Authors: *L. ANKOL REDLICH, A. IONESCU, T. GRADUS PERY, E. PERLSON;
Physiol. and Pharmacol., Tel Aviv Univ., Tel Aviv, Israel

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurological disease characterized by muscle atrophy and motor neuron degeneration. Despite some progress, there is currently no effective treatment available for ALS. Although the disease's etiology is not fully understood, it involves a non-cell-autonomous mechanism and alterations in RNA metabolism. In an unbiased screen for miRNAs, we identified the downregulation of miR126-5p levels in ALS models' motor neuron axons and muscles. Overexpression of miR126-5p in primary MNs cultures from SOD1^{G93A} and TDP-43 ALS mice models increases axon growth and survival. We employed a unique compartmental MN-Muscle co-culture system that models the motor unit; and showed that miR126-5p rescues muscle contraction and NMJ activity in SOD1^{G93A} and TDP-43 ALS models. Moreover, using in vivo models, we demonstrated that overexpression of miR126-5p in the spinal cord of SOD1^{G93A} mice reduced MN loss by inhibition of the activated Caspase 3. Further, we identified and verified some miR126-5p targets that encode vital regulatory proteins. Finally, overexpressing miR126-5p in SOD1^{G93A} mice via AAV infection suggests improved behavioral motor activity, weight loss and survival. These findings support the idea that miR126-5p can regulate key degenerative processes, including axon growth and muscle innervation, suggesting that miR126-5p can be targeted for future therapeutic development.

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Poster

453. Therapeutics: ALS and SMA

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Program #/Poster #: 453.13

Topic: C.06. Neuromuscular Diseases

Support: Annexon Biosciences

Title: C1q inhibition reduces neurodegenerative damage, preserves neuromuscular junctions, and improves compound muscle action potential in the SOD1G93A mouse model

Authors: *J. VEREEN, A. TASSONI, V. MATHUR, C. HUYNH, L. KUHN, S. SANKARANARAYANAN, E. CAHIR-MCFARLAND, L. MATTHEAKIS, T. YEDNOCK, Y. ANDREWS-ZWILLING;
Annexon Biosci., Brisbane, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is a sporadic or genetic disease associated with peripheral loss of synaptic connectivity at the neuromuscular junction (NMJ) and central loss of motor neurons. The SOD1G93A mouse model has been widely used to study a familial form of ALS. C1q, the initiating molecule of the classical complement cascade, marks synapses in the central nervous system for glial elimination during normal development, but it also triggers aberrant synapse loss in neurodegenerative disorders. In ALS, C1q also tags the NMJ within the peripheral nervous system prior to its removal by macrophages. We hypothesized that excessive synaptic pruning initiated by C1q contributes to motor deficits in ALS and that pharmacologically inhibiting this process would be beneficial. We treated adult SOD1G93A mice with a C1q-blocking antibody (anti-C1q) from 7 to 16 weeks of age. Treatment for 9 weeks resulted in reduction in C1q level in plasma and spinal cord and muscle tissue, along with inhibition of downstream classical complement activation. Treated mice showed significant preservation of NMJ density, as measured by bungarotoxin labelling of the gastrocnemius muscle, and improvement in the amplitude of compound muscle action potential, demonstrating that C1q inhibition leads to increased synaptic connectivity at the NMJ. Furthermore, anti-C1q treatment reduced Nf-L levels in the cerebrospinal fluid and plasma of SOD1G93A mice compared to those observed in untreated mice, indicating reduced neuronal damage in this model. These findings suggest that inhibition of the classical complement pathway results in reduced Nf-L levels, NMJ preservation, and improved muscle nerve conduction following anti-C1q therapy treatment in the SOD1G93A mouse model of ALS. A Phase 2 study of ANX005, an anti-C1q therapy, in ALS patients is ongoing (ClinicalTrials.gov: NCT04569435).

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holder, excluding diversified mutual funds); Annexon Biosciences. **A. Tassoni:** A. Employment/Salary (full or part-time);; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **V. Mathur:** A. Employment/Salary (full or part-time);; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **C. Huynh:** A. Employment/Salary (full or part-time);; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **L. Kuhn:** A. Employment/Salary (full or part-time);; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **S. Sankaranarayanan:** A. Employment/Salary (full or part-time);; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **E. Cahir-McFarland:** A. Employment/Salary (full or part-time);; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **L. Mattheakis:** A. Employment/Salary (full or part-time);; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **T. Yednock:** A. Employment/Salary (full or part-time);; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **Y. Andrews-Zwilling:** A. Employment/Salary (full or part-time);; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences.

Poster

453. Therapeutics: ALS and SMA

Location: SDCC Halls B-H

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Program #/Poster #: 453.14

Topic: E.09. Motor Neurons and Muscle

Support: 2018R1A5A2025964
2019M3C7A1031867

Title: Transforming Growth Factor- β Inhibitor, Halofuginone has dual effects on amyotrophic lateral sclerosis: inhibition of joint contracture and motor neuron degeneration

Authors: *D.-Y. LEE^{1,2}, J.-J. SUNG^{1,2,3};

¹Dept. of Neurol., Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; ²Dept. of Neurol.,

³Neurosci. Res. Inst., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: As persistent elevation of transforming growth factor- β (TGF- β) promotes fibrosis of muscles and joints, and accelerates disease progression in amyotrophic lateral sclerosis (ALS), we investigated whether inhibition of TGF- β would be effective against both exacerbations. The effects of TGF- β and its inhibitor on myoblasts and fibroblasts were tested in vitro and confirmed in vivo, and the dual action of a TGF- β inhibitor in ameliorating the pathogenic role of TGF- β in ALS mice was identified. In the peripheral neuromuscular system, fibrosis in the muscles and joint cavities induced by excessive TGF- β causes joint contracture and muscular degeneration, which leads to motor dysfunction. In an ALS mouse model, an increase in TGF- β in the central nervous system (CNS), consistent with astrocyte activity, was associated with M1 microglial activity and pro-inflammatory conditions, as well as with neuronal cell death. Treatment with the TGF- β inhibitor halofuginone could prevent musculoskeletal fibrosis, resulting in the alleviation of joint contracture and delay of motor deterioration in ALS mice. Halofuginone could also reduce glial cell-induced neuroinflammation and neuronal apoptosis. These dual therapeutic effects on both the neuromuscular system and the CNS were observed from the beginning to the end stages of ALS; as a result, treatment with a TGF- β inhibitor from the early stage of disease delayed the time of symptom exacerbation in ALS mice, which led to prolonged survival.

Disclosures: **D. Lee:** None. **J. Sung:** None.

Poster

453. Therapeutics: ALS and SMA

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Topic: C.06. Neuromuscular Diseases

Support: MDA grant #603852
The University of Pittsburgh Momentum Funds

Title: Targeting Presynaptic Neuromuscular Function as a Therapeutic Approach for Amyotrophic Lateral Sclerosis

Authors: ***Y. BADAWI**, K. FETZER, Y. LI, Q. ERICKSON-OBERG, S. D. MERINEY;
Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that results in the progressive deterioration and loss of function of the motor neurons leading to paralysis. Studies indicated that synaptic transmission at neuromuscular junctions (NMJs) is reduced in early stages of the disease - before denervation and motoneuron death. Since NMJ synaptic dysfunction precedes denervation and motor neuron death, ALS has been hypothesized to be a “dying-back” neuropathy. To date, there are no treatments for ALS that target improving neuromuscular transmission, which would improve quality of life for ALS patients by enhancing neuromuscular strength. Here, we propose to test a novel Cav2-specific voltage-gated calcium channel gating

modifier that we have developed (GV-58), which we hypothesize will improve neuromuscular function in SOD1^{G93A} mice, a rodent model of ALS. The evidence that neuromuscular activation can be beneficial in preventing the development and progression of neurological disorders comes from studies highlighting the beneficial effect of exercise on the neuromuscular system. Exercise maintains NMJs and improves NMJ recovery from peripheral nerve injury and degenerative changes. Exercise intervention in advanced stage ALS patients is difficult and is hindered by the debilitating symptoms of the disease. Therefore, our strategy is to substitute exercise-based interventions with a pharmacological intervention that enhances the activation of the neuromuscular system. First, we show significant denervation in the epitrochleoanconeus (ETA) muscle of SOD1^{G93A} mice (which has not previously been documented as vulnerable) at the early symptomatic stage (P90) and progressed at the mid-symptomatic stage (P120). Our results also demonstrate impaired magnitude of transmitter release in the ETA muscle of SOD1^{G93A} mice, at the symptomatic stage (P90) (~40% below control levels). Interestingly, we found that treating *ex vivo* nerve muscle preparations from the SOD1^{G93A} mice with GV-58, significantly increased quantal content. This GV-58-mediated increase in synaptic activity may reduce denervation and provide better support for the NMJ. Therefore, we are testing the hypothesis that GV-58 could prove to be a new intervention approach to strengthen synaptic transmission, delay the loss of motor skills, increase the quality of life, and potentially prolong the life-span of ALS patients.

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Poster

453. Therapeutics: ALS and SMA

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Program #/Poster #: 453.16

Topic: C.06. Neuromuscular Diseases

Support: CIHR
HBHL
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Faculty of Medicine - McGill

Title: Exploring ASO therapeutic strategies in treating the ALS-causing FUS^{R521H} variant using zebrafish

Authors: *C. J. RAMPAL¹, K. PALACEK², G. A. B. ARMSTRONG¹;
¹McGill, McGill Univ. Integrated Program in Neurosci., Montreal, QC, Canada; ²McGill Univ., Montreal, QC, Canada

Abstract: Amyotrophic Lateral Sclerosis (ALS) is characterized by the relentless loss of motor neurons in the brain and spinal cord for which there is no effective treatment to slow or halt the disease progression. Dominantly inherited missense mutations in the gene encoding *Fused in*

Sarcoma (FUS), have been identified where FUS is mislocalized from the nucleus to the cytoplasm, often into aggregates. Among the commonly found mutations in *FUS* are the missense mutations occurring in the Nuclear Localization Sequence (NLS) at amino acid position R521. Synthetic antisense oligonucleotides (ASOs) are modified single stranded oligos that work by reducing the expression of target mRNA through endonuclease-mediated transcript knockdown. With success in other neurodegenerative diseases such as Spinal Muscular Atrophy [Nusinersen US Label. FDA. 2016], we believe ASOs may hold great promise for attenuating the devastating disease progression of ALS-associated missense mutations in *FUS*.

Through the use of the CRISPR/Cas9 system, we have generated the analogous FUS^{R521H} variant (fus^{R536H} knockin (KI) mutation in a *Danio rerio* (Zebrafish) model as well as a knockout model ($fus^{-/-}$). We present data characterizing the degenerative phenotype in our adult homozygous R536H variant zebrafish. Comparing motor phenotypes through free swim and forced swim experiments we find at 3 years of age our homozygous fus^{R536H} zebrafish display a significant motor deficit in comparison to both wildtypes (WT) and knockouts. This data is further supported by an autoregulation age-related deficit seen in our homozygous variants found through immunoblot experiments that show an increase of Fus protein in comparison to WT. RNA sequencing was performed on 1-year old WT, $fus^{R536H/R536H}$ and $fus^{-/-}$ spinal cords and mRNA misregulation of synaptic factors was found to be present in mutant spinal cords. Our results, supported by data in ALS-FUS literature, has led us to believe that knocking down mutant Fus in our animals will attenuate the phenotype in our mutants to the same level as our knockouts. We have generated potential *fus*-targeting ASOs and have shown significant knockdown through rt-PCR analysis in larvae. These ASOs will be delivered into the CNS of adult “symptomatic” mutant fish via novel cerebrointraventricular injections and phenotype regression will be analyzed.

These results indicate a degenerative phenotype in our R536H variants that is unique from the behaviour, expression and analysis seen in our wildtype and knockout lines leaving promise for this model to be tested in different experimental therapies and to further our understanding of the biological nature of FUS-associated ALS.

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Poster

453. Therapeutics: ALS and SMA

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Topic: C.06. Neuromuscular Diseases

Support: ERC STARTING GRANT 805426-FutureTrophicFactors
ERC POC 101069256 - Regenera

Title: Mesencephalic astrocyte-derived neurotrophic factor variant delays disease onset and increases survival in a *sod1-g93a* mouse model of amyotrophic lateral sclerosis

Authors: *L. BECKETT, J. KORPIKOSKI, K. VALKONEN, M. H. VOUTILAINEN;
Fac. of Pharm., Univ. of Helsinki, Helsinki, Finland

Abstract: Background: Amyotrophic Lateral sclerosis (ALS) is a progressive neurodegenerative disease characterised by the selective death of upper and lower motor neurons (MNs) in the brain and spinal cord respectively, leading to patient death within 3-5 years. Superoxide dismutase 1 (SOD1) gene mutation is causative in 12% of familial and 2% of sporadic cases of ALS, and is the most studied animal model of ALS. One key component of ALS and the SOD1 gene mutation is the pathological induction of endoplasmic reticulum (ER) stress. Mesencephalic astrocyte-derived neurotrophic factor (MANF) acts as a glucose-regulated protein 78 (GRP78) co-factor and promotes the survival of ER stressed neurons. MANF also acts at the neuroplastin receptor to inhibit the generation of pro-inflammatory cytokines. One key drawback of MANF is that it cannot pass the blood-brain barrier (BBB). Here we have developed a MANF variant (vMANF) which is capable of passing the BBB and is protective both *in vivo* and *in vitro*.

Methods: SOD1 transgenic mouse line B6SJLTg (SOD1*G93A)1Gur/J were grouped in sex, litter and rotarod-behaviour matched groups. Baselines were taken at week 14 and micro-osmotic pumps filled with vehicle (n=15) or vMANF (n=14; 3µg/day) were subcutaneously implanted. To test lumbar motor neuron (LMN) protection a group were sacrificed at week 17 (n=6), paraffin fixed spines were sectioned from L3-5 and choline acetyltransferase (ChAt) positive LMN cell bodies were counted. To assess the *in vitro* effect of vMANF, the NSC-34 cell line were used. Toxin concentration required to kill 50% of the cells (TC50) was used in combination with vMANF.

Results: vMANF treated transgenic mice showed a delay in symptom onset of 17 days, a significant protection at weeks 16 and 17 (p=0.0065 & 0.0004 respectively; Kruskal-Wallis test with Dunn's post-hoc) and a median survival increase of 9 days (p=0.165; Mantel-Cox test). Counting of ChAt positive LMNs showed a significant protection in vMANF treated transgenic mice (p=0.0013; unpaired t-test). *In vitro*, vMANF provides significant protection against toxin-induced apoptosis from 5ng/ml to 5µg/ml (p<0.001 repeated measure ANOVA with Sidak's post-hoc test). vMANF had no effect on naïve cells.

Discussion: Here we show that vMANF is as a neuroprotective compound, capable of passing the BBB. Further to this, we show that subcutaneous infusion of vMANF prolongs life in a mouse model of ALS and delays the onset of clinical symptoms. We show that vMANF protects LMN from SOD1-linked degeneration, and that vMANF has no effect on naïve cells. vMANF shows potential as a promising treatment for ALS, a disease critically lacking in therapeutic options.

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Poster

453. Therapeutics: ALS and SMA

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Program #/Poster #: 453.18

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS069726
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AHA 16GRNT31280014

Title: Impact of NMN supplemented diet on the motor function of SOD1^{G93A} ALS mice

Authors: *S. LUNDT, N. ZHANG, L. POLO-PARADA, S. DING;
Univ. of Missouri, Columbia, MO

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult on-set neurodegenerative disease. ALS causes the degeneration of motor neurons in the motor cortex and spinal cord. Patients with ALS initially experience muscle weakness which leads to paralysis and then death. Nicotinamide adenine dinucleotide (NAD) is one of the most prevalent metabolites in the human body. NAD is most widely recognized for being involved in energy metabolism, such as in glycolysis, the tricarboxylic acid cycle and oxidative phosphorylation, but is also involved in hundreds of different cellular reactions. Importantly, NAD levels are known to decline with age and in neurodegenerative diseases, such as Alzheimer's disease (AD) and ALS. NAD levels can be increased through the administration of intermediate metabolites of NAD biosynthesis, such as nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN). Treatments involving NR or NMN are being investigated for a possible role in treating neurodegenerative diseases, including ALS. We were interested in determining whether supplementing the diet of SOD1^{G93A} (ALS) mice with NMN had any impact on motor function, neuromuscular junction structure and function, and skeletal muscle mitochondrial morphology. ALS mice given an NMN-supplemented diet (ALS^{+NMN}) exhibited delayed motor dysfunction, with impairments to rotarod and hanging wire performance occurring two weeks later than ALS mice fed normal chow. In walking gait tested at 60, 90, and 120 days old, ALS^{+NMN} had increased stride length for both the fore- and hind-limbs compared to ALS mice. When we recorded evoked (EPP) and miniature (mEPP) end-plate potentials from semitendinosus muscles at 18 weeks old, we found that ALS^{+NMN} mice had larger EPP amplitudes and facilitation ratios following stimulation. While quantal content was not affected by an NMN diet, mEPP amplitude and frequency were elevated in ALS^{+NMN} mice. In skeletal muscle, mitochondrial morphology in ALS^{+NMN} mice was significantly improved compared to ALS mice. In summary, these results indicate that dietary consumption of NMN can suppress motor and mitochondrial deficits caused by ALS.

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Poster

453. Therapeutics: ALS and SMA

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Program #/Poster #: 453.19

Topic: C.06. Neuromuscular Diseases

Support: Center for Immunology Grant

Title: Investigating the role of IL-10 and CCL5 in SMA astrocyte-mediated pathology

Authors: *R. L. ALLISON, A. D. EBERT;

Cell Biology, Neurobiology, and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Spinal muscular atrophy (SMA) is a leading genetic cause of infant mortality and is caused by a mutation or deletion of the survival of motor neuron 1 (SMN1) gene, resulting in reduced expression of the ubiquitous SMN protein. Motor neurons (MNs) are particularly impacted by decreased SMN protein levels, and MN loss is the primary phenotypic outcome in SMA patients. SMN deficient MNs show intrinsic deficits in splicing and function, but these defects alone are not sufficient to induce overt MN loss. Our lab has found that astrocytes differentiated from SMA patient-derived induced pluripotent stem cells (iPSCs) secrete high levels of pro-inflammatory ligands into their media (ACM) compared to healthy control (HC) astrocytes and that SMA ACM is capable of inducing MN loss. We also found that SMA iPSC-derived microglia display increased reactive morphology and phagocytic ability when exposed to SMA ACM compared to HC ACM. Within SMA ACM, CCL5 was the most upregulated cytokine compared to HC ACM. CCL5 has been shown in other diseases to activate microglia and induce dysfunction in neurons. In a different MN disease context, we found that microglia conditioned medium with high levels of anti-inflammatory cytokine IL-10 can ameliorate astrocytic effects on neuronal pathology. Based on these data, we hypothesize that reducing CCL5 while increasing IL-10 signaling will ameliorate astrocyte-driven glial activation and MN loss in SMA. In support of this, we found a trend for decreased levels of IL-10 in SMA patient spinal cord compared to HCs. Within astrocytes specifically, we see decreased levels of IL-10 as well as its downstream effectors (phosphorylated STAT3, SOCS3). We also see trends for upregulated transcripts for the CCL5 receptors CCR1 and CCR5 in SMA microglia compared to HCs. Within postnatal day 3 (PND3) spinal cord sections from SMN Δ 7 mice, we found decreased SOCS3 staining compared to HC mice which appears to inversely correlate with GFAP and pNF κ B staining and worsens by PND9. Together, these data support the idea that astrocyte-targeted CCL5/ IL-10 treatments may decrease astrocyte-mediated microglial activation and MN loss in SMA to allow for an extended therapeutic window.

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Poster

453. Therapeutics: ALS and SMA

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Topic: C.06. Neuromuscular Diseases

Support: NSF ASCENT: 610 – 4731000 - 60056702

Title: Intimate and unobtrusive integration of conductive polymer within decellularized nerve

Authors: *A. S. MEHTA, X. XIE, J. TROPP, J. RIVNAY;
Northwestern Univ., Northwestern Univ., CHICAGO, IL

Abstract: Peripheral nerve damage is a common injury affecting 20 million Americans. Nerve gap when unrepaired leads to neuroma and eventually muscle atrophy. Thus, full, and proper nerve regeneration across the nerve gap becomes imperative. Previously designed conduits have shown therapeutic potential with significant limitations e.g., fascicle and bioelectrical mismatch. To overcome such challenges, we developed biohybrid grafts composed of decellularized nerve (DN) and conductive polymer (CP) that can provide both active and passive cues to promote peripheral nerve regeneration (PNR). Rat (female) sciatic nerves were harvested, decellularized, and modified via *in situ* polymerization with CP- poly(3,4, -ethylenedioxythiophene) (PEDOT). Few reports have utilized PEDOT-based composites for peripheral nerve repair, and design rules for such composites remain nascent. Therefore, we first investigated the impact of polymerization conditions on the conductivity of biohybrids (DN+PEDOT(DNP)) with different ratios of ferric chloride (FeCl_3) relative to the monomer EDOT. Four composites (DNP1:0.5), (DNP1:1), (DNP1:2), & (DNP1:4), with increasing concentration of FeCl_3 ranging from 0.5 M to 4 M were fabricated. We found that the 1:1 molar ratio condition provided the biohybrid (DNP1:1) with the highest conductivity of the investigated composites (0.167 mS cm^{-1}). X-ray fluorescence assessed residual iron to be lowest for the composite DNP1:1 (0.548 ppm). Mechanical testing revealed a small increase in young's modulus, DNP1:1 = 46.9 kPa vs. pristine DN = 32.9 kPa. Both Scanning electron microscopy and Fourier-transform infrared spectroscopy demonstrated intact morphology and microstructure. To improve conductivity, DNP1:1 was subjected to more cycles of *in situ* polymerization ranging from 2 to 5 (DNP1:1C2, DNP1:1C3, DNP1:1C4, DNP1:1C5). Compared to DNP1:1, conductivity increased dramatically after cycle 2 (DNP1:1C2= 2.6 mS cm^{-1}). While subsequent cycling increased the conductivity of the biohybrid, cycling had deleterious impacts on stiffness (e.g. DNP1:1C2 = 62.11 kPa vs DNP1:1C5 = 325.79 kPa). We also tested our composites for hemo- and biocompatibility; all tested samples were highly compatible. Taken together we found DNP1:1C2 to be an ideal nerve conduit for peripheral nerve regeneration. Numerical data are presented as mean \pm SD (n=3); statistical significance was evaluated by student *t*-test. This study provides a model example of a biohybrid component that can provide multi-modal cues to promote nerve regeneration. Currently, we are investigating axon projections across the optimized nerve conduit (DNP1:1C2) using 3D cell culture setup.

Disclosures: A.S. Mehta: None. X. Xie: None. J. Tropp: None. J. Rivnay: None.

Poster

453. Therapeutics: ALS and SMA

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 453.21

Topic: C.06. Neuromuscular Diseases

Support: Alcyone Therapeutics, Inc
Sims Family Foundation
Nationwide Children's Hospital

Title: Ssaav9-ighmbp2 gene therapy significantly improves motor performance in severe smard1-like mouse model, nmd^{em3}, and cmt2s mouse model, nmd^{em5}

Authors: ***S. HOLBROOK**^{1,3}, A. HICKS², P. B. MARTIN², G. A. COX^{2,3}, K. C. MEYER⁴;
¹Univ. of Maine/ The Jackson Lab., The Jackson Lab., Mount Desert, ME; ²The Jackson Lab., Bar Harbor, ME; ³The Univ. of Maine, Orono, ME; ⁴Res. Inst. Nationwide Childrens Hosp., Res. Inst. Nationwide Childrens Hosp., Columbus, OH

Abstract: Autosomal recessive mutations in IGHMBP2, a ubiquitously expressed DNA/RNA helicase, have been linked to a spectrum of neuromuscular degenerative diseases (NMDs). C57BL/6J-Ighmbp2^{EM3Cx} is a SMARD1-like mouse strain, or Spinal Muscular Atrophy with Respiratory Distress, created via CRISPR-Cas9 targeting of the *Ighmbp2* gene and hereafter referred to as EM3. SMARD1 is characterized by muscle weakness starting in the distal extremities and diaphragmatic paralysis leading to respiratory failure. Most patients are diagnosed in early infancy and die in early childhood. The EM3 mouse model has an average lifespan of ~3 weeks and displays neuromuscular degeneration in the hindlimbs, intercostals, and diaphragm. C57BL/6J-Ighmbp2^{EM5Cx} is a Charcot-Marie-Tooth disease type 2S model, hereafter referred to as EM5, with a separate mutation in the same gene. EM5 does not have a decreased lifespan but does show impacted motor and sensory function beginning around the 4 week timepoint. In collaboration with the Meyer lab at Nationwide Children's Hospital, we are testing 2 different ssAAV9 vectors expressing human cDNA of *IGHMBP2*. Each has a different promoter with one having a Chicken β -Actin (CBA) Promoter [higher expression levels than endogenous levels] and the other having a truncated Methyl-CpG binding protein 2 (MECP2 aka P546) promoter [expression levels close to endogenous levels expressed by neurons]. We performed postnatal day 1 (p1) intracerebroventricular (ICV) injections on EM3 and EM5 mutants and unaffected sibling pups to determine the efficacy of each treatment, respectively, and if there are toxic effects associated with overexpression of IGHMBP2 in wild type mice. Using a variety of assays to determine strength and neuromuscular degeneration such as wirehang, muscle fiber and nerve analysis of the hindlimbs, and neuromuscular junctions occupancy, we determined that the P546 promoter is more effective in EM3 mice and that either virus causes the EM5 mice to show no significant difference between mutant mice and wildtype mice. Treatment has also been shown to be sensitive to dose in both models with high variability in motor recovery in smaller doses.

Disclosures: **S. Holbrook:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Alcyone Therapeutics, Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Nationwide Children's Hospital. **A. Hicks:** None. **P.B. Martin:** None. **G.A. Cox:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Alcyone Therapeutics, Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Nationwide Children's Hospital. **K.C. Meyer:** B. Contracted Research/Research Grant (principal investigator

for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Alcyone Therapeutics, Inc.

Poster

453. Therapeutics: ALS and SMA

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 453.22

Topic: C.06. Neuromuscular Diseases

Support: Funded by NeuroSense Therapeutics

Title: Shifting the paradigm - PrimeC: A potential disease modifying treatment for neurodegenerative disorders driven by novel biomarkers measuring mechanism of action

Authors: *S. ZIMRI¹, A. PUSHETT¹, N. RUSSEK-BLUM¹, E. EITAN², S. PAGANONI³, J. D. BERRY³;

¹NeuroSense Therapeut., Herzelia, Israel; ²NeuroDex Inc., Boston, MA; ³Sean M. Healey & AMG Ctr. for ALS at Mass Gen. and Neurolog. Clin. Res. Institute, Dept. of Neurol. Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: Neurodegenerative diseases such as ALS have a complex underlying pathophysiology, indicating that a multi-factorial strategy targeting multiple pathways may be needed to achieve positive therapeutic effects. PrimeC is a novel formulation composed of unique doses of ciprofloxacin and celecoxib, aiming to synergistically inhibit the progression of ALS by addressing three key pathologies: microRNA dysregulation, iron accumulation, and neuroinflammation. Ciprofloxacin, a fluoroquinolone antibiotic, is an iron chelator, and a regulator of Dicer activity, a key enzyme in the microRNA processing pathway. Celecoxib, an NSAID, regulates neuroinflammation through COX-2 dependent and independent pathways. Synergistic effect between these two drugs has been previously demonstrated in ALS preclinical models Based on the therapeutic effect of each compound and the synergistic effect of their combination we set out to test the therapeutic effect of PrimeC in people with ALS. The relevance of PrimeC's postulated mode of action in the pathophysiology of ALS was assessed in neuron derived exosomes (NDEs). NDEs have been shown to cross the BBB and enter the blood circulation, enabling their easy isolation from blood samples. They carry neural molecular signatures echoing the content of cells from which they originated, and thereby serve as "real-time" health reflectors. The effect of PrimeC on NDE markers was evaluated clinically in a 12-month, open-label, phase-IIa study in 15-patients with ALS dosed with PrimeC and compared to matched longitudinal non-treated ALS blood samples. Significant differences in ALS-related biomarkers were detected in ALS patients compared to controls at baseline including changes in a cassette of biomarkers that correspond to inflammatory pathology (COX2, NRF2), the neuroinflammatory marker Prostaglandin2, miRNA regulators (DICER, AGO2), and a marker of iron accumulation (Ferroportin1). Furthermore, markers representing autophagy (LC3) and

lysosomal trafficking (CatD) were significantly different in ALS samples compared to controls at baseline. Importantly, significant changes were seen in several of these ALS-related biomarkers following treatment with PrimeC (e.g TDP43 P=0.002, PGJ2 P<0.001 CATD P=0.015, LC3 P=0.05), indicating biological activity of PrimeC on a variety of ALS-related biomarkers. The present study demonstrates that PrimeC mode of action is relevant for the pathophysiology of ALS and that these pathologies can be modulated by PrimeC treatment. Additionally, this study highlights the important role of biomarkers as informative tools to increase the efficiency of ALS clinical studies.

Disclosures: **S. Zimri:** A. Employment/Salary (full or part-time); full time employ, NeuroSesne Therapeutics. **A. Pushett:** A. Employment/Salary (full or part-time); full time employ, NeuroSesne Therapeutics. **N. Russek-Blum:** A. Employment/Salary (full or part-time); part-time, NeuroSesne Therapeutics. **E. Eitan:** None. **S. Paganoni:** None. **J.D. Berry:** None.

Poster

453. Therapeutics: ALS and SMA

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 453.23

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant AI093504
DoD Grant CB10721

Title: Antidotal treatment of botulism using an FDA-approved small molecule

Authors: ***P. MCNUTT**, C. ONDECK, S. O'BRIEN;
Wake Forest Inst. for Regenerative Med., Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: Botulinum neurotoxin (BoNT) is a highly potent, select agent toxin that inhibits neurotransmitter release at motor nerve terminals, causing death by respiratory asphyxiation. There is no specific treatment for botulism symptoms. Botulism patients require intubation and supportive care until recovery, which can take weeks or longer. In previous studies, we found the FDA-approved drug 3,4-diaminopyridine (3,4-DAP) reverses early botulism symptoms and prolongs survival in lethally intoxicated mice. However, the symptomatic benefits of 3,4-DAP are limited by its rapid clearance. Here we investigated the effects of sustained 3,4-DAP treatment in rats challenged with 2.5 LD₅₀ BoNT serotype A (BoNT/A). First, we confirmed repeated injections of 3,4-DAP reduced toxic signs and prolonged survival for over 24 versus vehicle. Rebound of toxic signs and death occurred within hours after the last 3,4-DAP treatment. We next tested whether therapeutic benefits were sustained by continuous infusion of 3,4-DAP. Three infusion dose rates (0.5, 1.0 and 1.5 mg/kg-h) were identified that produced steady-state serum levels of 3,4-DAP consistent with clinical dosing. Dose-dependent effects of 3,4-DAP infusion were then compared in rats given 2.5 LD₅₀ BoNT/A. In contrast to vehicle, which had 100% mortality, infusion of 3,4-DAP at ≥ 1.0 mg/kg-h from 1-14 d after intoxication

produced 94.4% survival, without rebound of toxic signs after infusion was stopped. In contrast, withdrawal of 3,4-DAP infusion at 5 d resulted in death within 12 h. We exploited this novel survival model of lethal botulism to explore neurophysiological parameters of diaphragm paralysis and recovery. While neurotransmission was nearly eliminated at 5 d, neurotransmission was significantly improved at 21 d in 3,4-DAP-infused survivors, although still depressed compared to naïve rats. In conclusion, continuous infusion with 3,4-DAP produced symptomatic and antidotal effects at clinically relevant blood levels in rats challenged with 2.5 LD₅₀ BoNT/A. 3,4-DAP is the first small molecule to acutely reverse paralysis and promote survival in animal models of botulism, thereby meeting a critical treatment need. These data contribute to a growing body of evidence supporting the use of 3,4-DAP to treat clinical botulism.

Disclosures: P. McNutt: None. C. Ondeck: None. S. O'Brien: None.

Poster

453. Therapeutics: ALS and SMA

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 453.24

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant 4R33NS106719-03
MDA Grant 516532

Title: A novel fumarate-based prodrug improves survival and neurobehavioral scores in the leigh syndrome mouse

Authors: *C. MONTGOMERY¹, L. ADCOCK¹, S. DUGAR², G. CORTOPASSI¹;
¹Univ. of California, Davis, Davis, CA; ²Myto Therapeut., Davis, CA

Abstract: Background and Rationale: Leigh syndrome (LS), also known as sub-acute necrotizing encephalomyopathy, is an orphan inherited infantile neurodegenerative condition of mitochondria with no approved therapy, and the NDUFS4 KO mouse is one of the first and the best characterized mouse model of this disease. Leukocytic inflammation is thought to drive pathogenesis in this mouse LS model (PMID: 35050903). The monomethyl fumarate prodrug Tecfidera®, a known Nrf2/Nfe2l2 activator, has been clinically approved for the neuroinflammatory condition Multiple Sclerosis (MS) since 2013. Under MTA we received novel Monomethyl Fumarate (MMF) prodrug MYT-109 from Myto Therapeutics, with a markedly improved pharmacokinetic profile as compared to Tecfidera®. We hypothesized that the improved pharmacokinetic exposure to MMF provided by MYT-109 would improve its pharmacodynamic potency as a Nrf2 inducer and its efficacy as neuroprotective molecule and have tested both in the Leigh Syndrome NDUFS4 KO mice. **Objectives:** Can improved MMF pharmacokinetics result in improved Leigh Syndrome outcomes? Specifically, does improved MMF PK increase pharmacodynamic potency resulting in greater survival than Tecfidera®? **Methods:** NDUFS4 Knockout mice were dosed once daily in peanut butter with either no drug

or added MYT-109 (232mg/kg) starting at 21 days. A subset of animals had tissue harvested at 43 days of age for biochemical analysis. All survival animals stayed on treatment until reaching UC Davis IACUC humane endpoints, with at least eight animals per study group. Tissues were flash frozen and pulverized for all assays. **Results:** NDUFS4 KO mice had deficiencies of Hmox1 and BDNF gene expression in brain. MYT-109 potently induced Nrf2 downstream genes Hmox1 and Nqo1, and also induced BDNF in brain. Significantly increased open field activities (total distance, time in center zone) were observed in MYT-109 dosed LS mice. Furthermore, MYT-109 dosed LS mice had statistically significant improvement in median survival, while Tecfidera® showed no extension in median survival. **Conclusion:** The data above support MYT-109 as a novel therapeutic for the orphan disease Leigh Syndrome.

Disclosures: **C. Montgomery:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Receipt of drug from Myto Therapeutics/Sphaera Pharma. **E. Ownership Interest** (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Myto Therapeutics. **L. Adcock:** None. **S. Dugar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Myto Therapeutics. **G. Cortopassi:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Receipt of drugs from Myto Therapeutics/Sphaera Pharma. **E. Ownership Interest** (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Myto Therapeutics.

Poster

453. Therapeutics: ALS and SMA

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 453.25

Topic: C.06. Neuromuscular Diseases

Title: Repurposing of Trametinib in the SOD1-G93A mouse model of amyotrophic lateral sclerosis

Authors: *S.-Y. LEE, Y. CHUN, H. KIM, S. HAN;
Genuv, Genuv Inc., Seoul, Korea, Republic of

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the selective degeneration of upper and lower motor neurons. Previously, we selected a MEK inhibitor, trametinib (SNR1611), as the most neuroregenerative and neuroprotective substance among FDA-approved small molecule drugs through a phenotype-based screening using neural stem cells. Here, we provide preclinical evidence supporting therapeutic use of trametinib for ALS. Oral administration of trametinib to SOD1-G93A ALS model mice increased motor performance and grip strength and improved survival rate. Trametinib also reduced motor neuronal loss and cell death in the spinal cord. In spinal motor neurons, autophagy was enhanced upon trametinib treatment. Moreover, trametinib reduced the activation of astrocytes and

microglia, indicating its anti-neuroinflammatory capacity in the spinal cord. Finally, we observed that trametinib treatment reduced atrophy in gastrocnemius muscle. The combined data provide clear evidence that MEK inhibition by trametinib slows disease progression of the ALS model mice by enhancing autophagy and thus protecting motor neurons, as we have shown in AD models. Currently, trametinib is a repurposed drug candidate in clinical trial (Phase I/IIa) for the indication of ALS in Korea (ClinicalTrials.gov identifier: NCT04326283).

Disclosures: **S. Lee:** A. Employment/Salary (full or part-time);; Genuv, Inc.. **Y. Chun:** None. **H. Kim:** A. Employment/Salary (full or part-time);; Genuv, Inc. **S. Han:** A. Employment/Salary (full or part-time);; Genuv, Inc..

Poster

454. Neuronal Injury and Death I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 454.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R21NS081182
R01NS097903
R35NS127253
R37NS033123

Title: Ataxin-2 modulates p53-dependent apoptosis

Authors: ***M. M. GANDELMAN**, S. PAUL, W. DANSITHONG, K. P. FIGUEROA, D. R. SCOLES, S. M. PULST;
Dept. of Neurol., Univ. of Utah, Salt Lake city, UT

Abstract: The transcription factor p53 activates complex responses to cellular stress, including neuronal death. p53 increases are common in neurodegenerative diseases, observed both in animal models and human patients, however the triggers and modulators of p53-mediated cell death in neurodegeneration remain poorly understood. Expansions in the polyglutamine tract of Ataxin-2 (ATXN2) cause spinocerebellar ataxia type 2 (SCA2), and intermediate ATXN2 expansions increase risk of amyotrophic lateral sclerosis (ALS) up to 10-fold. Given the initial success in targeting *ATXN2* for treating ALS, we seek to understand the related pathogenic and neuroprotective mechanisms. We characterized the transcriptomic landscape resulting from *ATXN2* knockdown (KD) in HEK293 cells using an siRNA targeting *ATXN2* (siATXN2). Our analysis showed 2187 differentially expressed genes, with a cutoff of 0.5 log₂fold change and a 5% FDR. Hallmark gene set enrichment analysis showed the p53 pathway was negatively enriched after *ATXN2* KD. Ingenuity pathway analysis (IPA, Qiagen) showed that *ATXN2* KD modified the abundance of 90 out of the 98 transcripts annotated in the p53 pathway. Interestingly, IPA adjudicated a highly significant negative z-score to this pathway, indicating its activation would be inhibited, and IPA upstream analysis suggests a role for E2F1 in controlling

p53 signaling in ATXN2 KD conditions. Because *ATXN2* KD is protective in multiple neurodegeneration models, we tested whether siATXN2 could prevent p53-dependent apoptosis triggered by etoposide, a chemotherapy drug that induces DNA damage and causes p53-dependent cell death. Treatment of HEK293 cells with etoposide triggered p53 phosphorylation and an increase in cleaved caspase 3, which were prevented by siATXN2. Similarly, in HEK293 cells modified by CRISPR-Cas9 to express one expanded *ATXN2* allele (*ATXN2*-Q58) *ATXN2* KD prevented p53 apoptosis caused by etoposide. These experiments indicate that targeting either WT or mutated *ATXN2* can decrease p53-dependent apoptosis, and this connection could potentially be harnessed to treat p53-mediated neurodegeneration.

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Poster

454. Neuronal Injury and Death I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 454.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NeurATRIS ANR-11-INBS-0011
ANR-21-CE13-0009-03

Title: Role of SFXN1 in neuronal cell death

Authors: *G. LIOT^{1,2,3}, M.-C. GAILLARD¹, G. AUREGAN¹, M. GUILLERMIER¹, M. GAUDIN¹, S. BERNIER¹, F. PETIT¹, P. GIPCHTEIN¹, C. JAN¹, M. SRIWARAN¹, C. GARDIER¹, N. DUFOUR¹, C. JOSÉPHINE¹, N. BONNEFOY⁴, M.-P. GOLINELLI⁵, A. BEMELMANS¹, K. CAMBON¹, N. LE FLOCH-LELEU⁶, E. BROUILLET¹;

¹CEA MIRCen, Fontenay aux roses, France; ²CNRS UMR9199, Fontenay aux Roses, France;

³Univ. Paris Saclay, Orsay, France; ⁴Inst. of Integrative Biol. of the Cell (I2BC), UMR9198,

CEA/CNRS /UPSaclay, Gif-sur-Yvette, France; ⁵Inst. de Chimie des Substances Naturelles (ICSN) UPR2301 CNRS, Gif-sur-Yvette, France; ⁶Lab. of Genet. and Biol. of the Cell (LGBC)

UR4589 UVSQ/UPSaclay /EPHE, Montigny-le-Bretonneux, France

Abstract: In human, five different sideroflexins (SFXN) form a family of mitochondrial proteins. Despite their involvement in some mitochondrial human diseases, their functions are still not well characterized, especially in the brain. Recently, two functions have been described for these SFXN: a role of mitochondrial serine transporter in the one carbon metabolism pathway and a role in iron homeostasis regulation. Interestingly, in neurodegenerative diseases (Alzheimer, Parkinson and Huntington's diseases), iron accumulation was observed as well as features of ferroptosis, a regulated cell death process involving iron homeostasis deregulation. Our aim is to study if one member of this SFXN family (SFXN1) is implicated in neuronal physiology and neuropathology. We used a mouse neuronal hippocampal cell line, HT22, in

which we induced ferroptotic stress by treating these cells in dose response with high concentrations of glutamate or with an inhibitor of glutathione peroxidase 4 (GPX4), RSL3. A co-treatment with ferroptotic inhibitors (deferrioxamine, ferrostatin-1) or radical oxygen species (ROS) scavenger (N-acetyl-cysteine) blocks the neuronal cell death induced by glutamate or RSL3. To study the role of SFXN1 in these models, we generated stable cell lines by transduction with lentivirus expressing a shRNA control or a shRNA directed against endogenous murine SFXN1. Glutamate toxicity in these stable HT22 cell lines expressing the shRNA SFXN1 was partially blocked compared to HT22 cell lines expressing the shRNA control. To understand which cellular mechanisms are involved in the neuroprotection induced by SFXN1 knock down, we performed western blot. We observed that NRF2 and GPX4, two key molecules involved in ROS detoxification and ferroptosis, were less underexpressed upon glutamate treatment in shRNA SFXN1 expressing cells compared to shRNA control expressing cells. In the laboratory, we developed *in vivo* mouse models of Alzheimer's disease (tauopathy) and Huntington's disease by stereotaxic injection of adeno-associated virus (AAV) in the hippocampus and lentivirus (LV) in the striatum respectively. Our aim is to study the impact of SFXN1 downregulation by shRNA in these two models. First, we verified that SFXN1 is expressed in both structures, hippocampus and striatum. The next step will be to perform *in vivo* experiments to assess the efficacy of SFXN1 downregulation using a lentivirus system and its effect on neurodegeneration. In conclusion, SFXN1 downregulation was shown to be protective against neurodegeneration *in vitro* and will be validated *in vivo* in the near future.

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Poster

454. Neuronal Injury and Death I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 454.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2019R1A2C1011083
KBRI basic research program (22-BR-02-02)

Title: Role of CHFR in neuronal DNA damage responses

Authors: *K. KIM¹, J. SONG², E. LEE³, J. SEOL², M. KIM¹;
¹Korea Brain Res. Inst., Daegu, Korea, Republic of; ²Seoul Natl. Univ., Seoul, Korea, Republic of; ³Ajou Univ., Suwon, Korea, Republic of

Abstract: Neurodegenerative disorders are often associated with uncontrolled DNA damage responses and neuronal dysfunction, including abnormal neuronal apoptosis and neurite shrinkage. Neurons are known to re-enter the cell cycle upon DNA damage, leading to neuronal cell death. However, little is known about how neurons respond to DNA damage conditions. Here, we have identified that CHFR, also known as a cell-cycle checkpoint, is substantially expressed throughout the brain. Based on the high expression of CHFR in neurons, we have investigated whether the stability of CHFR is modulated under DNA damage conditions. We showed that neuronal cell death was augmented in a CHFR-dependent manner, and the expression of death-related genes was also increased under DNA damage conditions. Interestingly, neuronal senescence was elevated in the presence of both CHFR and DNA damage-inducing drug doxorubicin in a dose-dependent manner shown by SA- β -gal (senescence-associated beta-galactosidase) analyses. CHFR methylation levels were decreased by the DNA double-strand break-inducing decitabine treatment, analyzed by the Infinium MethylationEPIC array. We showed that the neurite length was shortened when CHFR was more introduced into mouse primary cortical neurons. Moreover, the neurite length was shorter when pan cyclin-dependent kinase (CDK) inhibitor roscovitine was treated in the presence of CHFR. Therefore, our results suggest that CHFR plays an important role in neuronal DNA damage responses to maintain neuronal integrity. This work was supported by Mid-career Research Program (NRF-2019R1A2C1011083, NRF-2022R1A2C1004326), KBRI basic research program (22-BR-02-02) funded by the Korea government (MSIT).

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Poster

454. Neuronal Injury and Death I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 454.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Exposure to ozone increases caspase 3 and 8, and downregulates Bcl-2 in hippocampus, cerebellum and cortex of rats

Authors: *E. URIBE¹, P. RODRIGUEZ¹, M. RUBIO-OSORNIO², Z. MORALES¹, V. MENA¹, L. HERNANDEZ¹, W. MORENO¹, N. GALLARDO¹, D. FLORES¹, C. RUBIO¹; ¹Neurophysiol., ²Neurochemistry, Inst. Nacional de Neurologia y Neurocirugia, Mexico City, Mexico

Abstract: Ozone (O₃) is one of the main air pollutants on Earth. It is formed by a photochemical reaction of volatile organic compounds (VOC) and nitric oxide synthase (NOS). According to the World Health Organization (WHO), O₃ levels above 100 $\mu\text{g}/\text{m}^3$ for an exposure time of 8 hours or more are harmful. O₃ causes oxidative stress, causing lipid peroxidation, inflammation, metabolic and cell signaling changes, and possibly the onset of cell death in sensitive brain areas. However, its effector mechanisms have not been fully described. It is known that inflammation

and oxidative stress can induce cell death, mostly in the apoptosis pathway. Apoptosis is the programmed cell death, which activates caspases, DNA fragments and forms apoptotic bodies. This process occurs through 2 pathways: intrinsic and extrinsic, in which various proteins interfere. This study aims to recognize the expression of the pro-apoptotic proteins and Bcl-2 in the acute O₃ exposure context in rat's cerebral cortex, cerebellum and hippocampus. Twenty male Wistar rats (250-300g) were divided into two groups. For 12 hours, the control group (n=10) was exposed to pollution-free air, whereas the experimental group (n=10) was exposed to 1ppm of O₃. The rats were sacrificed after the exposure for immunofluorescence and Western Blot analysis. A t-test was used for independent samples and ImageJ processing program for obtaining the arbitrary units and optic density. Pro-apoptotic proteins and Bcl-2 from both groups were compared, finding significant differences in caspase 3 and 8, Bcl-2 and TUNEL (p= 0.000 bilateral), concluding that acute O₃ exposure initiates apoptosis mainly through an extrinsic pathway in the hippocampus, cerebellum and cortex of rats.

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Poster

454. Neuronal Injury and Death I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 454.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ANR-19-CE14-0036 STRESS

Title: Bdnf can cross the cell membrane to control the keap1-nrf2 cytoprotective system

Authors: J. FATH¹, F. BROUILLARD¹, *C. BERNARD³, J.-J. BENOLIEL^{4,2}, C. BECKER¹; ¹T3S, ²T3s, INSERM, U1124, Paris, France; ³INSERM U1106, INSERM U1106, Marseille cedex 05, France; ⁴Service de Biochimie Endocrinienne et Oncologie, APHP GH Sorbonne Université, Site Pitié-Salpêtrière, Paris, France

Abstract: In addition to its well-known receptor-mediated function in cell survival, differentiation and growth, we report here that extracellular brain-derived neurotrophic factor (BDNF) can also control the intracellular KEAP1 (Kelch-like ECH-associated protein 1)-NRF2 (nuclear factor erythroid-derived 2-like 2) cytoprotective system in a receptor-independent manner. Since BDNF crosses the cell membrane at low temperature, energy-dependent entry mechanisms (endocytosis, receptor activation) are ruled out. Extracellular BDNF can cross the cell membrane because its amino acid sequence includes a protein-translocation domain, also known as cell-penetrating peptide (CPP). The CPP sequence of BDNF is necessary and sufficient to confer its cell membrane crossing properties since 1- it enables otherwise impermeant molecules to enter the cell, including peptides; 2- the depletion of this sequence precludes it.

Once in the cytosol, BDNF can directly interact with the KEAP1-NRF2 system, enabling the nuclear translocation of NRF2. This nuclear translocation of NRF2 induced by BDNF is functional since it induces the activation of NRF2 target genes involved in detoxification, drug response, catabolism, and antioxidant defenses. This new property of BDNF to cross the cell membrane and act on KEAP1-NRF2 system has been proved in different cell types, suggesting a ubiquitous mechanism. BDNF is thus more than a neurotrophin acting on cognate receptors. We here unravel its novel function, which is cytosolic, via its protein-protein interaction properties, and which can directly trigger the translocation of NRF2 to the nucleus and the subsequent activation of cytoprotective response. Targeting BDNF action on NRF2 may be of considerable relevance due to the critical role of oxidative stress in most, if not all, pathological states. This study challenges dogma and establishes a new concept in which a protein known to exert its effects by activating its receptors, can also cross the cell membrane to induce a direct cytosolic action. This novel form of communication, whereby a receptor ligand-protein exerts a biological activity by crossing the cell membrane, opens new avenues for cell signaling.

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Poster

454. Neuronal Injury and Death I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 454.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIA/NIDA Intramural Research Programs
Diversity in Aging Research Pipeline Program

Title: Aldehydic products of lipid peroxidation induce cell death in human neural stem cells, neurons and astrocytes

Authors: *E. CALZADA¹, K. DEMEULENAERE¹, P. MIRANDA TAPIA², B. LAING², Y. APONTE², C. RAMSDEN¹;

¹Natl. Inst. on Aging, Baltimore, MD; ²Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: α , β -unsaturated aldehydes are highly reactive compounds present in cigarette smoke, car exhaust, thermally-stressed vegetable oils, and produced via peroxidation of polyunsaturated fatty acids and lipids. Substantive studies in rodents and tissue cultures have demonstrated that aldehyde exposure increases cellular inflammatory responses, impairs memory, and can result in premature death. In Alzheimer's disease (AD) brains, α , β -unsaturated aldehydes, including acrolein and crotonaldehyde (CRA), are detected in the vicinity of amyloid β plaques and neurofibrillary tangles. Collectively, this suggests that reactive aldehydes could contribute to AD; however, there is a paucity of data on the effects of specific aldehydes on the viability of human brain cells implicated in AD pathogenesis. In the present study, we evaluated the impact

of acrolein and CRA exposure in induced pluripotent stem cell-derived neural stem cells (NSCs), neurons (iNeurons), and post-mortem astrocytes. Human induced NSCs were derived from CD34⁺ peripheral blood mononuclear cells that were previously re-programed via Sendai virus transduction of Yamanaka factors and selective growth in neural progenitor medium. NSCs were frozen after 5 passages and used within 10-17 passages for this study. iNeurons were generated from NSCs by growth in neuronal differentiation media for 2 weeks. Cell identity for each cell population was confirmed by western blot and immunocytochemistry of selective biomarkers, and electrophysiology measurement of tetrodotoxin-sensitive action potentials in iNeurons. Acrolein and CRA toxicity was tested by seeding cells in 96-well plates for 2 days or 14-16 days (for iNeurons) prior to incubation with sequential dilution of aldehydes (200-12.5 μ M) in basal media for 18hr. We observed that acrolein and CRA were toxic in all three lines. NSCs were highly sensitive to aldehyde-induced cell death with an LD₅₀ of ~12.5 μ M in acrolein and 100 μ M LD₅₀ in CRA. Surprisingly, iNeurons were more resistant to CRA with an LD₅₀ ~200 μ M and LD₅₀ of 100 μ M in acrolein. Post-mortem astrocyte cultures had an LD₅₀ of 50 μ M in acrolein and 200 μ M in CRA for comparison. Findings demonstrate potential toxic effects of acrolein and crotonaldehyde in three types of cells implicated in AD pathogenesis. Assessments of mechanisms underlying aldehyde induced death are underway.

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Poster

454. Neuronal Injury and Death I

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant 1R35NS116852-01
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Title: A dynamic balance between neuronal death and clearance after acute brain injury

Authors: *T. BALENA¹, K. P. LILLIS³, N. RAHMATI⁴, F. BAHARI¹, V. I. DZHALA¹, Y. BERDICHEVSKY⁵, K. J. STALEY²;

¹Massachusetts Gen. Hosp., Charlestown, MA; ²Massachusetts Gen. Hosp., Boston, MA;

³Harvard Med. School, MGH, Charlestown, MA; ⁴Harvard Med. Sch. / Massachusetts Gen. Hosp., Boston, MA; ⁵Lehigh Univ., Bethlehem, PA

Abstract: After acute brain injury, neuronal apoptosis may overwhelm the capacity for microglial phagocytosis, creating a queue of dying neurons awaiting clearance. The size of this queue should be equally sensitive to changes in neuronal death and the rate of phagocytosis, and thus a dynamic balance between death and clearance should exist. Altering the rate of initiation of apoptosis (i.e. the entry rate into the cell death pathway) or the efficiency of phagocytosis (i.e.

the exit rate from the cell death pathway) should therefore have significant effects on the number of visible dying neurons.

We evaluated the death of neurons in a chronically epileptic in vitro preparation in which serial multiphoton microscopy could be performed over a period of days or weeks. Organotypic hippocampal slice cultures were made from CLM1 (Clomeleon) and wild-type C57BL/6J mice on P6 and incubated in vitro. Slices were imaged with transgenic fluorophores to assess healthy neurons, and various bath-applied fluorophores to assess apoptotic neurons.

Serial imaging demonstrated that the capacity for microglial phagocytosis of dying neurons was overwhelmed for two weeks, based on an accumulation of neurons which stained positive for cell death markers such as propidium iodide. Altering phagocytosis rates, for example by poisoning the microglia with liposomal clodronate, dramatically changed the number of visibly dying neurons. Similar effects were generated when the visibility of dying neurons was altered by changing the membrane permeability for vital stains. Canonically neuroprotective interventions such as seizure blockade with kynurebate and neurotoxic maneuvers such as perinatal ethanol exposure were mediated by effects on microglial activity and the membrane permeability of apoptotic neurons, and had either no or opposing effects on healthy surviving neurons which expressed transgenic fluorescent proteins. Preliminary results from in vivo stroke experiments corroborate our findings that loss of fluorescent protein expression is a useful early biomarker for cell death.

After acute brain injury, microglial phagocytosis is overwhelmed by the number of dying cells. Under these conditions, the assumptions on which assays for neuroprotective and neurotoxic effects are based are no longer valid. Thus longitudinal assays of healthy cells, such as assessment of the fluorescence emission of transgenically-expressed proteins, provide more accurate estimates of cell death than do single-time-point anatomical or biochemical assays. More accurate estimates of death rates will increase the translatability of preclinical studies of neuroprotection and neurotoxicity.

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Poster

454. Neuronal Injury and Death I

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Program #/Poster #: 454.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Title: The Activating transcription factor-4 contributes to neurodegeneration following optic nerve injury

Authors: P. SOMASUNDARAM, M. M. FARLEY, M. A. RUDY, J. HEO, D. G. STEFANOFF, M. SHAH, P. GOLLI, S. WANG, S. KULKARNI, *T. A. WATKINS;
Dept. of Neurosurg., Baylor Col. of Med., Houston, TX

Abstract: The Activating transcription factor-4 (Atf4) contributes to neurodegeneration following optic nerve injury

Understanding how cellular stress signaling contributes to neuronal apoptosis may generate opportunities for neuroprotection in neurodegenerative conditions. Using a mouse model of CNS axon injury, we have identified the Activating transcription factor-4 (Atf4) as a prominent contributor to the death of distressed retinal ganglion cells (RGCs). Previous studies in the optic nerve crush model have demonstrated that MAPK kinase-dependent stress signaling, working through c-Jun and other transcription factors, is required for the death of injured RGCs. MAPK stress signaling also engages the Integrated Stress Response (ISR) through activation of the endoplasmic reticulum (ER) stress-responsive kinase Perk, and knockout of Perk is partially neuroprotective. To understand the mechanisms downstream of neuronal Perk, we utilized AAV2 to express Cre recombinase under the control of the human Synapsin-1 promoter in retinas of conditional knockout mice. Following knockout of Perk or either of two prominent ISR transcription factors downstream of Perk, Atf4 and the C/ebp homologous protein (Chop), we analyzed RGC survival using anti-RBPMS immunostaining 2 weeks after optic nerve crush and retinal gene expression by RNA-seq three days post-crush. Neuronal knockout of Atf4 results in 403 differentially expressed genes ($n=5$; adjusted p -value <0.05) and mimics the significant (but incomplete) neuroprotection ($n=5$; $p<0.01$) afforded by neuronal knockout of Perk ($n=5$; $p<0.05$). Moreover, Atf4-dependent gene expression changes exhibit significant overlap with those of Perk and unexpectedly include partial Atf4 dependence of injury-induced regeneration-associated genes (RAGs), such as *Sprr1a* and *Atf3*. In contrast, though germline Chop knockout mice have been demonstrated to exhibit RGC neuroprotection, the contribution of neuronal Chop to the response was modest, with only 29 differentially expressed genes ($n=5$; adjusted p -value <0.05) in the conditional knockout compared to the wildtype injured control. While these include known Chop target genes *Stbd1* and *Avil*, eight of the most highly significant expression changes occur in a cluster near the *Ddit3* gene that encodes Chop and are likely to be attributable to local effects of genomic modification. Together these results suggest that Atf4 is a more substantial contributor to the transcriptional response to CNS axon injury than previously appreciated and that it is the predominant pro-apoptotic transcription factor downstream of the Perk-activated ISR following optic nerve insults.

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Poster

454. Neuronal Injury and Death I

Location: SDCC Halls B-H

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Program #/Poster #: 454.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: FoRUM (Ruhr-University Bochum)

Title: Age-dependent effects in autoimmune mediated glaucoma?

Authors: *S. C. JOACHIM¹, C. ERB¹, C. THEISS², N. SCHONHOVEN¹, H. B. DICK¹, S. REINEHR¹;

¹Exptl. Eye Res. Inst., ²Dept. of Cytology, Inst. of Anat., Ruhr-University Bochum, Bochum, Germany

Abstract: Purpose: Age-related diseases like glaucoma, which is a leading cause of blindness, are having an upward trend due to an aging society. This leads to an immense socio-economic impact and enormous impairments for patients. Glaucoma is a multifactorial disease, where immune processes seem to be involved. Elevated antibody levels against heat shock protein 27 (HSP27) were detected in glaucoma patients. We previously noted glaucoma-like damage after an intravitreal HSP27 injection in rats. We now aimed to investigate if aged mice are more prone to this damage than younger ones. **Methods:** HSP27 was intravitreally injected in young (1-2 months) and old (7-8 months) mice. Controls of both ages received PBS and non-injected eyes served as native controls. Retinal thickness was analyzed via optical coherence tomography (OCT) after 4 weeks (n=5/group). Retinal ganglion cells (RGCs; RBPMS) and macroglia (GFAP) were analyzed via immunohistology (n=5-6/group). Also, RGCs (*Pou4f1*), microglia (*Iba1*), and *Il1b* (n=4/group) were evaluated with RT-qPCR. Optic nerve cross-sections were also evaluated (n=5/group). **Results:** OCT measurements showed no alterations at both ages. RGC number was significantly decreased in young HSP27 mice compared to native and PBS animals (p<0.001). Also, fewer RGCs were seen in old HSP27 mice compared to both controls (p<0.05). *Pou4f1* mRNA expression levels were significantly downregulated in young HSP27 mice compared to PBS and native ones (p=0.03), this was also the case in old HSP27 mice (p<0.009). The GFAP⁺ area was significantly increased in young HSP27 mice compared to native and PBS ones (p=0.03) and in old HSP27 mice compared to native controls (p=0.04). *Il1b* mRNA expression was significantly upregulated in young HSP27 mice compared to native and PBS ones (p<0.05). In old mice, significantly higher *Il1b* levels were noted in HSP27 and PBS mice in contrast to native ones (p=0.02). *Il1b* mRNA expression was higher in old than in young HSP27 mice (p=0.03). *Iba1* mRNA expression was significantly elevated in young as well as old HSP27 mice compared to respective native and PBS animals (p<0.01). In old HSP27 mice, *Iba1* mRNA expression levels were significantly lower than in young HSP27 mice (p<0.001). HSP27 animals of both ages had a higher optic nerve damage score than respective controls. **Discussion:** At both ages, intravitreal HSP27 injection led to RGC loss and inflammation. Some age dependent effects were noted. While microglia seem to respond more in young animals, inflammatory cytokines showed a more prominent reaction at advanced age. This emphasizes the pivotal role of HSP27 as well as of age-related factors in glaucoma.

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Poster

454. Neuronal Injury and Death I

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 454.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant RF1NS113840

Title: Inflammasome activation after peripheral nerve injury and its prevention by nuclear factor erythroid 2-related factor 2 (NRF2)

Authors: *F. CHERRY¹, M. S. RASHEED³, M. CHAVEZ¹, J. LI⁴, P. M. GRACE²;
²Symptom Res., ¹UT MD Anderson Cancer Ctr., Houston, TX; ³Symptom Res., M. D. Anderson Cancer Ctr., Houston, TX; ⁴The Univ. of Texas MD Anderson Cancer Ctr., Pearland, TX

Abstract: Inflammasome Activation in Peripheral Nerve Injury and its Interaction with Nuclear Factor Erythroid 2-Related Factor 2 (NRF2) Fisher R. Cherry, B.S., MD Anderson Cancer Center; Mohd Sami Ur Rasheed, Ph.D., MD Anderson Cancer Center; Melissa Chavez, B.S., MD Anderson Cancer Center; Jiahe Li, Ph.D., MD Anderson Cancer Center; Peter M. Grace, Ph.D., MD Anderson Cancer Center

Purpose: Peripheral nerve injury increases levels of interleukin-1 β (IL-1 β), which contributes to nociceptive hypersensitivity. IL-1 β is matured by inflammasomes; e.g., absent in melanoma 2 (AIM2) and NLR family pyrin domain containing 3 (NLRP3), which are activated by a variety of signals like reactive oxygen species (ROS). Inflammasomes also mature gasdermin D which creates pores in the cell membrane, allowing for release of IL-1 β . However, it is not fully known which inflammasomes are activated after peripheral nerve injury, where they are activated, and whether GSDMD-dependent pores form to allow IL-1 β release. In addition to addressing these questions, we further tested whether scavenging ROS by pharmacological activation of NRF2 would attenuate activation of inflammasomes. **Methods:** Male and female C57BL/6J mice underwent sciatic nerve chronic constriction injury (CCI). Sciatic nerve (SN), dorsal root ganglion (DRG), and spinal cord were harvested after 0, 1, 5, 7, 14, and 21 days after injury to measure transcription of inflammasome components. Propidium iodide was administered by i.p. injection one hour before taking tissue to measure pore formation. NRF2 was pharmacologically activated by CPUY192018 (30 mg/kg daily for three days; i.p.). Mechanical allodynia was quantified by von Frey tests. **Results:** There was an early increase in gene expression of inflammasome components after injury, with a preference towards NLRP3 in male mice and AIM2 in female mice. This trend was repeated at days 14 and 21. A high expression of caspase 1 and interleukin 1 beta (IL-1B) was observed throughout the SN and DRG, but propidium iodide staining indicated pyroptosis only occurred in the injured SN. Effects of CPUY192018 treatment will be presented. **Conclusion:** Different inflammasomes may be responsible for IL-1 β processing and release in males and females. Although inflammasome components are upregulated in DRG, there is little pore formation, suggesting additional regulation of IL-1 β release at this site.

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Poster

454. Neuronal Injury and Death I

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Program #/Poster #: 454.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Research Training Initiative for Student Enhancement (RISE) Program
NIH Grant

Title: Complex interactions between zinc, acidosis, and ASICs-mediated cell injury

Authors: A. HENRY-SMITH, T. YANG, T. LENG, *Z.-G. XIONG;
Morehouse Sch. of Med., Morehouse Sch. of Med., Atlanta, GA

Abstract: Stroke is a leading cause of death and long-term disabilities, and current treatments have limited success. It is therefore vital to search for new brain injury mechanisms and effective stroke intervention strategies. Zinc toxicity has been increasingly recognized in ischemic brain injury, but the detailed pathways/mechanisms are unclear. The current study investigates the interactions between zinc, acidosis, and acid-sensing ion channels (ASICs)-mediated cell injury. In NS20Y cells, which express ASICs, and CHO cells transfected with ASIC1a or ASIC2a, incubation with acidic solutions activated acid sensing ion channels and produced significant cell injury (analyzed with one way ANOVA) as indicated by increased LDH release and FDA/PI staining. Addition of 30-100 μ M zinc to the extracellular solution at pH 7.4 produced a dose-dependent increase in cell injury. The zinc-induced cell injury at pH 7.4 was replicated in wild-type CHO cells and in CHO-ASIC1a cells in the presence of ASIC1a inhibitor PcTX1, indicating ASIC1a-independent effect. Interestingly, addition of 30-100 μ M zinc to the pH 6.0 solution produced no change, a small increase, or even a decrease in cell injury depending on the cells that express different ASICs. Our findings suggest that there is a complex interaction between zinc, acidosis, and ASICs-mediated cell injury. Understanding the detailed interaction and the underlying mechanism may help in creating more effective stroke intervention strategies.

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Poster

454. Neuronal Injury and Death I

Location: SDCC Halls B-H

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Program #/Poster #: 454.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Characterization of the mitochondrial and lysosomal networks and oxidative stress of Infantile Neuronal Ceroid Lipofuscinosis dermal fibroblasts

Authors: J. LAGRAFF¹, T. PHAM², B. BALOUCH³, H. NAGORSKY⁴, *Q. CHU-LAGRAFF⁵;

¹Genet., Harvard Med. school, Boston, MA; ²Neurobio., Boston Children's Hospital- Harvard Med. Sch., Boston, MA; ³Drexel Sch. of Med., Philadelphia, PA; ⁴Temple Sch. of Med., Philadelphia, PA; ⁵Union Col., Union Col., Schenectady, NY

Abstract: Infantile Neuronal Ceroid Lipofuscinosis (INCL) is a pediatric neurodegenerative disorder caused by mutations in the palmitoyl protein thioesterase 1 (PPT1) gene. PPT1 deficiency leads to retinal and CNS deterioration due to the accumulation of autofluorescent granular osmiophilic deposits (GRODs) storage materials in the lysosome. Although these deposits collect in all cell types, their accumulation in the neurons preferentially lead to neurotoxicity, oxidative damage, and apoptosis. The underlying molecular mechanisms leading to INCL pathology remain poorly understood. Here we present a comprehensive cellular characterization of human PPT1-deficient fibroblast cells harboring Met1Ile and Tyr247His compound heterozygous mutations. Phenotypic characterization of this mutant fibroblasts revealed autofluorescence storage materials as expected, and distinct organellar abnormalities of the lysosomal and mitochondrial structures, which supports previous postulations about endoplasmic reticulum and mitochondrial-mediated pathologies. There was an abundance of vacuolar and lysosomal compartments in INCL fibroblasts, which suggests an upregulation of lysosomal biogenesis, and is known to be associated with endoplasmic reticulum stress. The mitochondrial network displayed a morphology consistent with mitochondrial dysfunction, and a heightened susceptibility to exogenous reactive oxygen species (ROS)-induced cell death, which suggests elevated basal levels of endogenous ROS in the cell. Finally, we present a preliminary assessment of the effect of dimethyl fumarate on INCL fibroblast vacuolar phenotype.

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Poster

454. Neuronal Injury and Death I

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: J.C. Bose Fellowship (JCB/2020/000037) from the Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India
NBRC Core funds

Title: Characterization of Acute flaccid paralysis in BALB/c mouse model

Authors: *M. BHASKAR¹, A. BASU²;

¹Mol. and Cell. Neurosci., Natl. Brain Res. Ctr., Gurugram, India; ²Mol. and Cell. Neurosci., Natl. Brain Res. centre, Gurugram, India

Abstract: Poliomyelitis-like-illness is a common manifestation associated with neurotropic infections. Functional loss and death of spinal motor neurons often lead to reduced muscle tone and paralysis, subsequently progressing to long-term motor sequelae among disease survivors. Regardless of the several reports demonstrating the molecular basis of encephalopathy, the pathogenesis behind virus-induced flaccid paralysis remained largely unknown. Therefore in our present study, we aim to elucidate the mechanism responsible for limb paralysis by studying two different neurotropic agents, responsible for causing clinical-AFP (Acute flaccid paralysis) in vast region of south-east Asia and Indian subcontinent. Experimental model for studying virus-induced AFP was generated by intraperitoneal injection of 10-day old BALB/c mice. The degree of induced paralysis was quantified accurately by performing a battery of behavioural tests to assess gait, neurodegeneration and locomotion deficits. Progressive decline in motor performance of the affected animal was observed when compared with age-matched control mice. Paralysis was correlated with the death of motor neurons (MN) by studying various cell death assays in both in vivo and in vitro settings. Furthermore, this study demonstrates that upon infection, MNs undergo the extrinsic arm of apoptosis in a RIG-I-dependent fashion via activation of transcription factors pIRF-3 and pIRF-7. Both gene-silencing experiments using specific RIG-I-short interfering RNA and in vivo morpholino abrogated cellular apoptosis, validating important role of pattern recognition receptor (PRR) RIG-I in MN death. Hence, from our experimental observations, we hypothesized that host innate response plays a significant role in the deterioration of motor functioning upon neurotropic infections.

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Poster

454. Neuronal Injury and Death I

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 454.14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ARUK Scotland Junior Small Grant
EPSRC EP/P030017/

Title: Willin/FRMD6-mediated mitochondrial dysfunction contributes to neuronal A β toxicity

Authors: *D. CHEN, L. AITKEN, F. GUNN-MOORE;
Sch. of Biol., Univ. of St Andrews, St Andrews, United Kingdom

Abstract: Mitochondrial dysfunction and progressive accumulation of amyloid beta (A β) have long been implicated in the pathogenesis of Alzheimer's disease (AD); however, the underlying mechanisms are not well understood. FERM domain-containing protein 6 (FRMD6), also known

as Willin, is an upstream regulator of Hippo signaling and modulates neuronal differentiation through ERK signaling. Initially identified as a novel binding partner of neurofascin 155 in the rat sciatic nerve, Willin/FRMD6 has recently been shown to play a role in axon myelination, neuropeptide exocytosis, neuronal differentiation, and peripheral nerve repair. These newly identified roles of Willin/FRMD6 in neuronal cells are of particular interest given that Willin/FRMD6 has been reported as a potential AD risk gene in a series of genome-wide association and neuroimaging studies. However, the mechanistic link between Willin/FRMD6 and AD pathogenesis remains unexplored. Here, we demonstrate the direct effects of A β on Willin/FRMD6 expression and position mitochondrial oxidative stress as a novel potential mechanism underlying the role of Willin/FRMD6 in AD pathogenesis. Using primary mouse neurons and mouse hippocampal HT22 cells, we demonstrate that A β and oxidative stress induce downregulation of Willin/FRMD6 protein expression. Next, we examine the functional consequences of decreased Willin/FRMD6 expression and demonstrate that Willin/FRMD6 knockdown results in mitochondrial perturbation and imbalanced mitochondrial dynamics leading to excessive mitochondrial fragmentation and oxidative stress, both of which are key early features of AD pathogenesis. Importantly, increasing Willin/FRMD6 expression ameliorates A β -induced mitochondrial abnormalities. Thus, enhancing Willin/FRMD6 expression holds potential as a therapeutic strategy for protecting against A β -induced mitochondrial and neuronal dysfunction. Taken together, these studies provide the first direct evidence that Willin/FRMD6 is involved in the regulation of mitochondrial morphology and function and highlight a potential novel biochemical pathway through which Willin/FRMD6 is involved in AD pathogenesis.

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Poster

454. Neuronal Injury and Death I

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 454.15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: PROSNI

Title: Curcumin inhibits hippocampal oxidative damage with increased activity in plasma antioxidant enzymes in rats after acute and chronic exposure to ozone

Authors: *A. A. RAMIREZ-MENDOZA¹, M. A. RAMIREZ-HERRERA², M. L. MENDOZA-MAGANA³, M. M. J. ROMERO-PRADO¹, A. R. TEJADA-MARTÍNEZ⁴, C. R. CORTEZ-ÁLVAREZ¹;

¹Univ. of Guadalajara, Guadalajara, Mexico; ²Univ. Guadalajara Ctr. Univ. Ciencias Salud, Guadalajara, Mexico; ³Univ. de Guadalajara, Guadalajara Jalisco, Mexico; ⁴IMSS, Guadalajara, Mexico

Abstract: Ozone (O₃) is a tropospheric pollutant with high oxidant power formed in highly populated cities. It has been demonstrated that O₃ affects the central nervous system and provokes neurochemical alterations, cognitive decline, headaches, dysfunction, degeneration, and neuronal death. These manifestations have been related to an extremely high lipid peroxidation and protein carbonylation with concomitant decrease in antioxidant enzymes: catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD). Curcumin (CUR) is a natural polyphenol isolated from the rhizome of the Asian plant *Curcuma longa* Lin with pharmacological activity such as anti-inflammatory, anticancer, antioxidant, antimicrobial, among others. The present work aims to analyse the effect of curcumin on the lipid peroxidation and protein carbonylation profiles in hippocampus homogenates as well as the activity of plasma antioxidant enzymes CAT, GPx, and SOD after acute and chronic exposure to O₃. Fifty male Wistar rats were divided into 5 experimental groups: I (intact control group), C (CUR fed control group), O (O₃ exposed control group), PC (preventive, CUR fed since 7 days before O₃ exposure), and TC (therapeutic, exposed to O₃ since 7 days before CUR fed) groups. All experimental groups were exposed to 0.7 ppm of O₃, except the I control, and the C control. CUR was dietary administrated at an approximate dose of 6.5 mg/Kg for the corresponding groups during the exposure time to O₃. Experiments were carried out through an acute (15 days) and a chronic (60 days) O₃ exposure phase. In the preventive and therapeutic groups, lipid peroxidation and protein carbonylation were simultaneously and significantly inhibited in both exposure phases and in both CUR administration modes. Meanwhile, the activity of plasma CAT, GPx, and SOD was significantly increased in both exposure phases with slight differences, and in both CUR administration modes. Our findings suggest that CUR could be used as an antioxidant and neuroprotector against tropospheric O₃ pollution in a preventive or therapeutic mode based on the facts that CUR is well tolerated and exerts no adverse effects.

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Poster

454. Neuronal Injury and Death I

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIA grant R01AG060919
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Title: Antagonistic roles of Ras-MAPK and Akt signaling in integrin-K⁺ channel complex-mediated cellular apoptosis

Authors: *E. FORZISI, W. YU, P. RAJWADE, F. SESTI;
Rutgers Univ., Rutgers Univ. Grad. Program In Neurosci., Piscataway, NJ

Abstract: The voltage-gated delayed rectifier potassium (K⁺) channel KCNB1 (Kv2.1) is commonly found in the cortical and hippocampal regions of the brain and it forms complexes with α 5-integrins, known as IKCs. These complexes transduce the electrical activity at the plasma membrane into biochemical events that impinge on cytoskeletal remodeling, cell differentiation, and migration. However, when cells are subject to stress of oxidative nature IKCs turn toxic and cause inflammation and death. Here, biochemical, pharmacological, and cell viability evidence demonstrates that IKCs activate an apoptotic Mitogen-activated protein kinase/extracellular signal-regulated kinase (Ras-MAPK) signaling pathway in response to oxidative insults. Simultaneously, wild-type (WT) KCNB1 channels sequester protein kinase B (Akt) causing dephosphorylation of BCL2-associated agonist of cell death (BAD), a major sentinel of apoptosis progression. In contrast, IKCs formed with C73A KCNB1 variant that does not induce apoptosis (IKCC73A), do not sequester Akt and thus are able to engage cell survival mechanisms. Taken together, these data suggest that apoptotic and survival forces co-exist in IKCs. Integrins send death signals through Ras-MAPK and KCNB1 channels simultaneously sabotage survival mechanisms. Thus, the combined action of integrins and KCNB1 channels advances life or death.

*These data have been published. Forzisi, E, Yu, W, Rajwade, P, Sesti, F. Antagonistic roles of Ras-MAPK and Akt signaling in integrin-K⁺ channel complex-mediated cellular apoptosis. FASEB J. 2022; 36:e22292. doi:10.1096/fj.202200180R

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Poster

454. Neuronal Injury and Death I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 454.17

Topic: C.08. Ischemia

Support: NS106901
P206GM109089

Title: Gene expression changes following spreading depolarization in a mouse stroke model

Authors: *M. DELL'ORCO¹, L. LI¹, J. WEISEND¹, N. PERRONE-BIZZOZERO¹, L. A. CUNNINGHAM¹, R. A. MORTON¹, A. P. CARLSON², C. W. SHUTTLEWORTH¹;
¹Neurosciences, ²Neurosurg., Univ. of New Mexico HSC, Albuquerque, NM

Abstract: Spreading depolarization (SD) is a slowly propagating wave of profound depolarization that sweeps through cortical tissue. SD is not harmful to healthy brain tissue, but can cause irrecoverable injury to metabolically compromised tissue and thus cause expansion of acute brain injuries. SDs occur in stroke brain, usually originating near ischemic foci and propagating relatively widely throughout peri-infarct and surrounding tissue leading to tissue loss. Despite clear deleterious consequences, previous studies have shown changes in expression

levels of genes known to regulate synaptic plasticity and neurogenesis following SD. The present study aimed to perform an extensive, unbiased analysis to identify a more complete range of biological pathways modified by SD. In initial studies, SDs were induced repetitively (4 SDs in 2 hours at 30 min intervals) in healthy, female mice and confirmed with intrinsic optical imaging. SD induction was with either focal application of KCl in C57Bl/6 mice (NaCl for sham controls, n=6) or with optogenetic stimulation (2 mW for 20 seconds) in Thy1-ChR2-YFP mice (n=6). In a second set of studies, SDs were induced repetitively with focal KCl (4 SDs at 30 min intervals) in stroke model (dMCAO in C57Bl/6 mice). Two hours after onset of the initial SD, cortical slices were collected and RNA was extracted from total hemispheres (to compare ipsilateral and contralateral), or from punches in 3 different regions of the ipsilateral hemisphere: 1) SD initiation site; 2) intermediate site > 3mm from initiation, 3) remote site >5 mm from initiation. RNA-seq and spatial genomics identified differentially expressed genes (DEGs). Consistent with previous studies, top DEGs include genes encoding the neurotrophic factor BDNF, intermediate early genes FOS, and JUNB. We also found significantly increased levels of other cell proliferation related genes as DUSP6; plasticity related genes as HOMER1a and c and ARC, and inflammation related genes as PTGS2, EGR2 and NR4A1. Pathway analysis revealed significant increases in the expression of genes associated with axogenesis, branching of axons, neuritogenesis, dendritic growth, and regeneration of neurites. We also found a significant decrease in expression in genes associated with cell death, apoptosis and neuronal degeneration. DEGs showed higher expression in the intermediate and distal regions, as compared to SD initiation sites. These results identified a range of novel targets that could be used to test whether clusters of SD may enhance plasticity or recovery in surviving peri-infarct tissue, in addition to the well-established role of SD in expansion of infarct core.

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Poster

454. Neuronal Injury and Death I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

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Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Grant # 1840 for HB-D and BM-B from CONACYT.
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Title: Analysis of cell stress-related nuclear spatial constraints during neuronal injury

Authors: *G. VARELA CASTILLO¹, A. BARRIENTOS BONILLA¹, R. NADELLA², B. BERNAL-MORALES¹, D. HERNÁNDEZ-BALTAZAR³;

¹Inst. de Neuroetología, Univ. Veracruzana, Xalapa, Mexico; ²Intl. collaboration (ID1840), Srikakulam, Andra Phadesh., India; ³Investigadoras e investigadores por México. Dirección Adjunta de Desarrollo Científico., Consejo Nacional de Ciencia y Tecnología (CONACYT)., CDMX, Mexico., Mexico

Abstract: Introduction. In cells, the degree of spatial confinement can be determined by their largest and stiffest organelle, the nucleus. The nuclear shape result of the balance between the chromatin organization and nucleoskeleton. Although there are various immunostaining techniques for diagnostic studies using haematoxylin-eosin, the analysis of neuronal nuclear patterns has been neglected till date. **Objective.** To determine the nuclear morphometric patters caused in diverse stress models. **Method.** Male adult Wistar rats were used. Cellular stress was induced by three different approaches (n = 3 rats *per* treatment): 1) lipopolysaccharide (LPS; 500 mg/Kg, i.p.), an immune response activator, 2) intranigral injection of staurosporine (Sta; 50 nM), an apoptosis enhancer, and 3) 30%-partial hepatectomy (30%-PH). The tissue sections (5-10 µm) were immunostained with Hoechst 33342 (nuclear marker), CD11b/c-OX-42 (microglial marker), tyrosine hydroxylase (dopaminergic phenotype marker) and caspase-3 (apoptosis marker). The evaluation of shape, size, number and hyper/hypochromic features of cell nuclei was performed by segmentation algorithm followed by an automatic thresholding analysis from ImageJ software. **Results.** During inflammation, degeneration and cell death processes in *substantia nigra*, particulars regarding the nuclear patterns were distinguished. LPS and 30%-PH induced nuclear spatial constraints during neuronal injury, while Stau alter nuclear integrity. **Conclusion.** Regardless of the stress and/or stimulus, the analysis of nuclear patterns contributes to the understand of cellular process such as cell communication, cell survival, and neuronal homeostasis in stress-based animal models.

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Poster

455. Neuroprotection II

Location: SDCC Halls B-H

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Program #/Poster #: 455.01

Topic: C.08. Ischemia

Support: National Research Foundation funded by the Korean government Mistry of Science and ICT, NRF-2018R1C1B6006145
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Title: High mobility group box 1 acts as an autocrine chemoattractant for oligodendrocytes through toll-like receptor 2 in white matter stroke

Authors: *H. KIM, J. CHOI, X. JIN, S. KOH, H. CHO, B. KIM;
Ajou Univ., Ajou Univ., Suwon, Korea, Republic of

Abstract: White matter stroke (WMS) is an important yet unaddressed clinical entity. There is no available treatment option that can directly rescue the ischemic white matter from demyelination and neurodegeneration. Strategies to capacitate remyelination are necessary to improve neurological function and ultimately provide a cure for WMS. Remyelination initiates as the ischemic demyelination is recognized, followed by the migration of oligodendrocyte progenitor cells (OPCs) to the damaged white matter and subsequent proliferation and differentiation of OPCs. In the present study, we highlight the role of high mobility group box 1 (HMGB1), a damage-associated molecular pattern released from dying oligodendrocytes, as an autocrine chemoattractant that promotes OPC migration in WMS. We measured the migratory capacity of primary-cultured OPCs with the Boyden chamber assay, and the presence of HMGB1 in the media substantially promoted OPC migration by approximately two-fold compared to the control. Co-administration of the HMGB1 antagonist glycyrrhizin reversed such effects to almost non-stimulated levels. For the determination of the downstream targets of HMGB1, two candidates, receptor for advanced glycation end product (RAGE) and toll-like receptor 2 (TLR2), were genetically knocked down by transfection of siRNA, and the migratory capacities were analyzed. The knockdown of TLR2 attenuated the OPC migration-promoting effect of HMGB1, while the knockdown of RAGE did not. To establish that HMGB1 is an autocrine chemoattractant in WMS, the conditioned medium (CM) from oligodendrocytes treated with oxygen-glucose deprivation (OGD), an *in vitro* model of ischemic insult, was collected. The OGD-CM promoted OPC migration, and such effect was not observed in TLR2(-/-) OPCs. The results suggest that HMGB1 is released from damaged oligodendrocytes and exerts a migration-promoting function in a TLR2-dependent manner. In order to validate the results *in vivo*, TLR2(-/-) C57BL/6 mice (N=5) were treated with the vasoconstrictive agent N5-(1-iminoethyl)-L-ornithine as the *in vivo* models of white matter stroke, and revealed a larger lesion size compared to the wild-type mice (N=5), yet fewer oligodendrocytes within the lesion in immunohistochemistry. Collectively, the *in vitro* findings suggest that the HMGB1-TLR2 axis critically contributes to OPC migration, and the *in vivo* results confirm that it is significantly involved in the extent of ischemic demyelination. In summary, we successfully showed that HMGB1 as an autocrine chemoattractant promoted OPC migration through TLR2, and elucidated its potential as a therapeutic target to achieve remyelination in WMS.

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Poster

455. Neuroprotection II

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Program #/Poster #: 455.02

Topic: C.08. Ischemia

Support: MIUR PRIN 2017LYTE9M

Title: Siah2-induced balancing between mitophagy and mitochondrial biogenesis as mechanism for neuroprotection in ischemic brain preconditioning

Authors: *A. SCORZIELLO¹, M. SISALLI², L. ANNUNZIATO³;
¹Neurosci., Univ. of Naples "Federico II", Naples, Italy; ²Neurosci., Federico II Univ. of Naples, Naples, Italy; ³SDN IRCCS, Naples, Italy., Naples, Italy

Abstract: Mitochondrial quality control is crucial for the homeostasis of the mitochondrial network. The balance between mitophagy and biogenesis is needed to prevent the cerebral ischemia induced cell death. Ischemic preconditioning (IPC) represents an adaptation mechanism of CNS, that increased tolerance to the lethal cerebral ischemia. It has been demonstrated that hypoxia-induced Siah2-E3 ligase activation influences mitochondrial dynamics promoting the degradation of mitochondrial proteins. Therefore, we investigated the role of Siah2 in the IPC-induced neuroprotection in an *in vitro* model of IPC. To this aim, cortical neurons were exposed to 30-min oxygen and glucose deprivation (OGD, sublethal insult) followed by 3hrs OGD plus reoxygenation (lethal insult). Our results reveal that the mitochondrial depolarization induced by hypoxia activates Siah2 at mitochondrial level and increases LC3-II protein expression, an effect counteracted by the reoxygenation phase. By contrast, hypoxia reduces the expression of PGC1-alpha, a marker of mitochondrial biogenesis, whereas its expression was increased after reoxygenation thus improving mitochondrial membrane potential, mitochondrial calcium content, and mitochondrial morphology, and leading to the neuroprotective effect of ischemic preconditioning. Collectively, these findings indicate that the balance between mitophagy and mitochondrial biogenesis due to the activation of the Siah2-E3 ligase is involved in IPC-induced neuroprotection.

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Poster

455. Neuroprotection II

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Title: Elucidation of Toll-like receptor 2 mediated protective signal pathways in oligodendrocytes: The role of NF-kB and cIAP2

Authors: *J. CHOI¹, X. JIN², H. KIM², S. KOH¹, B. KIM¹;
¹Departments of Brain Sci. and Neurol., ²Dept. of Brain Sci., Ajou Univ. Sch. of Med., Suwon-si, Korea, Republic of

Abstract: Myelinating glia, oligodendrocyte (OL), provides saltatory conduction and various support to the axon through axomyelinic synapse. White matter stroke (WMS) is an important clinical entity that comprises one-fourth of ischemic stroke and is the second most common cause of senile dementia but underestimated neurologic disorders. The main pathology of WMS is ischemic OL loss. Therefore, a therapeutic strategy for WMS should focus to reduce the ischemic OL death. Previously, our group showed Toll-like receptor 2 (TLR2) in OLs protects OLs from ischemic damage and one of the endogenous TLR2 ligands is high-mobility group box 1 released from dying OL. However, there is a need to uncover more detailed intracellular survival pathways mediated from TLR2 in oligodendrocytes. We investigated primary OL culture from P1 pups and oxygen-glucose deprivation as an in vitro ischemic model. Firstly, we examined the phosphorylation or activation of canonical TLR2 pathway signaling proteins, such as AKT, ERK1/2, p38, CREB, or NF-kB in OL after Pam3CSK4, a TLR2 agonist, application. TLR2 activation didn't phosphorylate AKT but phosphorylate ERK1/2, p38, and CREB and activate NF-kB. Secondly, we used a chemical inhibitor or siRNA to each signal pathway proteins and found p38 and NF-kB were essential for TLR2-mediated OL protection from ischemic insults but not ERK1/2 and CREB. To validate our findings, we performed bulk RNA seq in OL with or without Pam3CSK4 or OGD. According to RNA seq data, NF-kB is located in the central position of TLR2-mediated OL protection. Also, we found that cIAP2 and Bcl2 have upregulated in TLR2-NFkB dependent manner. After the acquisition of RNA seq, we transfected OL with various constitutive active plasmids or siRNA to manipulate signaling pathways. cIAP2 rather than Bcl2 was increased after transfection in either p38 or NF-kB active state. Interestingly, p38 constitutive activation with NF-kB knockdown couldn't increase cIAP2 and

failed protection OLs from OGD. On the contrary, NF- κ B constitutive activation with p38 knockdown could increase cIAP2 and protect OLs from OGD. Additionally, cIAP2 overexpression with NF- κ B knockdown also showed OLs protective effects but not vice versa. These findings show TLR2 in OLs exerts a protective effect through NF- κ B and cIAP2 and suggest a novel therapeutic target to overcome ischemic OL death and WMS.

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Poster

455. Neuroprotection II

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Program #/Poster #: 455.04

Topic: C.08. Ischemia

Support: NIH/NINDS R01NS115800
Postdoctoral Research Fellowship from American Epilepsy Society
Iowa Neuroscience Institute

Title: Cation-chloride cotransporters' role in neuronal swelling during oxygen-glucose deprivation in the neonatal neocortex

Authors: *Y. TAKEZAWA^{1,2}, R. LANGTON^{1,2}, S. M. BAULE³, M. B. ZIMMERMAN⁴, S. BAEK⁶, J. GLYKYS^{1,2,5};

¹Dept. of Pediatrics, ²Iowa Neurosci. Inst., ³Dept. of Biomed. Engin., ⁴Dept. of Biostatistics, ⁵Dept. of Neurol., The Univ. of Iowa, Iowa City, IA; ⁶Sch. of Data Sci., Univ. of Virginia, Charlottesville, VA

Abstract: Neonatal hypoxic-ischemic encephalopathy (HIE) can result in life-long disabilities. HIE causes cytotoxic neuronal swelling through the entry of water and ions, including Cl⁻. Neurons do not have water channels and have a low water permeability; thus, multiple water pathways have been proposed. The cation-chloride cotransporters (CCCs), including NKCC1 and KCC2, move water in different cells, but it is unclear if they do in neonatal neurons. We determined how modulating CCCs activity during prolonged and brief hypoxia alters neuronal swelling and intracellular Cl⁻ concentration [Cl⁻]_i in neocortical neurons (layer IV/V) during the neonatal period (post-natal day 9-13). We used acute brain slices from Clomeleon mice which encode a ratiometric fluorophore sensitive to Cl⁻ and exposed them to oxygen-glucose deprivation (OGD) while imaging neuronal size and [Cl⁻]_i by multiphoton microscopy. The neuronal area was measured using an automated neuronal morphology analysis framework based on convolutional neuronal networks. CCCs were modulated pharmacologically using bumetanide (NKCC1 blocker), VU0463271 (KCC2 blocker), CLP257 (KCC2 enhancer), or furosemide (broad-spectrum CCCs). A linear mixed model for repeated measures was used to test for significant changes in the neuronal area and [Cl⁻]_i. We observed neuronal swelling and Cl⁻ accumulation starting 10 minutes after OGD, which worsened in prolonged OGD or returned to

baseline during reperfusion. Compared to when no drug was perfused: (1) blocking NKCC1 reduced neuronal swelling during early but not after prolonged OGD, while it aggravated Cl⁻ accumulation during prolonged OGD, (2) blocking KCC2 did not worsen swelling but increased Cl⁻ accumulation during prolonged OGD, and aggravated neuronal swelling during reperfusion, (3) enhancing KCC2 did not alter neuronal swelling but prevented Cl⁻ accumulation during early OGD and reperfusion, and (4) blocking CCCs broadly with furosemide reduced both swelling and Cl⁻ accumulation in either prolonged and brief OGD. However, blocking simultaneously NKCC1 and KCC2 with their specific antagonists aggravated neuronal swelling during prolonged OGD. Our results indicate that NKCC1 is involved in water movement in neocortical neurons during early brain development. Yet, furosemide is more effective in preventing neuronal swelling than only blocking NKCC1 during prolonged hypoxia, suggesting additional water pathways at this age.

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Poster

455. Neuroprotection II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 455.05

Topic: C.08. Ischemia

Title: Hmgb1 induces hepcidin upregulation in astrocytes and causes acute local iron surge and subsequent ferroptosis in the post-ischemic brain

Authors: *D. DASHDULAM, S. SONG-I, O. SANG-A, K. SEUNG-WOO, L. JA-KYEONG; Anat., Inha Univ., Incheon, Korea, Republic of

Abstract: Since iron participates in a variety of vital functions in the brain, dysregulation of iron levels in the brain causes functional disturbances and damage to neurons. Among the molecules involved in the regulation of intracellular iron levels, hepcidin, a peptide hormone, plays a principal role in the regulation of ferroportin (FPN), the only known iron exporter, by triggering its internalization and lysosomal degradation. In the present study, we showed a rapid iron surge in both the cortical core and penumbra of the ischemic hemisphere, as early as 3 h after cerebral ischemia (middle cerebral artery occlusion, MCAO), which was maintained until 4 d post MCAO. Upregulation of local hepcidin expression occurred 1 h post MCAO in both the cortical core and penumbra of the ischemic hemisphere and significant hepcidin accumulation in the serum were also detected beginning 6 h post MCAO. High mobility group box 1 (HMGB1) is a prototypic danger associated molecular pattern and we previously reported that it is markedly accumulated in brain parenchyma, cerebrospinal fluid, and the serum after transient MCAO and plays a critical role in the aggravation of damage through its proinflammatory function. Here, treatment with recombinant HMGB1 stimulated astrocytes to induce hepcidin expression, with the TLR4-JNK and CXCR4-p38 signaling pathways involved in this process. Intracellular iron

accumulation after treatment with hepcidin was observed in both neurons and microglia, which coincided with the downregulation of FPN and upregulation of the ferritin heavy chain, ferritin light chain, and divalent metal transporter 1 in those cells. Moreover, we demonstrated that HMGB1-mediated local hepcidin upregulation and the subsequent local iron surge cause ferroptosis during the acute phase in the post-ischemic brain, and confirmed the critical role of HMGB1 during this process by the functional blocking of HMGB1, using the intranasal administration of HMGB1 A box or anti-HMGB1 antibody. These findings show that HMGB1, a well-known pro-inflammatory mediator in the ischemic brain, serves as a ferroptosis inducer by upregulating hepcidin in astrocytes, thereby aggravating acute damage in the post-ischemic brain.

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Poster

455. Neuroprotection II

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Topic: C.08. Ischemia

Support: RFAG042189

Title: Treatment with Tamoxifen, a Selective Estrogen Receptor Modulator, Exacerbates Astrocyte Activation and Mitochondrial Stress After Oxygen Glucose Deprivation

Authors: ***M. ZARDENETA**¹, **T. BRANYAN**³, **F. SOHRABJI**²; ²Neurosci. and Exptl. Therapeut., ¹Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX; ³Texas A&M Univ., Bryan, TX

Abstract: Introduction: Tamoxifen (TAM), a selective estrogen receptor modifier, is a first-line treatment option for hormone receptor positive breast cancer. TAM inhibits downstream estrogen receptor signaling in tumor cells. Stroke risk is increased in women with low estrogen levels, such as early-onset menopause, and doubles in women after the age of 55, which corresponds with the average age of menopause. Women with breast cancer who were treated with tamoxifen had an increased risk for ischemic stroke (82%). Since stroke severity is associated with increased blood brain barrier (BBB) damage, we investigated the effects of tamoxifen on activation and mitochondrial stress in astrocytes, which is a cellular component of the BBB. Methods: Cultured female human astrocytes were treated with tamoxifen (1uM), 17 β -estradiol (10nM), or vehicle (DMSO) for 24 hours. Cells were separated into two groups: normoxic (21% O₂, 25mM glucose) or oxygen and glucose deprivation (OGD) conditions 6 hours before analysis. ROS production was measured using the Cellular ROS Assay Kit (Red) and aquaporin-4 levels were visualized via immunocytochemistry. Mitochondrial function was assessed by using a Seahorse XFe96 Analyzer. Results: Tamoxifen treatment increased Aquaporin-4 protein

levels ($p < 0.0001$) in cultured astrocytes after OGD compared to both vehicle (DMSO) and 17β -estradiol treatment. While there was no significant effect, tamoxifen treated cells showed a trend toward ($p = 0.0732$) increased ROS production after OGD compared to vehicle. Additionally, tamoxifen reduced oxygen consumption rate ($p = 0.0197$) and basal respiration under normoxic conditions in astrocytes when compared with DMSO or 17β -estradiol treatment. Conclusions: Tamoxifen treatment altered the expression of a water channel protein and decreased the ability to respond to oxidative stress in astrocytes. Aquaporin-4, when localized to the astrocytic end feet, facilitates edema after stroke, and ischemic stroke by definition puts brain tissue under oxidative stress. Our findings suggest tamoxifen may increase edema, which is known to impair stroke recovery. These data suggest a mechanism by which breast cancer treatment may increase the risk for severe ischemic stroke.

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Poster

455. Neuroprotection II

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Topic: C.08. Ischemia

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Title: Mcao stroke causes an increase in hippocampal soluble amyloid beta and bace1 activity in a trpm2-dependent process

Authors: M. FALK¹, *J. BASAK¹, D. MITCHELL¹, B. WASSERMAN¹, N. QUILLINAN¹, P. HERSON²;

¹The Univ. of Colorado Sch. of Med., Aurora, CO; ²The Ohio State Univ. Col. of Med., Columbus, OH

Abstract: Post-stroke cognitive impairment and dementia (PSCID) is a phenomenon of increasing concern in survivors of large vessel ischemia. The molecular mechanisms underlying PSCID are poorly defined, but may involve perturbations in pathways involved in both neurodegeneration and oxidative stress. Specifically, synaptic dysfunction after stroke may be directly mediated by alterations in the levels of amyloid beta ($A\beta$), the peptide that accumulates in the brains of Alzheimer's disease (AD) patients. In this study, we used the transient middle cerebral artery occlusion (MCAo) model in mice to evaluate if a large vessel stroke increases soluble amyloid $A\beta$ levels in the brain in a process that depends upon TRPM2, an ion channel activated by oxidative stress. Soluble $A\beta_{40}$ and $A\beta_{42}$ levels were increased in the ipsilateral hippocampus in MCAo mice 7 days after the injury when compared to sham-treated mice (71.1% increase for $A\beta_{40}$, $p = 0.04$; 91.7% increase for $A\beta_{42}$, $p = 0.03$). Non-statistically significant increases in both soluble $A\beta_{40}$ and $A\beta_{42}$ were observed in the ipsilateral MCAo

cortex compared to the sham cortex. The increased hippocampal A β levels were notably only present in male mice, with no changes present in female mice. We also analyzed the level and activity of BACE1 (β -site APP cleaving enzyme 1), an enzyme that generates A β in the brain. We found that BACE activity is increased by approximately 50% in the ipsilateral hippocampus of the MCAo mice, with no changes in BACE expression levels. Finally, we measured A β levels and BACE activity after a MCAo stroke in TRPM2 knock-out mice and found no statistically significant increases in either of these parameters. Our data highlights that a transient MCAo stroke leads to increases in soluble A β 40 and A β 42 in the hippocampus of the injured brain via a mechanism that requires the presence of TRPM2. We also directly show that activation of BACE1 activity occurs after a MCAo stroke and may be a relevant pathway for increased A β production. Taken together, these data provide a potential molecular pathway linking ischemia to altered neurodegeneration and oxidative stress after a large vessel stroke and suggest that increased A β levels may lead to synaptic and cognitive deficits seen with PCSID.

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Poster

455. Neuroprotection II

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Topic: C.08. Ischemia

Support: NRF-2020R1A2B5B01001442
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Title: Astrocyte-derived OPN mediates the formation of corpora amylacea-like structures from degenerating neurons in the CA1 region of the rat hippocampus after ischemia

Authors: *T. RIEW^{1,2}, J.-W. HWANG¹, X. JIN³, H. KIM⁴, M.-Y. LEE^{1,2};

¹Dept. of Anat., Col. of Medicine, The Catholic Univ. of Korea, Seoul/Seocho-Gu, Korea, Republic of; ²Catholic Neurosci. Inst., Seoul, Korea, Republic of; ³Dept. of Radiology, Massachusetts Gen. Hosp., Boston, MA; ⁴Integrative research Support Center, Lab. of Electron Microscopy, The Catholic Univ. of Korea, Seoul/Seocho-gu, Korea, Republic of

Abstract: We previously demonstrated that osteopontin (OPN) is closely associated with calcium precipitation in response to ischemic brain insults. The present study was designed to elucidate the possible association between deposition of OPN and progressive neurodegeneration in the ischemic hippocampus. To address this, we analyzed the OPN deposits in the rat hippocampus after global cerebral ischemia in the chronic phase (4 to 12 weeks) after reperfusion using immunoelectron microscopy and correlative light and electron microscopy. We identified three different types of OPN deposits based on their morphological characteristics, numbered according to the order in which they evolved. Dark degenerative cells that retained

cellular morphology were frequently observed in the pyramidal cell layer, and type I OPN deposits were degenerative mitochondria that accumulated among these cells. Type II deposits evolved into more complex amorphous structures with prominent OPN deposits within their periphery and within degenerative mitochondria-like structures. Finally, type III had large concentric laminated structures with irregularly shaped bodies in the center of the deposits. In all types, OPN expression was closely correlated with calcification, as confirmed by calcium fixation and Alizarin red staining. Notably, type II and III deposits were highly reminiscent of corpora amylacea, glycoprotein-rich aggregates found in aged brains, or neurodegenerative disease, which was further confirmed by ubiquitin expression and periodic acid-Schiff staining. In addition, analysis by electron microscopy in combination with in-situ hybridization revealed distinct glial roles in the formation of OPN deposits. Astrocytes, but not microglia were shown to be involved in the production of type I OPN deposits while type II and III deposits were in direct contact with both astrocytes and microglia. Overall, our data provide a novel link between ongoing neurodegeneration and the formation of corpora amylacea-like structures and calcium deposits in the ischemic hippocampus, suggesting that astrocyte-derived OPN may play an important role in such processes.

Disclosures: T. Riew: None. J. Hwang: None. X. Jin: None. H. Kim: None. M. Lee: None.

Poster

455. Neuroprotection II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 455.09

Topic: C.08. Ischemia

Support: U.S. Dept. of Veterans Affairs; # 11O1 BX004884

Title: Hyperglycemia occurring in the days after stroke exacerbates brain damage by increasing microglial superoxide production.

Authors: *S. WON, R. FONG, S. GHOSH, J. WANG, A. ZHANG, N. J. BUTLER, O. TAMBOU NZOUTCHOUM, E. MOCANU, J. DAVIS, R. LAKKARAJU, A. HUYNH, K. KIM, R. A. SWANSON;
Neurol., VAMCSF & UCSF, San Francisco, CA

Abstract: It has been recognized for decades that elevated blood glucose (hyperglycemia) during ischemia/reperfusion greatly exacerbates injury; however, little is known about the effect of hyperglycemia that begins later, over the hours to days after ischemia. This often occurs after stroke as part of a systemic stress response, even in non-diabetic patients, but neither clinical evidence nor prior basic science studies provide guidance on how post-stroke hyperglycemia should be managed. Here we investigated this issue using a mouse photothrombotic model of permanent ischemia. Normoglycemia or hyperglycemia (blood glucose of 300-400 mg/dL; 15-20 mM) was maintained between 17 - 48 hours after ischemia onset. Post-stroke hyperglycemia was

found to increase the final infarct volume, increase hemorrhage formation, and exacerbate motor dysfunction. Hyperglycemia also increased superoxide formation in peri-lesional microglia/macrophages. Importantly, hyperglycemia did not have these deleterious effects in p47^{phox}^{-/-} mice, which cannot form an active superoxide-producing NADPH oxidase-2 complex, or in wild-type mice treated with a peptide inhibitor of NADPH oxidase-2 (gp91-TAT peptide). These results suggest that hyperglycemia occurring many hours after ischemia can increase oxidative stress in peri-infarct tissues by fueling NADPH oxidase activity in reactive microglia/macrophages, and by this mechanism may contribute to worsened outcome.

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Poster

455. Neuroprotection II

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Program #/Poster #: 455.10

Topic: C.08. Ischemia

Support: University of Wisconsin-School of medicine and public health startup funds

Title: Potential Role of Astroglial TLR4/ERK Signaling During Oxygen Glucose Deprivation and Focal Cerebral Ischemia

Authors: *S. TEERTAM¹, C. GENC², J. PANACKAL², C. MCBAIN², A. MCMILLAN², B. FAMA KIN²;

¹Univ. of Wisconsin, Madison, Madison, WI; ²Univ. of wisconsin, madison, WI

Abstract: Background: Astrocyte toll-like receptor-4 (TLR4) signaling occurs during in innate immune activation following focal cerebral ischemia. **Aim:** The involvement of astrocyte TLR4 signaling on BBB integrity is currently poorly understood following focal cerebral ischemia (FCI). The present study is aimed to investigate the role of HMGB-1 stimulation in astrocyte ERK/TLR4 signaling and in Oxygen Glucose Deprivation (OGD) in primary astrocytes. We also evaluated the role of astrocyte-specific TLR4 deletion on infarct size following Middle Cerebral Artery Occlusion (MCAo).

Methods: Wild type (WT) primary cortical astrocytes were stimulated with HMGB1 and pre-treated with TAK-242 (TLR4 specific inhibitor) to evaluate the role of HMGB-1 on TLR4/ERK signaling. We also performed 12h OGD in primary WT astrocytes and evaluated ERK signaling following OGD and OGD and reperfusion using immunoblotting. In addition, mice with astrocyte-specific TLR4 deletion (TLR4 CKO) and control mice (tamoxifen-treated) were subjected to transient middle cerebral artery occlusion (MCAo) and MRI was performed to assess the lesion volumes.

Results/Conclusion: Our study demonstrated that TAK-242 pre-treatment of cultured astrocytes

showed a trend towards decreased HMGB-1 induced ERK activation. In addition, ERK-phosphorylation was significantly increased following OGD and ERK-phosphorylation was reversed by reperfusion. Infarct volumes in mice with astrocyte-TLR4 deletion trended towards a decrease in infarct size compared to control tamoxifen-treated animals. These studies show that the TLR4/ERK signaling pathway plays an important role in astrocyte innate immune activation. Inhibition of TLR4/ERK signaling may play an important role in adjunctive treatment of stroke and inhibition of blood barrier permeability following stroke.

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Poster

455. Neuroprotection II

Location: SDCC Halls B-H

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Program #/Poster #: 455.11

Topic: C.08. Ischemia

Support: AFOSR Grant FA9550-18-1-0051
NIH Grant NS106138
NIH Grant NS097775

Title: Novel ATP-independent F-actin polymerization in neuronal dendrites undergoing ischemic stress

Authors: B. CALABRESE¹, R. MORTAZAVI¹, L. S. COTSIRILOS¹, C. E. ANDOLINA¹, S. RAVIPATI¹, A. Y. SHIH², *S. HALPAIN¹;
¹Neurobio., UC San Diego, La Jolla, CA; ²Seattle Children's Res. Inst., Seattle, WA

Abstract: Ischemia and excess NMDA receptor activation are known triggers of cytotoxic edema. Here, we show that, despite decreasing ATP levels, both hippocampal and cortical neurons respond to ischemia with a rapid and extensive accumulation of F-actin within the somatodendritic compartment (“actinification”). This is also accompanied by an extensive and simultaneous loss of dendritic spine structural integrity without a corresponding loss of presynaptic boutons. The sudden accumulation of F-actin in the dendritic shaft, where F-actin usually is relatively low, occurs both in vitro, after oxygen/glucose deprivation and in mouse brain in vivo after stroke. Hyperactivation of NMDA receptors drives actinification within minutes. Distinct from stress fibers, this specific actin assembly is myosin independent and, instead, it is driven by activity of the F-actin polymerization factor inverted formin-2 (INF2). The resulting long and linear filaments are highly decorated by drebrin and are resistant to latrunculin, suggesting that they turn over slowly, enduring while the ischemic stress persists. Indeed, upon stress removal, F-actin can readily disassemble and become re-concentrated within dendritic spines. When actinification is prevented, neurons become more vulnerable to NMDA-induced cell death in vitro and to increased ischemic infarct severity in vivo. These results

describe a novel neuron-specific, ATP-independent F-actin polymerization that is induced by cytotoxic edema and promotes cell survival post-ischemia.

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Poster

455. Neuroprotection II

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Topic: C.08. Ischemia

Support: Marie Lewis Research Foundation
NIH/NIGMS P20 GM109098
NINDS BINP R01 NS117754

Title: Chronic brain hypoperfusion produces Alzheimer's disease-like cognitive impairment and neuropathology in mice.

Authors: *K. KARELINA¹, D. R. CORBIN¹, B. L. CLARY¹, J. T. IVEY¹, R. OLIVERIO¹, C. OUDOMVILAY², C. ROMERO-BOHORQUEZ¹, B. WHITE¹, N. ZHANG¹, J. W. SIMPKINS¹, Z. M. WEIL¹, P. CHANTLER², C. M. BROWN¹;
¹Neurosci., ²Exercise Physiol., West Virginia Univ., Morgantown, WV

Abstract: Cerebrovascular disease plays a critical role in the pathogenesis of Alzheimer's disease and related dementias (ADRD). Indeed, reduced cerebral blood flow (CBF) due to intracranial arterial stenosis is an independent predictor of the development of ADRD. Further, numerous well-known AD risk factors including aging, hypertension, chronic inflammation, obesity, and brain injury share a common pathological mechanism: vascular pathology that promotes chronic brain hypoperfusion. However, until now, rodent models of acute permanent or transient hypoperfusion have been inadequate to study the mechanisms by which chronic hypoperfusion promotes ADRD. We recently adapted a mouse model of hypoperfusion with greater translational value, which produces a gradual reduction of CBF, in order to determine the effect of persistent cerebral hypoperfusion on cognitive decline and AD-associated brain pathology. Using the ameroid constrictor arterial stenosis (ACAS) model in C57Bl/6J mice, we restricted CBF to ~60% of baseline over a period of 30-60 days. Histological analysis of brains after 30 days of hypoperfusion revealed axonal degeneration (via silver staining) in the cortex, hippocampus, striatum, and corpus callosum, and cell death in the CA1 and dentate gyrus regions of the hippocampus (via Fluoro-jade B). Hippocampal qPCR revealed a trend toward increased miRNA 34a (linked to ADRD cognitive decline) in hypoperfused mice relative to shams. Finally, using pressure myography, we noted both endothelial dependent and independent dysfunction of the middle cerebral artery in ACAS vs sham mice. A separate cohort of animals underwent behavioral testing. Locomotor function was assessed via the rotarod, and revealed

significant impairments in hypoperfused mice relative to shams. Cognitive function was assessed via the hippocampus-dependent Barnes maze task. While all mice performed similarly during the 5 days of learning trials on the Barnes maze, the probe test revealed significant cognitive impairments with hypoperfused mice spending significantly less time in the target zone compared to shams. Taken together, these data replicate the clinical reports linking chronic brain hypoperfusion to the development of dementia, thus validating the utility of the ACAS model to study ADRD cognitive decline and neuropathology.

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Poster

455. Neuroprotection II

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Topic: C.08. Ischemia

Support: NIH Grant NS108484
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Title: Subventricular zone cytogenesis is a source of trophic support for neural repair after stroke

Authors: ***M. R. WILLIAMSON**¹, S. P. LE², R. L. FRANZEN², N. A. DONLAN², J. L. ROSOW², A. K. DUNN², T. A. JONES², M. R. DREW³;

¹Univ. of Texas at Austin, Austin, TX; ²Univ. of Texas at Austin, Austin, TX; ³Univ. of Texas At Austin, Austin, TX

Abstract: The subventricular zone (SVZ) contains neural precursor cells that produce new cells throughout life in a process called cytogenesis. Upon injury to the brain, cytogenesis increases substantially and newborn cells migrate towards the site of injury. We examined the identity and functions of newborn cells arising from the SVZ after stroke. With indelible lineage tracing, we show that the SVZ cytogenic response after cortical stroke in mice produces predominantly undifferentiated neural precursors that localize to peri-infarct regions. Reducing cytogenesis by ablating neural precursor cells or normal aging worsens motor recovery after stroke. Gain- and loss-of-function experiments demonstrate a crucial role of trophic signaling from SVZ-derived cells in driving neuronal and vascular plasticity and behavioral recovery after stroke. Thus, SVZ

cytogenesis provides a cellular source of trophic support for neural repair and recovery after stroke.

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Poster

455. Neuroprotection II

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Program #/Poster #: 455.14

Topic: C.08. Ischemia

Support: American Heart Association (16SDG30320001)
LSU Health Shreveport – Center for Applied Immunology and Pathological Processes

Title: Neuroimmune responses support neurogenesis and functional recovery following cerebral ischemia

Authors: B. AKHTER¹, N. AICH¹, M. BRADFORD¹, P. BOUDREAUX¹, T. BEAUDION¹, *K. RODGERS²;

¹Cell. Biol. and Anat., LSU Hlth. - Shreveport, Shreveport, LA; ²Cell. Biol. and Anatomy, Neurol. Dept., LSU Hlth. Shreveport, Shreveport, LA

Abstract: Introduction: Replacement of dead neurons following ischemia, either via enhanced endogenous neurogenesis or stem cell therapy, has been highly sought, but the low survival rate of newly generated neurons has left doubt about the therapeutic potential of adult neurogenesis. However, stroke in the young injured brain reveals a greater degree of plasticity and capacity for repair, along with enhanced post-ischemic functional outcomes compared to the adult. Our findings suggest that a possible mechanism is the immune response in the acute phase of stroke, which has a powerful age-related influence on neurogenesis and functional recovery. Our preliminary studies reveal an anti-inflammatory microglial signature during the acute phase of stroke in juveniles and proinflammatory in adults. While microglial responses have been shown to be both neuroprotective and neurodegenerative following brain injury, it has been generally accepted that activation of microglia during the acute response to stroke is detrimental for neurogenesis and neuronal replacement. However, our findings indicate that early microglial responses are key to survival of newborn neurons in juveniles. **Objective:** We utilized a juvenile stroke model to study the paracrine signaling triggered by ischemia in activated microglia and its consequence on newborn neuron survival and post-ischemic functional outcomes. **Methods:** Electroencephalography (EEG) was recorded from cortical surface electrodes and striatal depth electrodes implanted following transient middle cerebral artery occlusion (MCAO). Microglia were attenuated with Ibudilast (10 mg/kg), a glial cell activation inhibitor. Neurogenesis and inflammatory markers were examined and functional outcomes assessed with neurobehavioral

measures and ECoG recordings. **Results:** Short-term inhibition (4d) of microglial activation during the acute phase after stroke resulted in reduced neurogenesis and reversal of motor recovery and neuroplasticity in MCAO-injured juveniles. In contrast, treatment in MCAO-injured adults improved neurogenesis and functional outcomes revealing age-related differences in post-ischemic immune responses. **Conclusions:** These striking findings provide evidence of a supportive role for microglia in neurogenesis and improved functional recovery following stroke in juveniles. This novel insight into early, anti-inflammatory microglial responses after stroke may alter strategies for effective stroke treatment, promoting substantial post-ischemic neuronal replacement and functional recovery in both juveniles and adults.

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Poster

455. Neuroprotection II

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Topic: C.08. Ischemia

Title: The roles of astrocytic NAMPT in mediating astrocyte metabolism and neuronal survival after ischemic stroke

Authors: ***Z. ZHANG**¹, S. DING², N. ZHANG¹;

¹Univ. of Missouri-Columbia, COLUMBIA, MO; ²Dept. of Biomedical, Biol. and Chem. Engin., Univ. of Missouri, Columbia, MO

Abstract: The roles of astrocytic NAMPT in mediating astrocyte metabolism and neuronal survival after ischemic stroke Zhe Zhang, Nannan Zhang, Shinghua Ding NAD⁺ is an important co-factor in cellular metabolism include glycolysis, TCA cycle and oxidative phosphorylation. Nicotinamide phosphoribosyl transferase (NAMPT) is the rate-limiting enzyme in the salvage pathway of mammalian NAD⁺ biosynthesis. In our previous studies, we demonstrated that neuronal NAMPT is protective in in vivo and in vitro ischemia. Astrocyte is a predominant glial cell type in the central nervous system (CNS) and plays an important role in regulating of neuronal function under normal conditions and promote neuronal survival after ischemic stroke. Here we found that NAMPT was highly upregulated in reactive astrocytes in both in vivo and in vitro ischemic models, suggesting NAMPT in reactive astrocytes may play an important role in bioenergetics and neuronal survival and brain repair after stroke. Thus, in this study, we knocked down NAMPT in primary astrocytes using siRNA and studied its effects on astrocyte metabolism and neuronal survival after stroke. We show that NAMPT knockdown significantly reduced NAD⁺, NADH and ATP levels in primary astrocytes. Moreover, we found that NAMPT knockdown reduced extracellular acidification rate (ECAR) remarkably but has little effect on oxygen consumption rate (OCR). We also show that astrocytic NAMPT is essential for the activation and proliferation of astrocytes after ischemia. More importantly, using conditional

medium, we demonstrated that astrocytic NAMPT can promote neuronal survival after glutamate excitotoxicity. Finally, using photothrombosis-induced ischemia model, we showed that astrocyte-specific conditional knockout mice exhibit increased brain infarction and neuronal death compared with wildtype mice. Collectively, our study demonstrates that reactive astrocytes play a critical role in regulating metabolism in astrocytes and promoting neuronal survival and brain repair after ischemia through enhanced expression of NAMPT.

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Poster

455. Neuroprotection II

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Topic: C.08. Ischemia

Support: Barrow Neurological Foundation

Title: The evolution of NETosis biomarkers and their relationship with peripheral immune cells in a preclinical model of ischemic stroke

Authors: *M. WU¹, J. YIN², T. MOHSEN², A. KINDELIN³, S. AHMAD², A. F. DUCRUET², A. S. AHMAD², M. WATERS²;

¹Barrow Neurolog. Inst., phoenix, AZ; ²Barrow Neurolog. Inst., Phoenix, AZ; ³Arizona State Univ., Tempe, AZ

Abstract: Neutrophil extracellular traps formation (NETosis) facilitates thrombosis and contributes to reperfusion resistance, a major challenge encountered during the thrombolytic treatment of acute ischemic stroke. However, the effect of stroke on NETosis biomarkers in plasma is not fully understood. Identifying the precise evolution of NETosis biomarkers and their potential effect on cerebral infarct expansion and their relationship with peripheral immune cells may provide new insights into the therapeutic options for acute ischemic stroke. Three month old C57BL/6 male mice were subjected to middle cerebral artery occlusion for 60 minutes followed by reperfusion (Ischemia-Reperfusion, IR). Control (ctrl) mice did not undergo IR. Peripheral blood was collected on days 1, 2, 3, 5, and 7 after IR and complete blood counts with differentials (CBCD) were measured and the ratios between whole blood cell populations were determined. Plasma was isolated and collected from the remaining whole blood and the concentrations of NETosis biomarkers [Citruinated histone 3 (CitH3), Neutrophil Elastase (NE), Myeloperoxidase (MPO), and deoxyribonucleic acid (DNA)] were measured via enzyme-linked immunosorbent assay and the relationship between the plasma concentrations of NETosis biomarkers and CBCDs were determined. Compared to ctrl mice, IR-subjected mice exhibited a drastic increase in plasma CitH3 and NE on day 1 ($p < 0.05$) while DNA and MPO reached their peak levels on day 3. IR-subjected mice also showed a significant increase in peripheral neutrophils and decline in peripheral leukocytes, lymphocytes, and monocytes on day 1 and day

2 ($p < 0.05$). The ratios of neutrophil to lymphocyte, neutrophil to leukocyte, lymphocyte to monocyte, and platelet to lymphocyte dramatically increased, while the ratio of platelet to neutrophil decreased on day 1 ($p < 0.05$) after IR. Correlation analysis showed that plasma NE level was positively correlated with neutrophil percentage but inversely correlated with lymphocyte percentage ($p < 0.05$). Plasma CitH3 level was positively related with neutrophil percentage ($p < 0.05$). Plasma MPO was positively correlated with neutrophil percentage but inversely related with lymphocyte percentage ($p < 0.05$). Our findings indicate that there are time dependent changes in plasma NETosis biomarkers concentrations and CBCDs after IR. We propose that targeting these biomarkers before they reach their respective peaks may offer potential therapeutic options to prevent the expansion of cerebral infarction and functional deterioration. Further investigation is needed to confirm this hypothesis and its clinical implications.

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Poster

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Topic: C.08. Ischemia

Support: DFG RU 2795

Title: Synaptic and perisynaptic glutamate signaling during the onset of metabolic stress

Authors: *S. PASSLICK¹, V. ZAVIALOV¹, J. A. FERIA-PLIEGO¹, P. UNICHENKO¹, N. KIANI¹, C. HENNEBERGER^{1,2};

¹Inst. of Cell. Neurosciences, Univ. of Bonn, Bonn, Germany; ²German Ctr. for Neurodegenerative Dis. (DZNE), Bonn, Germany

Abstract: Disruption of glutamate homeostasis is considered a central step in the pathophysiology of ischemia and stroke. Lack of energy causing breakdown of ion gradients, increased glutamate release and impaired glutamate clearance are thought to promote the accumulation and spread of glutamate in the extrasynaptic space leading to excitotoxic damage via overactivation of N-methyl-D-aspartate receptors (NMDARs). However, the sequence of events and the specific mechanisms that lead to perturbed glutamate signaling during varying durations of acute metabolic stress remain largely unclear. Therefore, we combined electrophysiology and pharmacological intervention with two-photon excitation imaging of synaptic glutamate release, Ca²⁺ signaling and astrocyte morphology to study the effect of moderate and strong metabolic stress on glutamate homeostasis in acute hippocampal slices. We found that strong metabolic stress resulted in a persistent failure of synaptic transmission, whereas moderate stress led to a transient failure, followed by a potentiation of synaptic

transmission. While the former was accompanied by strong increases in extracellular $[K^+]$, the latter was associated with small $[K^+]$ increases. Likewise, monitoring of extracellular glutamate levels or astrocytic Ca^{2+} transients revealed that long periods of metabolic stress induced a transient strong surge of extracellular glutamate and a prolonged increase in somatic $[Ca^{2+}]$, whereas short periods did not alter resting glutamate levels or astrocytic Ca^{2+} transients. However, we observed an unexpected persistent increase in glutamate transients evoked by synaptic stimulation although paired-pulse properties remained unchanged. Further experiments revealed that the increased glutamate transients were not due to changes in glutamate uptake dynamics, in astrocyte morphology or in the fraction or tortuosity of the extracellular space. However, the potentiation of synaptic transmission was sensitive to NMDAR inhibition and sensor imaging of the NMDAR co-agonists glycine and D-serine revealed increases in both in response to strong metabolic stress. Current experiments aim at investigating the mechanisms leading to increased synaptic glutamate transients after brief episodes of metabolic stress.

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Poster

455. Neuroprotection II

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Title: Reactive astrocyte-derived LCN2 in neurodegeneration after stroke

Authors: *O. CAPUK¹, R. LIU¹, J. WANG¹, Y. CHEN¹, J. COLLIER^{2,4}, S. JIN⁵, M. SUN³, S. MONDAL⁶, T. WHITESIDE⁶, D. STOLZ³, Y. YANG⁵, G. BEGUM¹;

¹Dept. of Neurol., Pittsburgh Inst. for Neurodegenerative Diseases, Univ. of Pittsburgh, Pittsburgh, PA; ²Dept. of Neurobio., ³Dept. of Cell Biol., Univ. of Pittsburgh, Pittsburgh, PA; ⁴Dept. of Neurol., Pittsburgh Inst. for Neurodegenerative Dis., Pittsburgh, PA; ⁵Dept. of Neurosci., Tufts Univ. Sch. of Med., Boston, MA; ⁶Dept. of Pathology, Univ. of Pittsburgh and UPMC Hillman Cancer Ctr., Pittsburgh, PA

Abstract: Reactive astrocytes secrete lipocalin-2 (LCN2) glycoprotein under inflammatory conditions that promotes cell death and degeneration. Elevated levels of LCN2 are considered as a biomarker of brain injury, however, the underlying regulatory mechanisms of its expression and release are not well understood. In this study, we investigated the role of astrocytic Na^+/H^+ exchanger 1 (NHE1) in regulating reactive astrocyte LCN2 secretion and neurodegeneration

after stroke. Astrocyte specific deletion of *Nhe1* in *Gfap-Cre^{ER+/-};Nhe1^{ff}* mice reduced astrogliosis and astrocytic LCN2 and GFAP expression, which was associated with reduced loss of NeuN⁺ and GRP78⁺ neurons in stroke brains. *In vitro* ischemia in astrocyte cultures triggered a significant increase of secreted LCN2 in astrocytic exosomes, which caused neuronal cell death and neurodegeneration. Inhibition of NHE1 activity during *in vitro* ischemia with its potent inhibitor HOE642 significantly reduced astrocytic LCN2⁺ exosome secretion. In elucidating the cellular mechanisms, we found that stroke triggered activation of NADPH oxidase (NOX)-NF- κ B signaling and ROS-mediated LCN2 expression. Inhibition of astrocytic NHE1 activity attenuated NOX signaling and LCN2-mediated neuronal apoptosis and neurite degeneration. Our findings demonstrate for the first time that reactive astrocytes use NOX signaling to stimulate LCN2 expression and secretion. Blocking astrocytic NHE1 activity is beneficial to reduce LCN2-mediated neurotoxicity after stroke. Support: NIH R01 NS110755-01A1 (G.B); S10OD016236 (S.W.), R01NS118747 and R01NS125490 (Y.Y.).

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Poster

455. Neuroprotection II

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Topic: C.08. Ischemia

Support: American Heart Association
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Cognitive Neuroscience of Development and Aging (CoNDA) Center
LB692 Nebraska Biomedical Research Development Fund

Title: TREM1-mediated neuroinflammation in global ischemia pathology

Authors: *R. URQUHART, H. KIM, G. P. JADHAV, J.-Y. HWANG;
Pharmacol. and Neurosci., Creighton Univ., Omaha, NE

Abstract: Comorbid with cardiac arrest, global ischemic stroke is caused by loss of cerebral blood flow, which induces hypoxia affecting the whole brain. Global ischemia promotes selective, delayed neurodegeneration of hippocampal CA1 pyramidal neurons, contributing to hippocampal-based learning and memory deficits. Understanding the molecular mechanisms driving global ischemia pathology is of urgent necessity for development of novel therapeutic strategies to address global ischemia-induced neurodegeneration and cognitive deficits. RNA-seq and Ingenuity Pathway analysis of rat CA1 subjected to global ischemia via 4-vessel occlusion revealed the triggering receptor expressed on myeloid cells-1 (TREM1) pathway is activated at

48 hr after ischemia. TREM1 is an innate immune receptor responsible for initiating and amplifying inflammation via synergism with immune-response related Toll-like receptors. TREM1 has an established proinflammatory role in myocardial ischemia, sepsis, and focal ischemia, but its role in global ischemia pathology is unclear. Thus, it was hypothesized that TREM1-mediated neuroinflammation promotes global ischemia-induced neurodegeneration, and TREM1 inhibition can rescue hippocampal integrity and function. In our validation experiments, RT-qPCR and Western blot analyses of rat CA1 reveals that TREM1 expression is significantly elevated within 3 hr of ischemic insult, and this increase is maintained for 48HR. Additional targets associated with TREM1 activation and signaling, including signaling partners (DAP12 (TYROBP), SYK), signal transduction targets (Nf-kB, STAT3), and downstream inflammatory cytokines (IL-1B, IL-18, IL-6) are differentially expressed within 3-48HR of ischemic insult. To examine a causal relationship between global ischemic insult and activation of TREM1 signaling, we tested if TREM1 inhibition can prevent global ischemia-induced neurodegeneration and cognitive deficit. LR12, a TREM1 inhibitory peptide, was stereotaxically administered directly into hippocampal CA1 in rats immediately after ischemic or sham surgery. Data is currently being collected to determine the neuroprotective effect of LR12. If LR12 administration can prevent TREM1 signaling and ameliorate loss of neurons and hippocampal-based learning and memory, this research will have established the importance of TREM1-mediated neuroinflammation and the therapeutic potential of TREM1 inhibition in global ischemia.

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Poster

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Support: NIH Grant R01MH086638

Title: Multiscale simulations of spreading depolarization with ischemic effects

Authors: *A. NEWTON¹, C. KELLEY^{1,3}, R. A. MCDOUGAL^{4,5,6,7}, W. W. LYTTON^{1,8,9,2},
¹Dept. of Physiol. and Pharmacol., ²Dept. of Neurol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; ³NYU Tandon Sch. of Engin., New York, NY; ⁴Ctr. for Med. Informatics, ⁵Dept. of Biostatistics, ⁶Program in Computat. Biol. and Bioinformatics, ⁷Wu Tsai Inst., Yale Univ., New Haven, CT; ⁸Dept. of Neurol., Kings County Hosp. Center., New York, NY; ⁹The Robert F. Furchgott Ctr. for Neural and Behavioral Sci., New York, NY

Abstract: Disruption of homeostasis is a characteristic of many pathologies including; ischemia, migraine aura, and epilepsy. We modeled challenges to ion homeostasis at the subcellular and tissue scale using NEURON/NetPyNE, focusing on spreading depolarization (SD) in the

presence or absence of ischemia. SD is characterized by a breakdown in the homeostatic maintenance of intracellular and extracellular ion concentrations, resulting in a wave of depolarization that propagates at ~2-7 mm/min with a period of neuronal silence lasting minutes. Our modeling looked at single-neuron effects as well as the tissue phenomenon (cells in extracellular space -- ECS), covering scales from local ion concentrations to slice.

Our single neuron simulations examined subcellular distributions of ions within a morphologically detailed CA1 pyramidal neuron. We used RxD with evolutionary algorithms (BluePyOpt) to obtain parameters that produce realistic electrophysiological responses while maintaining the homeostasis of K^+ , Na^+ , Ca^{2+} , and Cl^- , simulating electrical and ionic challenges in normoxic vs hypoxic conditions. With either stimulus, hypoxia produced an influx of Na^+ , increasing Na^+/K^+ pump activity, depleting ATP reserves, and gradually reducing K^+ and Na^+ Nernst potentials. Accumulation of Ca^{2+} (from both ECS and endoplasmic reticulum) and Cl^- followed the resulting depolarization. We varied basal oxygen concentrations and found an abrupt transition between normoxic and hypoxic behavior. Our model predicts dendrites will be more vulnerable to hypoxia. Dendrites' larger-surface volume ratio leads to a more rapid loss of homeostasis, with an increase in potentially excitotoxic Ca^{2+} and Cl^- relevant for dendritic beading and dendritic pruning.

Tissue simulation simulated a slice with realistic neuronal density ($90,000/mm^3$), which included K^+ , Na^+ , Cl^- ions together with oxygen-dependent Na^+/K^+ pumps. Simulated SD propagated at 2-4 mm/min, which increased by as much as 50% in models incorporating the effects of hypoxia or propionate. Our model made the following testable predictions; 1. SD can be inhibited by enlarging ECS volume; 2. SD velocity will be greater in areas with greater neuronal density, total neuronal volume, or larger/more dendrites; 3. SD is all-or-none: initiating K^+ bolus properties have little impact on SD speed; 4. Slice thickness influences SD due to relative hypoxia in the slice core, exacerbated by SD in a pathological cycle; 5. SD and high neuronal spike rates will be observed in the core of the slice. Cells in the periphery of the slice near an oxygenated bath will resist SD.

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Poster

455. Neuroprotection II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 455.21

Topic: C.08. Ischemia

Support: NIH NHLBI T32 HL125242
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Title: Cyclic Ion Mobility Mass Spectrometry Analysis of Cardiolipin Neuronal Subspecies in Primary Culture, Mice and Pigs

Authors: *K. J. EMAUS^{1,2}, D. M. MAKEY³, J. M. WIDER^{1,4}, E. GRULEY¹, J. MATHIEU¹, G. M. FOGO^{1,2}, B. T. RUOTOLO³, T. H. SANDERSON^{1,2,5,4,6};
¹Emergency Med., Univ. of Michigan, ANN ARBOR, MI; ²Neurosci. Grad. Program, ³Dept. of Chem., ⁴Max Harry Weil Inst. for Critical Care Res. and Innovation, ⁵Dept. of Mol. and Integrative Physiol., ⁶Frankel Cardiovasc. Ctr., Univ. of Michigan, Ann Arbor, MI

Abstract: Perinatal ischemia can result in hypoxia and severe neuronal damage to the infant brain. Reestablishing blood flow (reperfusion) is crucial for survival. However, reperfusion induces an accumulation of reactive oxygen species (ROS) produced by the mitochondria, culminating in high oxidative stress and irreversible tissue damage. This reveals a demand for therapeutics and treatments that address the larger neonatal hypoxia-ischemia encephalopathy (HIE) pathology. Current studies suggest the mitochondrial phospholipid cardiolipin (CL) converts to monolysocardiolipin (MLCL) when experiencing an environment high in oxidative stress. The accumulation of MLCL is thought to contribute to disruption of the healthy mitochondrial network, leading to insufficient ATP production, and programmed cell death. The physical structure of CL itself consists of a glycerol head and four fatty acid tails. During biosynthesis of CL a remodeling process occurs which alters the degree of saturation of the fatty acid tails. This remodeling process has clinical implications as seen in Barth Syndrome; wherein an X linked mutation resulting in mitochondrial dysfunction and dilated cardiomyopathy. While this remodeling pathway has been studied extensively in cardiomyocytes, its role in neuronal injury is largely unknown. We hypothesize the CL remodeling pathway to be integral to mitochondrial dynamics and overall neuronal health following oxidative injury. In the brain, there are several subspecies of CL, making analysis and quantification difficult. Using cyclic ion mobility-mass spectrometry (cIM-MS), we have been able to analyze CL isomers at higher resolution than previous ion mobility-mass spectrometry methods. Due to its circular traveling wave ion mobility path, the cIM-MS allows samples to undergo multiple passes rather than a single pass as seen in linear traveling wave ion mobility path. With each pass around the circular path the ions separate more, resulting in significantly increased resolution. Using this technology, we have identified CL subspecies in primary neuron cell culture and from mice and pig brain biopsies. Future studies will utilize this technology to analyze CL subspecies and MLCL in large and small animal models of neonatal HIE. The higher resolution achieved with the cIM-MS can provide a better understanding of the mechanistic role of CL in injury progression and potentially uncover targets for therapeutic intervention.

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Poster

455. Neuroprotection II

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Program #/Poster #: 455.22

Topic: C.08. Ischemia

Support: JSPS Grant 20J21472

Title: Extracellular DJ-1 Induces Sterile Inflammation In Ischemic Brain

Authors: ***K. NAKAMURA**^{1,2}, **R. KOYAMA**², **S. SAKAI**², **J. TSUYAMA**², **T. SHICHITA**²;
¹Dept. of Computat. Biol. and Med. Sci., The Univ. of Tokyo, Tokyo, Japan; ²Stroke Renaissance Project, Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan

Abstract: -Objective

Post-ischemic inflammation plays a key role in the progression of ischemic stroke pathologies. However, the detailed molecular mechanisms underlying the activation of infiltrating immune cells which trigger sterile post-ischemic inflammation have not been sufficiently clarified. We tried to identify the previously unknown damage-associated molecular patterns (DAMPs) which were inflammotogenic self-molecules derived from damaged tissue.

-Methods

Among the candidate proteins which were detected from brain lysate by mass spectrometry, recombinant proteins were generated and added to the culture of bone marrow-derived macrophages (BMMs) to examine the expression of inflammatory cytokines. To determine the important peptide sequence for DAMP activity, deletion mutant peptides were generated. We finally examined the extracellular release of candidate DAMPs by using a mouse model of transient middle cerebral artery occlusion (MCAO). Neutralizing antibodies or KO mice were used for compromising DAMP activity in ischemic stroke.

-Results

We successfully identified DJ-1 (Also known as Park7) as a novel DAMP in brain lysate. Recombinant DJ-1 protein activated BMMs only through TLR2 and TLR4. The expression of inflammatory cytokines was induced in a DJ-1 dose-dependent manner in vitro. DJ-1 had a unique peptide sequence, which was not related to its antioxidant activity, to trigger the production of inflammatory cytokines.

In the ischemic brain, the induction of DJ-1 expression was observed within only ischemic neuronal cells 6 to 12 hours after stroke onset. Twenty-four hours after stroke onset, DJ-1 was passively released into extracellular space from necrotic brain cells and directly contacted with the surface of infiltrating myeloid cells. DJ-1 deficiency significantly reduced the expression of inflammatory cytokines after the stroke. Administration of DJ-1-neutralizing antibody suppressed the expression of inflammatory cytokines and reduced the infarct volume and improved neurological deficits.

-Conclusion

DJ-1 was released into extracellular space in the ischemic brain and functioned as the previously unknown DAMP that directly activated infiltrating myeloid cells and induced sterile inflammation. Thus, extracellular DJ-1 would be a prominent therapeutic target for ischemic stroke.

Disclosures: **K. Nakamura:** None. **R. Koyama:** None. **S. Sakai:** None. **J. Tsuyama:** None. **T. Shichita:** None.

Poster

455. Neuroprotection II

Location: SDCC Halls B-H

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Program #/Poster #: 455.23

Topic: C.08. Ischemia

Support: Boag Family Endowment in Neuroscience
R. S. McLaughlin Fellowship
New Frontiers in Research Fund

Title: Effects of reactive oxygen species and antioxidants upon ischemic spreading depolarization in mouse hemi-brain slices

Authors: *P. C. P. S. GERMANO¹, R. D. ANDREW², J. A. HELLAS²;
¹Queen's Univ., Kingston, ON, Canada; ²Dept Biomed. & Mol. Sciences,,
Queen`s Univ., Kingston, ON, Canada

Abstract: Reactive oxygen species (ROSs) are highly reactive molecules generated the oxidative stress of brain ischemia which induce neuronal damage. ROS levels in the brain can be neutralized by the action of antioxidants. The standard `text-book` story is that during stroke or traumatic brain injury (TBI), excess glutamate release over-excites neurons, elevating Ca⁺² influx that damages mitochondrial handling of oxidative stress. But immediately upon ischemic stress, spreading depolarization (SD) is consistently initiated in gray matter (independent of glutamate) and recurrent SD could in fact generate and be affected by ROSs. Therefore, this study examined the effects of ROSs and their potential neutralization by antioxidants upon SD. We imaged intrinsic changes in light transmittance (LT) in mouse coronal hemi-brain slices during SD induced by oxygen/glucose deprivation (OGD) to discern the effects upon SD by ROS exposure. Artificial cerebrospinal fluid (aCSF) superfused the slices and was used as a vehicle for one of two ROSs (1 mM H₂O₂ or 20 μM Rotenone) individually or following antioxidant pretreatment. The ROSs H₂O₂ or rotenone alone did not affect the time to SD onset but dramatically increased the speed of SD propagation through both neocortex and hippocampal CA1 as compared to the control group (OGD alone). When the antioxidants TEMPO (500nm or 10 μM) or ascorbic acid (20 μM) in aCSF were bath-applied for ~30 minutes prior to ROS exposure, TEMPO significantly delayed SD onset in the neocortex and ascorbic acid significantly delayed SD onset in CA1. TEMPO and ascorbic acid pretreatment reversed the elevated speed of SD propagation induced by rotenone or H₂O₂, indicating that ROSs can speed up the SD front. Furthermore, the antioxidants reduced peak light transmittance compared to the control group and the H₂O₂ or rotenone groups. We suggest that during periods of recurrent SD in ischemic gray matter, ROS accumulation promotes the imitation and propagation of SD. Future field potential studies in these slices will confirm longer-term ROS damage to neurons as well as neuroprotective reversal by antioxidants.

Keywords: reactive oxygen species; antioxidant; spreading depolarization.

Disclosures: P.C.P.S. Germano: None. R.D. Andrew: None. J.A. Hellas: None.

Poster

455. Neuroprotection II

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Program #/Poster #: 455.24

Topic: C.08. Ischemia

Support: Diversity Supplement NS046072

Title: Sexually Dimorphic Amygdala Dysfunction in a Mouse Model of Global Cerebral Ischemia

Authors: *J. J. VIGIL¹, E. TIEMEIER¹, N. E. CHALMERS¹, P. S. HERSON², N. QUILLINAN¹;

¹Anesthesiol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO; ²Neurosurg., Ohio State Univ., Columbus, OH

Abstract: Modern medical advances have greatly increased the chance of surviving an ischemic event such as cardiac arrest or stroke. With more people surviving and recovering from these ischemic insults, it is becoming apparent that survivors experience long-term effects as it relates to brain function. We have previously identified cognitive dysfunction in a mouse model of global cerebral ischemia (GCI) which has been attributed to hippocampal neurodegeneration and impaired hippocampal plasticity. However, no study has attempted to identify amygdala dysfunction after GCI, despite clinical evidence of emotional dysfunctions, such as anxiety and PTSD. Given these clinical findings, it is important to identify the effect that GCI has on the amygdala, the emotional center of the brain. I hypothesize GCI results in deficits in amygdala dependent learning tasks and circuit specific deficits of long-term potentiation (LTP). Experimental GCI was induced in adult (8-12 week) C57BL6 mice via cardiac arrest and subsequent cardiopulmonary resuscitation (CA/CPR). CA/CPR was induced for 8 minutes and subsequent resuscitation by epinephrine injection, ventilation and mild chest compressions. Neuronal injury was evaluated at 3 days after CA/CPR by Fluorojade staining in coronal brain sections. Seven days post GCI, the amygdala-dependent delay fear conditioning paradigm was used to assess amygdala-dependent learning and memory. Synaptic plasticity was evaluated by performing LTP recordings in the basolateral amygdala. We observed no acute cell death within the amygdala. Behavioral testing revealed that only male mice displayed a background contextual fear deficit (Male:74.05% sham freezing vs. 47.2% CACPR freezing). Also, only male mice displayed a diminished cued fear response (52.4% sham freezing vs. 26.6% in CACPR). Similarly, plasticity involving cortical inputs to the basolateral amygdala was impaired only in males (143.6% of baseline in controls vs. 110.9% of baseline in CACPR). Interestingly intra-amygdala recordings revealed no disruption of LTP in this circuit. These results support the role of the amygdala in cognitive-affective impairments after CA despite a lack of neuronal cell death in this brain region. We have revealed a sexually dimorphic deficit in amygdala-dependent fear learning and memory that provide new insights into the role that biological sex plays in mediating brain dysfunction following CA. Our results also suggest a sex- and circuit-specific

deficit in synaptic plasticity within the amygdala that correlates with behavioral outcomes in males. We will continue to unravel the mechanisms by which this sexually dimorphic impairment occurs.

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Poster

455. Neuroprotection II

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Program #/Poster #: 455.25

Topic: C.08. Ischemia

Support: NSERC Grant RG203596-13

Title: Selective GPER agonist G-1 protects from global cerebral ischemia-induced impairments of glucocorticoid receptor and 5-HT1A receptor regulation in the hippocampus CA1 in ovariectomized Wistar rats

Authors: ***A. MORIN**, E. BRES, M. LAUZON, M. POITRAS, A. DOIRON, J. TUBIN, C. CUSHMAN, H. PLAMONDON;
Behavioural Neurosci., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Global cerebral ischemia (GCI), stemming from cardiac arrest, results in severe neuronal death and alterations in expression of affect-mediating markers in brain regions like the hippocampus CA1. Menopause is characterized by increased vulnerability to cardiovascular disease due to decreasing estrogen levels. G-1 is a selective agonist to G protein-coupled estrogen receptors (GPER) which has been shown to provide neuroprotection in ischemia models during menopause. This study assessed putative protection conferred by acute or repeated G-1 injections on glucocorticoid receptors (GR), serotonin receptors 1A (5-HT1A), and tyrosine hydroxylase (TH) expression following GCI. Ovariectomized Wistar rats were injected intraperitoneally for 7 consecutive days with only saline (vehicle), 6 days of saline and 1 day of G-1 (50 µL/kg; acute), or only G-1 (repeated) before undergoing 10-min GCI by four-vessel occlusion or sham surgery. Brain tissue was collected for thionine staining to determine neuronal injury and for immunofluorescent detection of GR and 5-HT1A in the CA1, basolateral amygdala (BLA), and paraventricular nucleus of the hypothalamus (PVN) and both GR and TH in the ventromedial prefrontal cortex (vmPFC). GCI led to significant neuronal death in the CA1, which was prevented by both G-1 administration regimens. Similarly, both GR and 5-HT1A were downregulated in the CA1 while G-1-exposed rats showed similar levels to sham-operated rats for both markers. No changes were found in the BLA, PVN, and vmPFC. These results highlight the ability of G-1, whether administered acutely or repeatedly, to protect from neuronal death and dysregulated GR/5-HT1A receptor expression in the vulnerable CA1.

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Poster

455. Neuroprotection II

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Topic: C.08. Ischemia

Support: NIH (NS094507)
Leducq Foundation (Stroke-IMPACT)

Title: The role of type 1 conventional dendritic cells in controlling the gut-brain axis in ischemic stroke

Authors: *A. COGO¹, C. IADECOLA², J. ANRATHER³;

¹Cornell University: Weill Cornell Med. Col., New York, NY; ²Weill Cornell Med., New York, NY; ³Weill Med. Col. Cornell Univ., New York, NY

Abstract: Ischemic stroke induces local and systemic immune responses which impact stroke outcome. Our laboratory has previously shown that antibiotic-induced alterations of the intestinal flora reduced ischemic brain injury in mice via dendritic cell (DC) mediated induction of intestinal regulatory T cells (Treg) and subsequent suppression of local IL17-producing $\gamma\delta$ T cells that participate in the inflammatory response after stroke by migrating from the gut to the meninges during the hyperacute phase. Type 1 conventional DCs (cDC1) are involved in Th1 polarization but also control the induction of Treg, whereas type 2 conventional DC (cDC2) are critical for Th2 and Th17 cell polarization for example. Here we focused on the role of the cDC1 in ischemic brain injury, utilizing *Batf3*^{-/-} mice that are selectively deficient in cDC1. In a model of transient focal cerebral ischemia, we show that ischemic brain injury is more severe in *Batf3*^{-/-} mice compared to the wild type (WT) mice (35.58±13.7 mm³ vs 21.27±4.64 mm³; p<0.05). Interestingly, altering intestinal microbiota by antibiotic (amoxicillin/clavulanate) treatment did not result in neuroprotection in *Batf3*^{-/-} mice while WT mice showed reduced infarct volume (15.77±6.8 mm³ vs 36.17±16.66 mm³; p<0.05). Compared to WT mice, *Batf3*^{-/-} mice showed increased Th17 cells in the lamina propria of the small intestine and the mesenteric lymph nodes. Antibiotic-induced microbial alteration reduced Th17 and IL17⁺ $\gamma\delta$ T cells in the lamina propria of the small intestine in WT mice but failed to do so in *Batf3*^{-/-} mice. Our results suggest that in ischemic brain injury cDC1 exert a neuroprotective effect by promoting Treg polarization and downregulating Th17 and IL17⁺ $\gamma\delta$ T cells in the intestine. The results also suggest that the neuroprotection observed in mice with an altered microbiome is dependent on cDC1 which might constitute the main link between gut microbiota and intestinal T cell immunity.

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Poster

455. Neuroprotection II

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Program #/Poster #: 455.27

Topic: C.08. Ischemia

Support: NINDS NS111767

Title: Differential regulation of glutamate transporters following ischemia

Authors: *S. K. GILL, K. L. REEB, A. C. K. FONTANA;
Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Stroke is one of the leading causes of death in the United States, however adequate treatment is still lacking. Moreover, the pathological mechanisms involved are still not fully understood, highlighting the need for further studies. In this work, we employed the oxygen glucose deprivation (OGD) *in vitro* model of ischemic stroke, to study the regulation of excitatory amino acid transporters (EAATs). These transporters help control extracellular concentrations of glutamate in the brain through its uptake. Glutamate dysregulation, such as following stroke, can lead to a neuronal death through a mechanism known as excitotoxicity. EAATs therefore play a crucial role excitotoxic outcomes by mitigating glutamate imbalances. Our goal was to understand how these transporters are regulated following different severities of ischemic insult as well the mechanisms that control this regulation. Our results demonstrated that increasing severities of insult resulted in decreased rates of glutamate transport, suggesting an inability of these transporters to effectively clear excess glutamate. We also found that the expression of glial transporters EAAT1 and EAAT2 are differentially regulated: EAAT1 is downregulated after ischemia, with a more profound effect after moderate insults, whereas EAAT2 is upregulated after mild and moderate OGD before decreasing below control levels after severe insult. These results suggest that these transporters may work together, likely as a compensatory mechanism. Furthermore, recently collected data using biotinylation approaches also show a similar pattern of surface expression of these transporters suggesting either their internalization or increased trafficking to the surface following insult. We next began to investigate the mechanisms that lead to the differential regulation of EAAT1 and EAAT2. As of recent we have collected data on possible mechanisms regulating EAAT2 expression following ischemia, one of the key players being the transcription factor NF- κ B. Expression levels of activated NF κ B was found to follow the same expression pattern as EAAT2, with upregulation after mild and moderate insults followed by downregulation after severe insults, providing evidence that NF- κ B may influence the increased transcription of EAAT2 following ischemia. In support, data collected from chromatin immunoprecipitation (ChIP) studies followed by qPCR will provide direct evidence of NF- κ B regulation of the EAAT2 gene. In sum, these findings advanced our knowledge on EAAT regulation following different severities of ischemic stroke *in-vitro* and provide insight into possible regulatory mechanisms involved in the process.

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Poster

455. Neuroprotection II

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 455.28

Topic: C.08. Ischemia

Support: FY2021 CHOP/PENN Mitochondria Research Affinity Group CHOP Core Facility Grant

Title: Persistently altered mitochondrial profile in the brain despite successful resuscitation after cardiac arrest in a pediatric porcine model

Authors: *S. PIEL¹, M. J. MCMANUS¹, K. HEYE², C. CLAYMAN¹, F. BEAULIEU³, J. I. JANOWSKA¹, J. STARR³, H. GAUDIO¹, T. HALLOWELL¹, N. DELSO¹, H. FAZELINIA⁴, Y. LIN¹, J. K. EHINGER⁵, M. KARLSSON⁶, R. A. BERG¹, R. MORGAN¹, T. J. KILBAUGH¹; ¹Resuscitation Sci. Ctr., ²Div. of Neurol., ³Dept. of Pediatrics, ⁴Proteomics Core Facility, Children's Hosp. of Philadelphia, Philadelphia, PA; ⁵Mitochondrial Medicine, Dept. of Clin. Sci. Lund, Lund Univ., Lund, Sweden; ⁶Dept. of Neurosurg., Rigshospitalet, Copenhagen, Denmark

Abstract: Annually, over 15,000 children and over 200,000 adults in the US experience an in-hospital cardiac arrest (CA) and receive cardiopulmonary resuscitation (CPR), with the incidence likely to increase concurrently with advancements in life-prolonging medical therapy. Despite successful resuscitation, many survivors suffer from long-term neurological injury. Currently, there are no established clinical therapies that preserve neurologic function. Critical for development of effective therapies is a better understanding of the pathological processes leading to neurological injury and long-term cognitive dysfunction. Maintenance of mitochondrial health and reduction of oxidative stress in the brain following CA may be an important convergence point for cell survival and neurological recovery. Here, we performed an exploratory study to characterize alterations in cerebral mitochondrial health following successful resuscitation from CA in a pediatric swine model. To this end, female Yorkshire piglets (*Sus scrofa domestica*) representing toddler age underwent asphyxia, followed by ventricular fibrillation and hemodynamic-directed CPR to mimic a clinically relevant insult with high-quality CPR (n=5). Sham animals (n=5) underwent identical anesthesia protocols. After successful resuscitation, animals were survived for four days, subsequently euthanized, and their cerebral mitochondrial function, quantity and proteomic profile was analyzed using parametric or non-parametric tests as appropriate. Here, we demonstrate that mitochondrial function and mitochondrial DNA (mtDNA) copy number is persistently reduced in the brain four days following successful resuscitation after CA, which is concurrent with a trend towards increased oxidative damage. Proteomics analysis further revealed 231 proteins to be significantly changed between Shams and placebo-treated animals. Compared to sham animals, CA animals showed a downregulation of proteins of the OXPHOS system (mitochondrial complex I, IV and V) as well as mitochondrial ribosomes (MRPL47) and complex assembly factors (ATPAF2), mitochondrial carrier proteins (SLC25A3, SLC25A11, MPC1) and proteins of the mitochondrial structure

organization system (MICOS10). To conclude, the mitochondrial profile in the brain is persistently changed in function, quantity and gene expression pattern four days after successful resuscitation from CA. Further delineation of the proteomic profile of the brain of these animals can provide deeper understanding of pathological processes in the brain and may lead to generation of novel therapeutic strategies to reduce neurological injury following CA.

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Poster

455. Neuroprotection II

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 455.29

Topic: C.08. Ischemia

Support: CalciGenix, LLC

Title: Oxygen-glucose deprivation differentially modulates intrinsic excitability of male and female hippocampal CA1 neurons

Authors: *B. NATWORA¹, M. MASSMAN¹, I. MORLEY¹, J. R. MOYER, JR.²;
¹Psychology, ²Psychology and Biol. Sci., Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Current reports rank stroke as the fifth leading cause of death, accounting for roughly 1 of every 19 deaths in the United States. Approximately 87% of reported stroke cases are the result of an ischemic stroke, characterized by the occlusion of an artery that supplies oxygen-rich blood to the brain. Under these pathological conditions, local excitatory networks may be activated through excessive glutamatergic signaling, leading to increased excitability, and ultimately delayed neuronal death. Since the prevalence of stroke in the United States increases with advancing age and since females have a higher susceptibility to stroke, it is important to investigate neuronal membrane properties, excitability, and structural integrity of neurons following an ischemic insult in relation to sexual dimorphism. In the present study, hippocampal brain slices were prepared from adult female and male Fisher 344 rats and placed in oxygenated aCSF. Half the slices were then subjected to 5 min of oxygen-glucose deprivation (OGD) and the other half remained in oxygenated aCSF (control). Within a subset of control- and OGD-treated hippocampal slices from each rat, cell death was assayed by trypan blue exclusion. In both sexes, OGD-treated slices had a significantly greater number of trypan blue-labeled neurons compared to control-treated slices, confirming the effectiveness of our OGD protocol. Furthermore, there were no statistically significant differences in cell death between males and females. For the remaining slices, biocytin-filled electrodes were used to obtain whole-cell recordings (WCRs) from CA1 neurons to investigate the effect of OGD on their physiological and morphological

properties. Preliminary data from 43 WCRs suggest that OGD differentially affects intrinsic excitability of female neurons. Following OGD, female CA1 neurons fired fewer action potentials compared with female neurons from control slices. This was seen across a range of current injection amplitudes from 100-450 pA. In contrast, data from male rat CA1 neurons suggest a subtle increase, or no change, to their intrinsic excitability following OGD. Thus, neurons from female rats exhibited a more profound reaction to an *in vitro* model of ischemia, as measured by oxygen-glucose deprivation.

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Poster

455. Neuroprotection II

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 455.30

Topic: C.08. Ischemia

Support: R01 NS075930 (PI)
U24 NS113452 (PI)

Title: Differential Response to Substrate Deprivation and Treatment in the Neurovascular Unit

Authors: *A. BROOKSHIER¹, P. LYDEN²;

¹USC, Los Angeles, CA; ²Neurol., Zilkha Neurogenetic Inst., Los Angeles, CA

Abstract: Prospective therapeutics for stroke patients continue to fail to avert neurological deficits after ischemia despite promising preliminary results in experimental preclinical studies. A potential cause for this is the differential responses of the individual components of the neurovascular unit (NVU) to treatment. We determined the responses of endothelial cells and neurons following the treatment with potential therapeutics after being subject to oxygen glucose deprivation (OGD). Primary rat neurons or endothelial cells were seeded at plate density of 4×10^4 cells with six repeats per treatment group. Tranexamic acid was selected for study based on previous agnostic proteomics screening of astrocyte paracrine protective conditioned media. The cell cultures were subjected to (OGD) with various concentrations of tranexamic acid (500 uM, 100 uM, 50 uM, 30 uM, 10 uM, uM, 500nM, and 100 nM) for the duration it took to reach cell-type specific LD₈₀. The LD₈₀ (OGD duration to kill 80% of cells) for endothelial cells and neurons at 4 and 2 hours, respectively. After 24 hours reperfusion, the MTT assay was used to assess cell viability. Cell cultures that were subjected to OGD but did not receive drug treatment acted as a positive control. Cell cultures that did not undergo OGD or receive treatment were negative controls. The toxicity of tranexamic acid was established using cell cultures that did not undergo OGD and received the full range of doses. The effects of tranexamic acid was assessed with multiple comparisons 2way ANOVA. The percentage of cell viability of neurons subjected

to OGD had the greatest difference between no drug treatment and 50 uM, 30 uM, and 10 uM ($19.85\% \pm 5.57\%$ vs $58.55\% \pm 3.96\%$, $58.28\% \pm 3.64\%$, and $64.35\% \pm 2.46\%$, respectively; $P < 0.001$), while the difference between no drug treatment and 1 uM was not significant. In contrast, the endothelial cell control group and each drug treatment dose did not differ significantly in percentage of cell viability. The endothelial cells' viability differed significantly from neuron's viability at concentrations: 500 uM, 100 uM, 50 uM, 30 uM, 10 uM, 500 nM, and 100 nM ($P < 0.0001$). These findings illustrate that endothelial cells and neurons respond differently to a potential therapeutic. The same drug treatment that led to the preservation of neurons failed to save endothelial cells, proving a need to find treatments that will benefit more NVU cell types than simply neurons. Subsequent studies will determine how astrocytes and other components of the NVU respond to tranexamic acid and how different families of drugs effect cell-type specific behavior.

Disclosures: A. Brookshier: None. P. Lyden: None.

Poster

455. Neuroprotection II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 455.31

Topic: C.08. Ischemia

Support: Council of Scientific and Industrial Research
Academy of Scientific and Innovative Research
CSIR-Indian Institute Of Chemical Technology
CSIR-Center For Cellular and Molecular Biology

Title: Revealing sex-specific differential regulatory role of h3k9me2 in mediating focal cerebral ischemia induced neural damage and recovery

Authors: M. RADHAKRISHNAN^{1,2}, K. SOREN^{1,2}, V. VIJAY^{1,2}, A. KUMAR^{3,2}, *S. CHAKRAVARTY^{4,2};

¹Applied Biol., CSIR-Indian Inst. Of Chem. Technol., Hyderabad, India; ²Acad. of Scientific and Innovative Res., Ghaziabad, India; ³CSIR-Centre of Cell. and Mol. Biol., Hyderabad, India;

⁴Applied Biol., CSIR-Indian Inst. of Chem. Technol., Hyderabad, India

Abstract: Cerebral ischemic stroke stands to be one of the leading causes of death and disability worldwide. Therapeutic interventions to mitigate acute ischemia induced neural damage are limited due to an inadequate understanding of the underlying molecular mechanisms. Recent studies have implicated epigenetic mechanisms, especially histone lysine acetylation and deacetylation, in ischemia-induced neural damage and death. However, the role of lysine methylation/demethylation, another abundant epigenetic mechanisms recently reported by us and few other labs to be involved in acute ischemic stroke induced cerebral damage, has not been studied in detail. Moreover, in light of the fact that sex of the individual appears to have an

influence on the post-stroke neurological outcome, here we also included both male and female CD1 mice while using our recently developed Internal Carotid Artery Occlusion (ICAO) model. Earlier employing magnetic resonance imaging (MRI), TUNEL and histopathological staining, we have shown that ICAO induces mild to moderate level of ischemia-induced cerebral damage. Here, first we report that female mice recover faster than male individuals after analysing the data obtained from neurological deficit score (NDS), grip strength test, rotarod test and open field test (OFT), at different time point post-ICAO. Then, the epigenetic and other molecular investigations led us to uncover the gender-specific differential regulation of some inflammatory, and apoptotic markers and the associated dysregulation of a number of histone lysine demethylases (KDMs) and methylases (KMTs) as well, post-ICAO. Specifically, we observed a significant attenuation in the global level of transcriptionally repressive epigenetic mark H3K9me2 in the male mouse striatal region affected by the ischemic insult, while an increase in its level in the same brain region of female mouse. Considering our previous report where we have shown a significant post-ischemic improvement by using Dimethyloxalylglycine (DMOG), an inhibitor of KDM4 or JMJD2 class of histone lysine demethylases in male animals, it would be interesting to see the duration-dependent (post-ICAO) effect in H3K9me2 levels, across the sexes. Overall, our results appear to reveal that the epigenetic mark H3K9me2 plays crucial role in mediating sex specificity or difference in post-ischemic sequential events during neural damage and repair which might also be partly associated with autophagy regulation.

Disclosures: **M. Radhakrishnan:** None. **K. Soren:** None. **V. Vijay:** None. **A. Kumar:** None. **S. Chakravarty:** None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NASA 80NSSC21K0273
NIEHS R21 S031211-01A1
NIGMS 1P20GM134974-01A1

Title: Utilization of a viral proxy for the detection of the DNA damage response permits the observation of space radiation-induced neurodegeneration, brain genomic instability, and behavioral deficits

Authors: ***E. KNOTT**¹, **K. M. KEYS**², **J. CHANCELLOR**³, **L. HARRISON**⁴, **X. LU**⁵;
¹Louisiana State Univ. Hlth. Scienc Pharmacology, Toxicology & Neurosci., Shreveport, LA;
²Louisiana Univ. Shreveport Hlth. Sci. Cen, Shreveport, LA; ³Louisiana State Univ., Baton Rouge, LA; ⁴LSU Hlth. Shreveport, Shreveport, LA; ⁵LSU Hlth. Sci. Ctr. Cell Biol. & Anat., Shreveport, LA

Abstract: The risk to brain health as a result of exposure to galactic cosmic radiation (GCR) is one of NASA's primary concerns as they evaluate the dangers of space exploration. In particular, exposure to such high-energy ionizing radiation may induce or exacerbate neurodegeneration and cognitive decline. Interestingly, it has been observed that, among the immune system alterations experienced by astronauts, many latent viruses can be reactivated following both short- and long-term spaceflights. Our novel genetically-encoded *in vivo* biodosimetry sensor, Probe with a viral proxy for the Instability of DNA surveillance/repair in Somatic brain Mosaicism (PRISM), is ideal for investigating the mechanisms and neurological consequences of GCR-induced neurodegeneration. PRISM utilizes the DDR-induced suppression of this immune system-like pathway, along with the viral transduction and the instability of hypermutable mononucleotide repeat regions, to promote expression of membrane-tethered fluorescent proteins in the presence of neuronal genotoxic stress. This probe may consequently be used to address the existing difficulties in visualizing neuronal damage *in vivo* with high spatiotemporal resolution. We here detail the use of Cre recombinase-driven expression of PRISM as a valid proxy for identifying the presence of DNA damage resulting from both chemical radiation mimics and simulated GCR. rAAV-PRISM was retro-orbitally injected into transgenic C57BL/6J mice. By utilizing reporter mice in which the expression of Cre is restricted to genetically-defined cell types associated with neurodegenerative diseases (NDD) (pyramidal (CamkIIa-Cre) and dopaminergic (TH-Cre) neurons, as well as those expressing dopamine receptors (Drd1-Cre and Drd2-Cre)), we are able to visualize NDD-relevant brain regions at a single-cell resolution. PRISM also permits the observation of neurons undergoing varying degrees neurodegeneration, with a resolution sufficient to identify changes in dendritic spines morphology. Our ongoing efforts to assess the neuronal DNA damage induced by a newly-developed method of recreating the intravehicular (IVA) radiation environment expected on spaceflight vehicles and extraterrestrial habitats aim to evaluate the risks that the space environment poses to brain genomic stability and, consequently, brain structural and functional changes.

Disclosures: E. Knott: None. K.M. Keys: None. J. Chancellor: None. L. Harrison: None. X. Lu: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01AG064078

Title: Inhibition of Hippo signaling pathway via genetic ablation enhances resistance of hippocampal neurons to ferroptotic neurodegeneration

Authors: *R. C. EVANS, N. DAR, R. NA, L. CHEN, Q. RAN;
Cell Systems and Anat., Univ. of Texas Hlth. San Antonio, San Antonio, TX

Abstract: The death and degeneration of neurons is the primary cause of neurodegenerative conditions such as Alzheimer's Disease (AD). Recent studies have indicated that ferroptosis, a form of regulated cell death driven by iron-dependent accumulation of lipid peroxides, likely plays a significant role in the neurodegenerative pathology observed in AD. Additionally, new evidence has suggested that the Hippo signaling pathway, which is known to regulate cell growth and proliferation, also regulates the expression of pro-ferroptotic genes in some cell types. While the effects of the Hippo pathway on neurons and their sensitivity to ferroptosis is unknown, we have shown that markers of Hippo signaling activity are elevated in brain tissue taken from the 5xFAD mice, a widely used animal model of AD, implicating the pathway as a potential new target for therapeutic intervention. The current study investigates whether direct inhibition of Hippo pathway activity in hippocampal neurons is protective against ferroptosis-driven neurodegeneration. To test this, we developed a mouse model that allows for conditional ablation of LATS1 and LATS2, key regulators of Hippo pathway activity, and utilized this to generate primary hippocampal neuronal cultures. Following treatment with 4-HT to induce genetic ablation, we confirmed the knockout LATS1 and LATS2, as well as downstream inhibition of key Hippo pathway proteins, in our primary cultures. Our results indicate that inhibition of the Hippo pathway in these neuronal cultures promotes the downregulation of select pro-ferroptotic genes and provides cellular resistance to treatment with chemical inducers of ferroptosis, but not inducers of other forms of cell death. The present findings suggest that Hippo pathway activity may play a role in neurodegenerative disease pathology and in regulating the resistance of hippocampal neurons to ferroptosis.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 4VA-Foundation

Title: Investigating the effects of Trpml1 mutation on gut morphology

Authors: *N. L. CUBBAGE, Q. F. DANEHY, B. T. VELAYUDHAN, M. T. WALKER; James Madison Univ., Harrisonburg, VA

Abstract: Purpose: The gut-brain axis in the human body is an essential pathway for bidirectional signaling to communicate changes in digestive activity within the gut. The vagus nerve is a major conduit in this network between the gut and brain. In some neurodegenerative diseases where patients suffer progressive somatomotor function loss it is unclear if there is a similar impact on visceromotor pathways. Our research tests the impact on the gut-brain axis in a neurodegenerative disease model, Mucopolysaccharidosis type IV (MLIV). MLIV patients demonstrate

neurological deficits, progressive somatomotor function loss, and eventual paralysis. We use a MLIV mouse model to test and measure changes in stomach wall morphology, cell population, and vagal innervation. **We hypothesize that the hypergastrinemia and achlorhydria in MLIV patients are driven in part by signaling defects along the gut-brain axis.** **Methods:** To test our hypothesis we harvest stomach tissue from 6 week and 7 month old mice. We measured changes in the layers of the stomach wall and cell morphology. We use RT-PCR and immunohistochemistry to measure changes in cell population. We also labeled vagal inputs to the stomach to observe any changes in axonal targeting within the layers of the stomach wall. **Results:** MLIV mice showed early onset morphological changes in stomach wall morphology. Mutant mice exhibit a significant enlargement of the stomach wall mucosa layer thickness. The cells in the mucosa have increased vesicle storage and there is a significant change in parietal cell morphology in the MLIV mutants. In addition, MLIV mice show an expansion of vagal axons into the mucosal layer. **Conclusion:** Overall, these results suggest that MLIV disorder may alter the structural and functional integrity of different layers of the stomach wall which lends additional insight into how neurodegenerative disorders affect visceromotor functions in mammals.

Disclosures: N.L. Cabbage: None. Q.F. Danehy: None. B.T. Velayudhan: None. M.T. Walker: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH).

Title: Dysregulated lysosomal cholesterol homeostasis mediates mTORC1-activation contributing to neurodegeneration in a mouse model of Infantile Neuronal Ceroid Lipofuscinosis

Authors: *A. P. APPU, M. B. BAGH, N. PLAVELIL, A. MONDAL, T. SADHUKHAN, A. B. MUKHERJEE;
NICHD, NIH, Bethesda, MD

Abstract: Infantile Neuronal Ceroid Lipofuscinosis (INCL) is a devastating neurodegenerative lysosomal storage disease (LSD) caused by inactivating mutations in the *CLN1* gene encoding palmitoyl-protein thioesterase-1 (PPT1). Despite this discovery, the mechanism of INCL-pathogenesis has remained elusive. Recently, lysosomal cholesterol has been reported to activate mTORC1 kinase, which suppresses autophagy and suppressed autophagy mediates neurodegeneration in most LSDs. We found that while the total cholesterol in the brain in *Cln1*^{-/-} mice, which mimic INCL, is significantly elevated, the lysosomal cholesterol level is

significantly lower than that in their WT littermates. Lysosomal cholesterol homeostasis is maintained by Niemann Pick C1 (NPC1)- and NPC2-proteins, which mediate cholesterol egress and import, respectively. Intriguingly, in *Cln1*^{-/-} mice, the lysosomal level of NPC1 was significantly lower compared with that in WT lysosomes. Previously, we reported that V0a1 subunit of vATPase, required dynamic S-palmitoylation (palmitoylation-depalmitoylation) for trafficking to the lysosomal membrane and Ppt1-deficiency in *Cln1*^{-/-} mice misrouted V0a1 to the plasma membrane (Bagh, M.B. et al. *Nat Commun.* 2017). We found that like V0a1, NPC1 also requires dynamic S-palmitoylation for trafficking to the lysosomal membrane and in *Cln1*^{-/-} mice, NPC1 is misrouted to the plasma membrane although NPC2, which is not S-palmitoylated, normally trafficked to the lysosome. Since endosomal trafficking of proteins to the lysosomal membrane requires sequential interaction with various adaptor proteins (APs), we investigated AP-mediated trafficking of NPC1 in WT and *Cln1*^{-/-} mice. We found that like V0a1, Ppt1-deficiency in *Cln1*^{-/-} mice, misrouted NPC1 to the plasma membrane. Consequently, in these mice cholesterol-egress from lysosomal lumen was impaired and increased cholesterol import by NPC2 dysregulated lysosomal cholesterol homeostasis. Along with this defect, elevated oxysterol binding protein, which increases cholesterol level on lysosomal surface, mediated mTORC1-activation and suppressed autophagy contributing to neurodegeneration. Our findings uncover a previously unrecognized role of *Cln1*/Ppt1 in lysosomal cholesterol homeostasis and reveal a pathway to INCL pathogenesis.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH)

Title: Dysregulated ER to Golgi trafficking mediates ER-stress and Unfolded Protein Response in a mouse model of Infantile Neuronal Ceroid Lipofuscinosis

Authors: *N. PLAVELIL, A. P. APPU, M. B. BAGH, K. ROY, A. B. MUKHERJEE; NICHD, NIH, Bethesda, MD

Abstract: Infantile neuronal ceroid lipofuscinosis (INCL) is a devastating neurodegenerative lysosomal storage disease (LSD) caused by inactivating mutations in the *CLN1* gene encoding PPT1, a lysosomal de-palmitoylating enzyme. Previously, we reported that INCL fibroblasts and *Cln1*^{-/-} mice, which mimic INCL, develop endoplasmic reticulum (ER)-stress mediating unfolded protein response (UPR) leading to neuronal apoptosis. The newly synthesized proteins are

transported from the ER to the Golgi via COPII vesicles and are subsequently transferred from the Golgi to the endosomal compartments. A retrograde transport (from the Golgi to the ER) may also occur via COP1 vesicles. We tested a hypothesis that in INCL either trafficking of proteins from the ER to the Golgi via COPII vesicles or retrograde transport from Golgi to the ER is dysregulated causing ER-stress. We found that in *Cln1*^{-/-} mice and in cultured INCL fibroblasts COPII-mediated export of proteins from the ER to the Golgi was dysregulated. Moreover, the sequential recruitment of the components of COPII complex, including the Sar1 GTPase, Sec23/Sec24 subcomplex, and Sec13/Sec31 subcomplex were significantly increased in *Cln1*^{-/-} mouse brain compared with those in WT mice. To further confirmation these results, we colocalized Sar1 GTPase, Sec 31, Sec 13, Sec 23, and Sec 24 in the ER of INCL fibroblasts and found that all these proteins were highly colocalized in the ER than those in normal fibroblasts. Similarly, we colocalized Sec 31 and Sec 24 in Golgi and found that more colocalization occurred in normal fibroblasts whereas the levels of Sec 13, Sec 23 and Sar1 GTPase do not have any significant change when colocalized with the Golgi marker. We found that there are no significant changes in the endogenous level of COPI proteins (e.g., β -COPI, α -COPI and ARF-1) although β -COPI is more colocalized in the Golgi and less colocalized in the ER of INCL fibroblasts than normal fibroblasts. Consistent with these results, the UPR markers IRE-1 alpha, Grp-78 and ATF-6 were significantly increased in *Cln1*^{-/-} mouse brain compared with those in WT mice. These results suggest that dysregulated ER to Golgi trafficking of newly synthesized proteins via COPII vesicles may cause excessive accumulation of these proteins in the ER leading to ER-stress, and uncontrolled ER-stress activates UPR leading to neuronal apoptosis in INCL. Our findings reveal a previously unrecognized pathway mediating ER-stress contributing to INCL pathogenesis.

Disclosures: N. Plavelil: None. A.P. Appu: None. M.B. Bagh: None. K. Roy: None. A.B. Mukherjee: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DA051450
AG070962

Title: The number of tyrosine hydroxylase-stained neurons in the ventral tegmental area is decreased after protracted abstinence from chronic methamphetamine administration

Authors: L. BOATNER, A. BHOWMIK, *S. M. GRAVES;
Dept. of Pharmacol., Univ. of Minnesota, MINNEAPOLIS, MN

Abstract: Methamphetamine (meth) is an addictive psychostimulant that is also neurotoxic. We recently reported that chronic 28-day administration of meth (5 mg/kg) results in degeneration of substantia nigra pars compacta but not ventral tegmental area (VTA) dopamine neurons (Du *et al.*, *Neuropharmacology* 200:108817, 2021). However, in rats trained to self-administer meth there is a loss of tyrosine hydroxylase-stained (TH⁺) VTA dopamine neurons but only after protracted abstinence from meth (Kousik *et al.*, *Eur J Neurosci* 40:2707, 2014). To determine whether VTA dopamine neurons in mice are similarly vulnerable after a period of abstinence, mice (approximately 8 weeks of age) were administered saline or meth (5 mg/kg; i.p.) for 28 consecutive days followed by 12 weeks of abstinence in the home cage; subjects were group housed with free access to food and water throughout the duration of the study. After 12 weeks of abstinence mice were euthanized and 4% paraformaldehyde fixed brain tissue collected. Brain sections (40 μm) spanning the entirety of the VTA were collected and stained for TH. The number of TH⁺ neurons in the VTA were stereologically quantified using the optical fractionator probe; every third section was counted by a blinded experimenter. Although chronic 28-day meth (5 mg/kg) administration fails to produce significant neuronal loss in the VTA (Du *et al.*, *Neuropharmacology* 200:108817, 2021), the number of TH⁺ neurons in the VTA were significantly decreased after 12 weeks of abstinence. These results, using non-contingent administration in mice, are consistent with results from rats trained to self-administer meth (Kousik *et al.*, *Eur J Neurosci* 40:2707, 2014). Together these studies indicate that while VTA dopamine neurons may be resistant to chronic meth-induced degeneration, they are not impervious to the deleterious consequences of chronic meth. Further investigation is needed to ascertain whether other monoaminergic neuronal populations are vulnerable to chronic meth-induced degeneration and whether potential degeneration progresses despite abstinence.

Disclosures: L. Boatner: None. A. Bhowmik: None. S.M. Graves: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5T32EY013934-19
NIH Grant 5R01EY020895-08

Title: Evidence of regulation of T148 phosphorylation on alphaA-crystallin by mTOR or PI3K

Authors: *Z. B. SLUZALA, P. E. FORT;
Univ. of Michigan, Univ. of Michigan, Ann Arbor, MI

Abstract: α-crystallin proteins have recently gained increased interest due to their demonstrated roles in neurodegenerative disorders, including diabetic retinopathy. Our lab has shown that one member of the α-crystallin family, αA-crystallin, is upregulated in the retina of diabetic donors.

This upregulation is accompanied with a substantial decrease in T148 phosphorylation, and impairment of α A-crystallin's neuroprotective abilities. While the molecular mechanisms underlying this T148 phosphorylation-dependent protection are getting clearer, the mechanisms of its regulation remain unknown. In the present study, we aimed to identify the kinase(s) responsible for T148 phosphorylation. R28 retinal neurons and MIO-M1 Müller glial cells were transfected with plasmids encoding either 3x-FLAG-tagged wild-type (WT) or 3x-FLAG-tagged T148C α A-crystallin and exposed to "diabetes-like" stress (2h incubation with 100ng/ml TNF α and 25mM glucose). These cell lines were chosen in part to elucidate differences in neuronal and glial mechanisms of cell protection. Kinase identification was performed using the chemoproteomic PhAXA assay which requires a chemical crosslinker to stabilize interactions formed between the newly introduced cysteine residue of the substrate and phosphorylating kinases. Kinase/substrate complexes were then immunoprecipitated and analyzed via western blot (WB) and liquid chromatography with tandem mass-spectrometry (LC-MS/MS). Specific complexes were identified by WB in the T148C+crosslinker condition, and several candidate kinases were identified in R28 retinal neurons by LC-MS/MS including mTOR, PI3K, WNK1, CK1 δ , Map4k4, Mapk3, and STK4. Of these, mTOR and PI3K demonstrated the greatest fold-change increase in protein abundance and peptide-spectrum matches between the WT+crosslinker and T148C+crosslinker conditions. Our lab has also previously shown decreased mTOR gene expression in human retina samples from patients with diabetic retinopathy in comparison to non-diabetic patient samples, and both mTOR and PI3K have been shown to be involved in diabetes and cell survival pathways, making these two candidate kinases particularly promising. These data demonstrate, for the first time, evidence of specific kinases regulating T148 phosphorylation on α A-crystallin.

Disclosures: Z.B. Sluzala: None. P.E. Fort: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Cyclin-dependent kinase inhibitor p21 dysregulation induces oligodendrocyte differentiation impairment in multiple sclerosis cerebral organoids

Authors: *N. DAVIAUD, S. A. SADIQ;
Tisch Multiple Sclerosis Res. Ctr. of New York, New York, NY

Abstract: Multiple sclerosis (MS) is an auto-immune neurological disorder characterized by inflammation, demyelination, and neural degeneration. The origin and evolution of MS are still poorly understood because of lack of animal models and relative inaccessibility to human brain tissue. Cerebral organoids represent an interesting tool to study neurological disorders as they recapitulate early human neurodevelopment, including the generation, proliferation and

differentiation of neural progenitors into glial cell and neurons. Members of the Cip/Kip family of cyclin-dependent kinases inhibitors (CDKi), such as p21, p27 and p57, are well characterized for their role as negative regulators of the cell cycle. Recent studies have shown that they play additional roles unrelated to cell cycle regulation, such as control of oligodendrocyte differentiation and maturation and thus, myelination. However, their importance in MS hasn't been thoroughly described. The purpose of this work was to use patient with MS induced pluripotent stem cells derived cerebral organoids to study the importance of CDKi in MS pathogenesis. We used this model to analyze the expression of CDKi in control and MS organoids, and study their effect on cell proliferation and differentiation capacity, after 42 days in vitro. We first analyzed p21, p27 and p57 expression in cerebral organoids derived from healthy controls and from patients with PPMS, RRMS and SPMS. P21⁺ and P57⁺ cells were localized in the lower cortical layers and colocalized with PAX6⁺ neural stem cells. P27⁺ cells were mostly expressed in the outer layers, and colocalized with CTIP2⁺ neurons. No ectopic cell location was found in the different types of MS compared to control organoids. Quantification revealed no difference for p27 and p57 expression in MS organoids compared to control, while a significant decrease was observed for p21 in every type of MS, particularly PPMS. Analysis of the cleavage plane angle revealed a transition from symmetric proliferative to asymmetric neurogenic division in MS samples, which was associated with p21 downregulation. We then analyzed Olig2⁺ oligodendrocyte population in organoids. A significant decrease of Olig2⁺ cell expression was detected in MS organoids, particularly PPMS and RRMS. In conclusion this work is a proof of principle, showing the c-organoids derived from patients with MS can be used as an innovative tool to better understand the genetic basis for phenotypic differences seen in MS. Using this model, we identified p21 as a new protein of interest in progressive MS pathogenesis.

Disclosures: N. Daviaud: None. S.A. Sadiq: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 1R01NS112706-01
R37NS045876
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Santen PhD Studentship

Title: Atp synthase c-subunit leak metabolism associated with abnormal mitophagic clearance

Authors: *B. PETRITI^{1,2,3}, S. SUBRAMANIAN¹, P. LICZNEFSKI¹, K. Y. CHAU³, G. LASCARATOS², D. F. GARWAY-HEATH², E. JONAS¹;

¹Intrnl. Med. and Neurosci., Yale Univ., New Haven, CT; ²Inst. of Ophthalmology, ³Inst. of Clin. Neurol., Univ. Col. London, London, United Kingdom

Abstract: The E50K mutation in the Optineurin gene is responsible for 16.7% of hereditary normal tension glaucoma (NTG), causing irreversible vision loss by progressive loss of retinal ganglion cells. Optineurin is an autophagy receptor in parkin-mediated mitophagy with defects leading to neurodegenerative diseases. To establish how the E50K mutation affects mitophagy and mitochondrial metabolism we studied fibroblast cell lines from participants with NTG known to have the E50K OPTN mutation and age-matched consanguineous healthy controls. Preliminary electron microscopy results showed that E50K mutant mitochondria were darker than controls, suggesting metabolic changes. We hypothesized that the mutant mitochondria are leaky, reversing ATP synthase to hyperpolarize the inner membrane potential. The leak may arise from depolarized mitochondria that have failed to undergo mitophagy. Indeed, we showed an increase in ATP synthase c-subunit in E50K mutant mitochondria versus controls whilst beta subunit remains relatively similar between lines resulting in an increase in c-subunit over beta subunit ratio. This suggests high ATP synthase c-subunit leak channel (ACLIC) activity. To measure ATP synthase reversal, we applied oligomycin and showed that, while control mitochondria hyperpolarised, E50K mitochondria depolarised, suggesting opposite sign to ATP synthase activity. Whilst trying to maintain their membrane potential by running ATP synthase in reverse, we expected to see an increase in oxidation in E50K mutant cells. We measured oxygen consumption rate using the Seahorse XFe24 analyser and found that basal OCR is higher in the E50K mutant cells compared to controls. An accelerated electron transport rate would require more Cox IV, and indeed we find Cox IV levels to be higher in the E50K mitochondria than controls. Previous work from our group showed that an increase in c-subunit leak could aberrantly elevate protein synthesis. We now show that there is increased c-subunit and overall protein synthesis in the E50K mutant cells. In conclusion, E50K mutant mitochondria have a mitochondrial inner membrane leak contributing to a “leak metabolism”; we suggest that increased c-subunit expression in the inner membrane may be a normal process of mitophagy.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH EY032908

Title: Characterization of a well-surviving retinal ganglion cell type using a gpr88-cre bac transgenic mouse line

Authors: *A. HALL, P. WILLIAMS, S. MCCRACKEN, Z. WANG, C. ZHAO;
Ophthalmology and Visual Sci., Washington Univ. in St. Louis, St. Louis, MO

Abstract: Retinal ganglion cells (RGCs) undergo irreparable degeneration in diseases such as glaucoma, which results in profound vision loss. Mice have 46 RGC types that survive on a continuum in response to degeneration, with some types surviving well and several that do not. Of the well-surviving types, one that expresses GPR88 has not been directly investigated or linked with a known RGC type. Therefore, we are characterizing these RGCs using a GPR88-Cre BAC transgenic mouse to explore their survival mechanisms and regenerative potential. To examine the types of RGCs labeled by the GPR88-Cre transgenic line, we delivered an adeno-associated virus (AAV) expressing a fluorescent reporter and categorized dendritic stratification in the retinal sublaminae. We found that the GPR88-Cre transgenic mouse line labels four types of RGCs based on dendritic morphology: monostratified, bistratified, high reaching bistratified, and reaching monostratified. We also observed axonal projections to the superior colliculus, lateral geniculate nucleus, medial terminal nucleus, and other accessory optic nuclei. To study survival and axon regeneration characteristics of GPR88-Cre RGCs, we used an optic nerve crush model, which damages all (RGC) axons in the optic nerve leading to death of 80% of RGCs and a loss of all axons in the optic nerve. Following optic nerve crush, we assessed the frequency of the four RGC types after injury, and the reaching monostratified type appears to exhibit the highest levels of survival against injury indicating that this type is likely the previously identified well surviving type. To assess the regenerative potential of GPR88 RGCs, AAV injection of shPTEN and rhCNTF in conjunction with optic nerve crush surgery was used. We found that some axons from GPR88 RGCs regenerate a moderate distance past the injury site.

Disclosures: A. Hall: None. P. Williams: None. S. McCracken: None. Z. Wang: None. C. Zhao: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS/Kakenhi C Research Grant #20K07458

Title: Roadblock1 regulates FMRP function by promoting its degradation

Authors: *S. EMAD EL-AGAMY¹, L. GUILLAUD¹, K. KONO², Y. WU^{3,4}, M. TERENCE¹;
¹Mol. Neurosci. Unit, ²Membranology Unit, Okinawa Inst. of Sci. and Technol., Okinawa, Japan; ³YCI Lab. for Next-Generation Proteomics, RIKEN Ctr. of Integrative Med. Sci., Yokohama, Japan; ⁴Fac. of Sci., Univ. of Geneva Sci. II, Geneva, Switzerland

Abstract: Cytoplasmic dynein is the main eukaryotic molecular motor mediating retrograde transport and plays a crucial role in highly polarized cells such as neurons. Roadblock 1 (DYNLRB1) is one of three light chains of the dynein complex and was shown to mediate survival signaling in sensory neurons. We used a proximity-dependent biotinylation approach coupled with mass spectrometry to identify DYNLRB1 interactors in adult dorsal root ganglia (DRG) neurons. Among the candidates identified, the Fragile X mental retardation protein (FMRP), an RNA-binding protein with implications in neurological diseases, was selected for further characterization. To assess the impact of DYNLRB1 on FMRP dynamics and function, we combined shRNA-mediated silencing of DYNLRB1 with pharmacological treatments that target the proteolytic machinery. Interestingly, DYNLRB1 knockdown reduced FMRP degradation. We found that knockdown of DYNLRB1 impaired FMRP recruitment to the dynein complex and caused axonal accumulation of the FMRP protein. Increased colocalization with LAMP1 positive lysosomes was also detected, suggesting a novel degradation route for the FMRP, which has not been previously explored in sensory neurons. The resulting increase in FMRP level promoted the formation of FMRP granules in the somatic compartment of DRG neurons, sequestering the FMRP-associated mRNA MAP1B, and reducing its translation. Our findings suggest that DYNLRB1-FMRP interaction controls FMRP function through the promotion of its targeted degradation. This mechanism could have a prominent role in the etiology of neurodegenerative diseases.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CHIR Canada grant
University of Toronto, Toronto, Canada

Title: A new paradigm into thinking of the pathophysiology of neurodegenerative diseases

Authors: L. HARBAUGH, *G. NAGRA;
Arkansas Col. of Osteo. Med., Fort Smith, AR

Abstract: Cerebrovascular diseases are believed to impair the periarterial interstitial fluid drainage pathways of the brain, leading to the accumulation of protein such as A-beta etc in the brain parenchyma of patients affected with neurodegenerative disorders. In any event, the combination of a cerebrospinal fluid (CSF) absorption deficit, the loss of CSF secretory capacity and the presence of cerebrovascular disease may contribute to a reduction in CSF turnover, and could provide the setting in which metabolic products toxic to the brain increase in concentration

and alter neurological function negatively in several diseases associated with the elderly. In this regard, several parameters associated with the cerebrospinal fluid (CSF) system show a change in the later stages of life, including elevated CSF outflow resistance. The latter implies a CSF absorption deficit. As a significant portion of CSF absorption occurs into extracranial lymphatic vessels located in the olfactory turbinates, the purpose of this proposal hypothesis is to highlight a significant study that determined whether any age-related impediments to CSF absorption existed at this location. In this regard, a quantitative method involving a rapid movement of the CSF tracer into the olfactory turbinates in young rats with the concentration of the tracer being much higher in the turbinates than in any other tissue measured was utilized. In this study, ¹²⁵I-human serum albumin was injected into the lateral ventricles of 3-, 6-, 12- and 19-month-old Fisher 344 rats. The animals were sacrificed at various times after injection of the radioactive tracer, and appropriate tissue samples were extracted. At 30 min post injection, the average tracer values expressed as per cent injected/g tissue were 6.68 ± 0.42 ($n = 9$, 3 months), 4.78 ± 0.67 ($n = 9$, 6 months), 2.49 ± 0.31 ($n = 9$, 12 months) and 2.42 ± 0.72 ($n = 9$, 19 months). The conclusion of the study was that lymphatic CSF transport declines significantly with age. In concert with the known drop in CSF formation, the reduction in lymphatic CSF absorption may contribute to a decrease in CSF turnover in the elderly leading to a hostile brain environment to hinder the clearance of toxic metabolites.

Disclosures: L. Harbaugh: None. G. Nagra: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

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Program #/Poster #: 456.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NS118177
Spastic Paraplegia Foundation

Title: Mechanisms of Neurodegeneration in SPAST-based models of Hereditary Spastic Paraplegia.

Authors: *E. PIERMARINI¹, S. AKARSU², A. KARABAY², L. QIANG¹, G. MORFINI³, P. W. BAAS¹;

¹Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; ²Istanbul Tech. Univ., Istanbul, Turkey; ³Dept. of Anat. and Cell Biol., Univ. of Illinois at Chicago, Chicago, IL

Abstract: The commonest variant of hereditary spastic paraplegia (HSP), termed *SPG4*-HSP, is caused by mutations of the *SPAST* gene, which encodes spastin. Like other variants of HSP, this one is characterized by gait defects resulting from degeneration of the corticospinal tracts. Over 200 different mutations of *SPAST* have been identified in patients, with the mutations found in various functional domains of the protein. *SPAST* has two start codons that produce a longer

isoform called M1 and a shorter isoform called M87 (or M85 in rodents). We have found that the principal disease mechanism for *SPG4*-HSP is gain-of-function toxicity of the mutant M1 isoform, although loss of spastin activity may also contribute. In various experimental models including genetic mice, *Drosophila*, cultured cells, and squid giant axon, we previously reported that different mutations of M1 aberrantly increase the activity of Casein Kinase 2 (CK2), which in turn results in diminished activity of molecular motors relevant to organelle transport. We also found, in neurons from the genetic mouse, that HDAC6 activity is increased, leading to microtubule deacetylation, which also negatively affects organelle transport. Here, we further pursued potential defects in axonal microtubules potentially arising from either reduced spastin or toxicity of mutant spastin. Using cultured neuroblastoma cells, we found that several different mutations of M1 all elicit these effects on CK2 and HDAC6, and that the effects on HDAC6 are prevented by drugs that inhibit CK2 activity. Moreover, in the mutant mouse but not the knockout mouse, we found that tau levels are notably increased, which may contribute to the degeneration of the corticospinal motor neurons. Using primary cultures from the mutant mouse or a heterozygous knockout mouse, we ectopically expressed fluorescently tagged EB3, which displays as comets at the plus end of microtubules during assembly. The shape, directional trajectory, and duration of the comets reveal the polarity orientation of microtubules as well as information on the rate and duration of their assembly. We found that the percentage of minus-end-directed decreased in the knockout group but increased in the mutant group. We are currently seeking to understand if the effects on microtubule orientation and tau are related to the CK2/HDAC6 pathway. Taken together these results enable us to start piecing together the disease pathways relevant to corticospinal axonal degeneration in *SPG4*-HSP so that effective therapies can be developed to stave off the degeneration of the corticospinal axons.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

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Program #/Poster #: 456.14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NS104078
NIH Grant MH101703

Title: Activation of lysosomal TRPML1 channel regulates lysosomal exocytosis and exosome release

Authors: *E. PENNA¹, M. BAUDRY², X. BI¹;

¹Col. of Osteo. Med. of the Pacific, ²Grad. Col. of Biomed. Sci., Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Emerging evidence has shown that lysosomes are more than just a degradation machinery; they perform various functions such as regulating cell signaling through lysosomal Ca²⁺ release. Many channels/transporters regulate lysosomal Ca²⁺ release and refilling, including the transient receptor potential mucolipin 1 (TRPML1). Our group recently showed that TRPML1-mediated Ca²⁺ release is critical for maintaining normal dendritic lysosomal trafficking, synaptic plasticity, and learning and memory¹. We also observed that lysosomes play an important role in Angelman Syndrome (AS)², a rare neurodevelopmental disorder currently without effective treatment. However, the role of TRPML1 in synaptic functions remains largely unknown. In this study, we aim to determine the involvement of TRPML1 in neuronal plasticity and pathogenesis in AS mice. To further analyze the role of TRPML1 in synaptic function, we used mouse brain synaptosomes, a well-known in vitro model of synaptic terminals. We prepared synaptosomal fractions from wild type (WT) and AS mice and incubated the synaptosomes in the presence of TRPML1 agonist or antagonist, ML-SA1 or ML-SI1 respectively, to test the effects on exosomes release (EVs). Our results show that TRPML1 activation with ML-SA1 elicited an increase in exosome release from synaptosomes from WT mice, but not from AS mice. Interestingly, lysosomal protease cathepsin-B (CTSB) was enriched in exosomes released from WT synaptosomes after TRPML1 stimulation, while no enrichment was observed in AS exosomes. Using EV immune-labeling analysis we observed the presence of CTSB in a specific exosomal subpopulation. Moreover, analysis of non-vesicular secreted proteins from WT synaptosomes showed a decrease of CTSB levels after TRPML1 stimulation, suggesting a different mechanism of CTSB secretion related to TRPML1 activation. These results indicate that TRPML1 regulates lysosomal exocytosis and exosomal release, and that this regulation is altered in AS mice. Further studies are needed to better understand the contributions of TRPML1 in synaptic plasticity and in neurodevelopmental disorders such as Angelman Syndrome.

¹Sun J, Liu Y, Hao X, Lin W, Su W, Chiang E, Baudry M, Bi X. LAMTOR1 inhibition of TRPML1-dependent lysosomal calcium release regulates dendritic lysosome trafficking and hippocampal neuronal function. *EMBO J.* 2022 Mar 1;41(5):e108119. doi: 10.15252/embj.2021108119. Epub 2022.

²Sun J, Liu Y, Jia Y, Hao X, Lin WJ, Tran J, Lynch G, Baudry M, Bi X. UBE3A-mediated p18/LAMTOR1 ubiquitination and degradation regulate mTORC1 activity and synaptic plasticity. *Elife.* 2018 Jul 18;7:e37993. doi: 10.7554/eLife.37993

Disclosures: E. Penna: None. M. Baudry: None. X. Bi: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Inhibiting upstream sterol synthesis reduces 7-DHC and prevents aberrant neuronal maturation in SLOS patient iPSC-derived neurons

Authors: *G. GAO¹, T. TRELEAVEN², Y. WANG², A. BYRNE², L. CHAI², L. GUO², M. GONCALVES², A. BIALAS², S. GIERA², B. WANG², B. ZHANG², E. DE RINALDIS², J. DODGE²;

¹Sanofi-Aventis, Cambridge, MA; ²Sanofi Aventis, Cambridge, MA

Abstract: Smith-Lemli-Opitz syndrome (SLOS) is a metabolic and developmental disorder caused by a deficit in cholesterol synthesis. Patients with SLOS have mutations in the 7-dehydrocholesterol reductase gene (*dhcr7*), which catalyzes the last step in cholesterol biosynthesis. As a result, cholesterol is reduced and 7-dehydrocholesterol (7-DHC), a toxic cholesterol precursor, accumulates throughout the body disrupting many developmental processes. The nervous system can be severely affected in SLOS given that *de novo* cholesterol synthesis peaks during brain development and is critical for neuronal maturation and myelination. Neurons are especially sensitive to 7-DHC and show accelerated differentiation and maturation in the presence of its oxysterol metabolite, DHCEO. In this study, we tested the therapeutic approach of lowering 7-DHC levels by targeting enzymes upstream of *Dhcr7* in the cholesterol synthesis pathway. First, we characterized two SLOS patient fibroblasts: GM03044 and GM05788. To gain insight into the mechanisms underlying neural phenotypes of SLOS, we generated SLOS patient iPSCs. Sterol analysis showed that SLOS iPSCs had higher 7-DHC and DHCEO than our two control iPSCs. All iPSCs were differentiated to NPCs, verified by Nestin and PAX6 staining. Analysis of sterol levels in SLOS fibroblasts and iPSCs cultured in cholesterol deficient mTeSR1 medium had lower cholesterol and higher 7-DHC compared to controls, consistent with published results. Differentiated neurons also showed elevated 7-DHC. During expansion in pluripotent conditions, control iPSCs maintained pluripotency in mTeSR1; however, SLOS iPSCs exhibited a spindled, neural progenitor-like phenotype. To analyze SLOS iPSC neural progenitors, neurospheres were generated in cholesterol depleted medium and plated onto matrigel-coated dishes in cholesterol deficient neural induction media. Extended differentiation assays revealed high β III-tubulin expression in SLOS iPSCs relative to controls. Analysis of iPSC-derived neuronal maturation by high content imaging revealed accelerated and elevated expression of β III-tubulin in SLOS patient-derived cells compared to controls, consistent with premature neuron differentiation. Notably, inhibiting upstream sterol synthesis significantly decreased 7-DHC levels and prevented early β III-tubulin expression. In summary, we have developed an in vitro model of disease that is amenable to high throughput screening for 7-DHC induced neurodevelopmental defects and identified cholesterol synthesis pathway enzymes upstream of *Dhcr7* as potential therapeutic targets for SLOS.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01NS104078
MH101703

Title: Contextual fear memory recall activates different biological processes in the hippocampus of Angelman syndrome model mice

Authors: *W. SU¹, X. HAO², M. BAUDRY¹, X. BI²;
¹GCBS, ²COMP, Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Angelman syndrome (AS) is a rare neurodevelopmental disorder with severe developmental delay, lack of language skills, severe cognitive impairment, motor dysfunction and sleep disorder, and AS patients often have comorbid epilepsy and autism. Genetic/genomic studies have attributed impaired expression of *UBE3A* in neurons as the cause of AS. Recently developed AS mouse models with Ube3a deficiency induced by various genetic manipulations recapitulate most of AS symptoms, including deficits in motor function and learning and memory, anxiety disorders, and altered social interactions. Contextual memory in the fear conditioning paradigm has been widely used to evaluate hippocampus-dependent learning and memory performance in various neurological disease models, including AS. Several research groups have reported that AS mice show impaired contextual memory recall, although the underlying mechanism remains unsettled. In the present study, we performed RNA-seq on hippocampal samples from both wildtype (WT) and AS mice in control conditions and after fear context recall. Principal component analysis showed that memory recall and genotype were the major drivers of sample variability. There were 281 recall-associated DEGs in WT mice and 268 DEGs in AS mice, when compared to their respective non-recall controls. KEGG Pathway analyses showed that among the top 10 enriched pathways for DEGs, 5 overlapped between WT and AS mice, while the other 5 were different. Interestingly, the PI3K-AKT pathway was shared by the two genotypes, but the MAPK signaling pathway was only present in AS mice, although both pathways play important roles in synaptic plasticity and memory functions. GO-Biological Process enrichment analysis showed that the extracellular structure/matrix organization term was prominently enriched in WT mice, while the RNA/Nuclear acid metabolic process was highly enriched in AS mice. Of these DEGs, 129 genes were shared between WT and AS mice. Bio-informatic analyses of the overlap and unique of these two gene sets showed that some processes known to be critical for synaptic plasticity and learning and memory are not activated in AS mice. These results suggest that contextual memory recall in AS mice recruits different transcriptional programs than in WT mice, which could be responsible for recall failure.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.17

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MH101703

Title: Ube3a deficiency induces enhanced autophagy in cerebellum of a mouse model of angelman syndrome and in cell lines

Authors: *X. HAO¹, M. BAUDRY², X. BI³;

¹Col. of Osteo. Med. of the Pacific, ²Grad. Col. of Biomed. Sci., Western Univ. of Hlth. Sci., Pomona, CA; ³Col. of Osteo. Med. of the Pacific, Western Univ. Hlth. Sci., Pomona, CA

Abstract: Angelman Syndrome (AS) is a neurogenetic disorder caused mainly by deletion of maternal chromosome 15q11-q13, wherein several imprinted genes, including the maternally expressed *UBE3A* gene, are encoded. Genetic studies have shown that while *UBE3A* deficiency causes AS, *UBE3A* over-expression has been linked to autism. *UBE3A* functions both as an E3 ligase in the ubiquitin-proteasome system, and as a transcriptional co-activator for steroid hormone receptors. Since there are extensive reciprocal interactions between the ubiquitin-proteasome and the autophagy-lysosomal systems, two major protein degradation pathways, this study investigated the effects of *UBE3A* deficiency on autophagy in cerebellum of AS mice and in primate and human cell lines. Immunofluorescent analysis indicated that the numbers of puncta immunopositive for LC3, a marker for autophagosomes, and for LAMP1, a lysosomal protein, were increased in Purkinje cells of AS mice, as compared to wild-type mice. Increased autophagic activity was confirmed by western blot analysis, which indicated an increased conversion of LC3I to LC3II. Levels of Beclin1, a factor involved in autophagy initiation, were also increased in Purkinje neurons of AS mice. *UBE3A* siRNA knockdown in either COS-1 or HEK293 cells resulted in increased numbers and size of LC3-immunopositive puncta, as compared to control siRNA-treated controls. Western blot analysis also showed that *UBE3A* knockdown increased LC3 II/I ratio in both cell lines. These results indicate that the lack of *UBE3A* leads to activation of autophagy in both in vivo and in vitro experiments. Increased autophagy is unlikely related to the mTORC1 pathway, which suppresses autophagy, as we previously showed that *Ube3a* deficiency results in enhanced mTORC1 activation. Further experiments are needed to identify the underlying mechanisms and potential roles of enhanced autophagy in the pathogenesis of AS.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.18

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant F32 HD105323
NIH Grant AG062593

Title: Neuroendocrine control of the proteostatic network by HPK-1 delays aging

Authors: *M. I. LAZARO-PENA¹, C. A. DIAZ-BALZAC², R. DAS¹, A. V. SAMUELSON¹;
¹Dept. of Biomed. Genet., ²Dept. of Medicine, Div. of Endocrinol., Univ. of Rochester,
Rochester, NY

Abstract: The progressive decline of cellular proteostasis is a hallmark of normal organismal aging, and is the basis for the onset and progression of a growing number of neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's disease. These diseases share a common characteristic: the accumulation of proteotoxic aggregates that result in cellular dysfunction and death. Proteotoxic disease is opposed by a cellular proteostatic network (PN) of approximately 1500-2000 proteins, which maintain the proteome by balancing rates of protein synthesis, degradation, folding, and sequestration. We have identified the transcriptional cofactor HPK-1 (homeodomain-interacting protein kinase) as an important PN component that preserves proteostasis and extends longevity. We find HPK-1 is primarily expressed in the nervous system during adulthood. Loss of neuronal *hpk-1* shortens lifespan, while neuronal overexpression of *hpk-1* is sufficient to increase lifespan. Neuronal HPK-1 is responsive to both acute heat shock and chronic nutritional stress, suggesting HPK-1 may act within the nervous system to integrate diverse signals and coordinate adaptive responses within the PN. We investigated whether HPK-1 acts cell autonomously in neurons to mediate stress response and proteostasis, or alternatively functions cell non-autonomously from neurons to regulate these processes in peripheral tissues. Neuronal loss of *hpk-1* hastens the collapse of both neuronal and muscle proteostasis. Conversely, neuronal overexpression of *hpk-1* delays the progressive decline of both neuronal and muscle proteostasis. Neuronal HPK-1 overexpression produces a paracrine signal to hyper-induce molecular chaperone expression locally and a neuroendocrine signal to induce autophagy in peripheral tissues. To further investigate the non-cell autonomous regulation of neuronal HPK-1, we expressed HPK-1 in different types of neurons and found that overexpression of HPK-1 in serotonergic and GABAergic neurons is sufficient to preserve proteostasis in the muscle. Interestingly, overexpression of HPK-1 in serotonergic neurons, but not in GABAergic neurons, is sufficient to increase heat stress resistance. In the contrary, overexpression of HPK-1 in the GABAergic, but not in serotonergic neurons, is sufficient to induce autophagy activity and extend lifespan. These suggests that HPK-1 exerts a different PN function in these types of neurons to preserve proteostasis. Collectively, our results position HPK-1 at a central regulatory node acting from the nervous system, upstream of the greater PN, by exerting distinct but complementary roles in different types of neurons.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.19

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Recovering behavioral impairment after Doxorubicin chemotherapy in rats

Authors: *B. POUDEL¹, J. L. CHEATWOOD²;

¹Southern Illinois Univ., Southern Illinois Univ., Carbondale, IL; ²SIU Sch. Med., SIU Sch. Med., Carbondale, IL

Abstract: Chemotherapy is one of the widely used treatments for patients with cancer. It has been proved as an effective therapy to lengthen the survival of cancer patients and improve their quality of life. However, more than half of the cancer survivors suffer from cognitive deficits following chemotherapy which is termed as “Chemo brain”. There is ample evidence on the side effects of chemo drug, the mechanisms of which seems to be ambiguous. Doxorubicin is a cytotoxic anthracycline antibiotic that has been reported to induce chemo brain via DNA damage, disruption of hippocampal neurogenesis, oxidative stress, inflammation, and dysregulation of apoptosis. Our study aims at assessing the behavioral deficits induced by Doxorubicin in motor tasks and anxiety-like behaviors. For this purpose, twelve young adult female hooded rats were assigned to either the Control (0.9% saline) or Dox groups (6mg/kg Intraperitoneal injection; once weekly for four weeks). Rats were assessed on several behavioral tests like the String-Pulling test, Bar walk test, and open field test as the standard measures of cognition. Two individuals blind to the treatment calculated the average time duration, number of attempts to pull the string, number of misses, before and after treatment for the string- pulling task, number of deep slips, slight slips, and the total number of slips for Bar walk test. Similarly, the number of entries to the center, number of entries to the corners, time spent at the center vs corner was calculated for the Open field test and compared between the groups. Student’s t-test was performed across the groups where no significant differences were detected between Dox-treated and Control rats which suggests that the Dox- treatment produced little or no significant peripheral neuropathy that disrupted performance on the motor task we used. Future studies of cognitive function using our model will likely not be confounded by Dox-associated motor impairment. Also, to understand the skilled movements and motor learning of hand movements in the String-Pulling task, frame to frame analysis using DeepLabCut™ is being carried out. The kinematics of the movement obtained from it can form a basis to explore the changes in the forelimb functions following Doxorubicin treatment which will support our data to develop animal models of neurological conditions. Furthermore, we plan to investigate the gene expression level of various markers of neurogenesis, and neuroinflammation in the brain samples of Doxorubicin treated and Control rats, and elucidate the mechanism associated with Doxorubicin- induced chemo brain.

Disclosures: B. Poudel: None. J.L. Cheatwood: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

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Program #/Poster #: 456.20

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5R01NS112327-02

Title: Ablation of neuronal galactosylceramidase results in neurodegeneration

Authors: *J. FAVRET¹, C. KREHER², N. I. WEINSTOCK⁴, L. WRABETZ^{2,3}, M. FELTRI^{2,3}, D. SHIN¹;

¹Dept. of Biotechnical and Clin. Lab. Sci., ²Inst. for Myelin and Glia Exploration, ³Biochem., Univ. at Buffalo, Buffalo, NY; ⁴Johns Hopkins, Baltimore, MD

Abstract: Krabbe Disease (KD) is a rare autosomal recessive lysosomal storage disease caused by mutations in the acid hydrolase Galactosylceramidase (Galc). Due to the function Galc serves in hydrolyzing a major myelin membrane component Galactosylceramide (GalCer) the demyelination and neurodegeneration has canonically been associated to the dysfunction of myelinating glia with neuronal pathology following secondarily. However, GALC is expressed in all brain cells ubiquitously, indicating a cell specific GALC function. Previously, using the neuron specific *Thy1-Cre/ER^{T2}* model *Galc* ablation was induced postnatally which resulted in delayed neuronal maturation albeit without accumulation of the cytotoxic Galc substrate Psychosine (Psy). In an effort to expand the in-vivo findings of neuronal specific KD pathology this study utilizes the constitutively active pan-neuronal *Syn1Cre* to induce a robust knockout in haplodeficient *Galc flox/-* mice. *Syn1Cre; Galc flox/-* mice exhibit neuronal specific expression of Cre recombinase which mediates an efficient loss of neuronal Galc and elicits Psy accumulation. While *Syn1Cre; Galc flox/-* mice had reduced bodyweight and a significant impairment of locomotive capabilities assessed via rotarod at both 2 and 6 months compared to control *Galc +/-* mice there was no impact on overall survival. Electron microscope morphological analysis of *Syn1Cre; Galc flox/-* mice revealed a significant increase in the number of degenerating and dying neurons; furthermore, G-ratio measurements showed a slightly thinner myelin sheath which was further validated by means of western blot analysis of myelin proteins. Lastly, analysis of inflammatory markers, namely GFAP and CD68 revealed a significant increase in astrocytosis and microgliosis respectively. Also, Parkinson's disease-like pathology in the neuron-specific Galc knockout such as lipofuscin accumulation may provide evidence of the connection between GALC dysfunction and the pathogenic mechanism of other neurodegenerative diseases. The study is the first of its kind to show that neurons are contributors to KD pathology in a cell-autonomous manner and thus require therapeutic intervention for disease management. A preliminary effort to further the understanding of KD neuronal pathology is underway utilizing the Translating Ribosome Affinity Purification technology to isolate relevant mRNA from the neurons of *Syn1Cre; Galc flox/-* mice. Findings from this cell specific RNA-seq experiment may lend insight into the mechanism in which neuronal pathology arises and influences the overall disease state.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

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Program #/Poster #: 456.21

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS intramural program (W.L.)
NCI intramural program (S.H.)

Title: Neuronal accumulation of peroxidated lipids promotes demyelination and neurodegeneration through the activation of the microglial NLRP3 inflammasome

Authors: *G. WANG¹, W. YIN², Q. TIAN¹, W. LU⁴, S. HOU³;
¹NINDS, ²NIDDK, NIH, Bethesda, MD; ³NCI, NIH, Frederick, MD; ⁴NINDS/NIH, NINDS/NIH, Bethesda, MD

Abstract: Peroxidated lipids accumulate in the presence of reactive oxygen species and are linked to neurodegenerative diseases. Here we find that neuronal ablation of ARF1, a small GTPase important for lipid homeostasis, promoted accumulation of peroxidated lipids, lipid droplets and ATP in the mouse brain and led to neuroinflammation, demyelination and neurodegeneration, mainly in the spinal cord and hindbrain. Ablation of ARF1 in cultured primary neurons led to an increase in peroxidated lipids in co-cultured microglia, activation of the microglial NLRP3 inflammasome and release of inflammatory cytokines in an Apolipoprotein E-dependent manner. Deleting the Nlrp3 gene rescued the neurodegenerative phenotypes in the neuronal Arf1-ablated mice. We also observed a reduction in ARF1 in human brain tissue from patients with amyotrophic lateral sclerosis and multiple sclerosis. Together, our results uncover a previously unrecognized role of peroxidated lipids released from damaged neurons in activation of a neurotoxic microglial NLRP3 pathway that may play a role in human neurodegeneration.

Disclosures: G. Wang: None. W. Yin: None. Q. Tian: None. W. Lu: None. S. Hou: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.22

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DEARC: P50AA0AA017823
NADIA: U01AA028710

Title: Adolescent Binge Ethanol Consumption Produces Dysregulation of Pro and Mature Neurotrophin Expression in a Region and Time-Specific Manner

Authors: *B. KIPP¹, L. M. SAVAGE²;

¹Binghamton Univ., Binghamton, NY; ²Psychology-Behavioral Neurosci., SUNY - Binghamton Univ. Behavioral Neurosci., Binghamton, NY

Abstract: Adolescent intermittent ethanol exposure (AIE) is a model of heavy adolescent binge drinking, that has been found to produce changes in neurobehavioral functioning long after the cessation of alcohol consumption. Cholinergic neurons within the basal forebrain are vulnerable to the toxic effects of ethanol and cholinergic phenotypic expression is significantly reduced following this model alongside reductions in cortical acetylcholine content. While the mechanisms driving the loss of basal forebrain cholinergic neurons are being investigated, the role that pro and mature neurotrophin activity may play in the development of this pathology has not been characterized. Target derived neurotrophins, such as NGF and BDNF which bind to TrkA and TrkB respectively, are crucial for the development and maintenance of cholinergic neurons. Immature forms of these neuropeptides, proNGF and proBDNF lead to axonal degradation, loss of cholinergic phenotype expression, and cell death through interactions with the co-receptor p75NTR. The relative expression of proNGF, NGF, proBDNF, BDNF, TrkA, TrkB, and p75NTR, as well as vesicular acetylcholine transporter (vAChT) and Choline Acetyltransferase (ChAT) were measured 2-hours, 24-hours, 3-weeks, and 6-months following 5g/kg 20% ethanol exposure from post-natal day 25-55. The nucleus basalis magnocellularis (NbM) and medial prefrontal cortex (mPFC) of Sprague Dawley male and female rats were examined for western blot expression of these markers. Two hours following the last ethanol exposure, mPFC expression of BDNF, and vAChT was significantly reduced in AIE-treated animals, alongside NbM reductions in NGF. During withdrawal, 24-hours following AIE, mPFC expression of proNGF significantly increased, while proBDNF was reduced in ethanol treated rats. In the NbM, NGF expression was reduced, while proBDNF expression significantly increased. Three weeks post AIE, there were no differences between ethanol and water treated animals were detected in the mPFC. In contrast, NbM expression of NGF and ChAT were reduced in AIE treated rats. Lastly, 6-months post AIE, mPFC expression of vAChT was reduced in ethanol treated animals, and the expression of ChAT and TrkA in the NbM were also lower than controls. Taken together, the dysregulation in pro and mature neurotrophin expression during repeated binge/withdrawal bouts of ethanol may promote the long-term neuropathological state following AIE.

Disclosures: B. Kipp: None. L.M. Savage: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

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Program #/Poster #: 456.23

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: AA025713
AG072894

AA020023
AA020024

Title: Neuroimmune and epigenetic mechanisms contribute to ethanol-induced reversible loss of cholinergic neurons in an *ex vivo* basal forebrain slice culture model

Authors: F. T. CREWS, *R. P. VETRENO;

Univ. of North Carolina at Chapel Hill, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Binge drinking and alcohol abuse are common during adolescence, and causes lasting cholinergic pathology. Using a preclinical rodent model of adolescent binge drinking (AIE; 5.0 g/kg, i.g., 2-days on/2-days off from postnatal day [P]25 to P54), we discovered reductions of basal forebrain cholinergic neurons (i.e., ChAT, TrkA, p75^{NTR}) that persist into adulthood. Recent studies from our laboratory link neuroimmune signaling and epigenetic silencing mechanisms to the persistent, but reversible, AIE-induced cholinergic pathology. In the current study, we developed an *ex vivo* basal forebrain slice culture (BFSC) model to investigate neuroimmune and epigenetic mechanisms underlying reductions of cholinergic neurons. BFSC tissue were prepared from P7 Wistar rat neonates and maintained in culture for 10 days prior to experimentation. In the present study, we extended our AIE cholinergic pathology data to include reductions of additional cholinergic phenotype (e.g., ChAT, VACHT, AChE, NGFR, TrkA) and cholinergic lineage (e.g., Isl1, Lhx8, Gbx2) genes in the adult basal forebrain following AIE. To validate the BFSC model, slices were treated with ethanol (100 mM; 4 days) and cholinergic neurons markers assessed. Ethanol treatment reduced ChAT+ neuron populations, caused neuronal shrinkage of the remaining cholinergic neurons, and decreased expression of cholinergic and lineage genes similar to the AIE model. Ethanol treatment of BFSC sections and AIE treatment both increased mRNA levels of proinflammatory signaling molecules (e.g. RAGE, IL-1 β , CCL2 [MCP-1]). Application of proinflammatory LPS (100 ng/mL; 24 hr) to BFSC slices mimicked ethanol-induced cholinergic pathology and neuroimmune induction that was blocked by treatment with anti-inflammatory drugs (i.e., glycyrrhizin [HMGB1 inhibitor; 500 μ M; 24 hr], BHT [anti-oxidant; 100 μ M; 24 hr]). In the *in vivo* AIE model, H3K9me2 methylation at promoter regions of cholinergic phenotype genes is associated with reversible epigenetic silencing of the cholinergic phenotype. In the BFSC model, treatment with G9a and REST inhibitors, which inhibit dimethylation of H3K9, blocked ethanol- and LPS-induced loss of ChAT+ cholinergic neurons. Together, these data indicate that the BFSC model provides a platform for studying AIE-induced cholinergic pathology and that inhibition of neuroimmune and REST-G9a-H3K9me2 epigenetic gene silencing blocks ethanol-induced loss of ChAT+ neurons further implicating neuroimmune-epigenetic mechanisms in the reversible loss of the cholinergic neuron phenotype.

Disclosures: F.T. Crews: None. R.P. Vetreno: None.

Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

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Program #/Poster #: 456.24

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RTI2018-095615-B-I00

Title: Pleiotrophin: A bridge between obesity and neurodegenerative disorders by promoting neuroinflammation and mitochondrial dysfunction

Authors: H. CAÑEQUE-RUFO, M. SÁNCHEZ-ALONSO, Á. ZUCCARO, J. SEVILLANO, M. RAMOS-ÁLVAREZ, *G. HERRADON;
Fac. of Pharmacy, CEU San Pablo Univ., Alcorcon, Spain

Abstract: Obesity is a chronic disease associated with the development of Metabolic Syndrome (MetS). Recent studies point to MetS as a risk factor for the development of neurodegenerative diseases, such as sporadic forms of Alzheimer's and Parkinson's disease. A mechanism that seems to be key in the connection between MetS and the development of neurodegenerative diseases is chronic inflammation associated with metabolic disorders, which underlies persistent neuroinflammation that contributes significantly to neurodegeneration. Recently, we identified pleiotrophin (PTN) as a novel neurotrophic factor that modulates neuroinflammation in different contexts, and as a key player in regulating energy metabolism and thermogenesis, suggesting that PTN could play an important role in the connection between MetS and obesity and neurodegenerative disorders. To test this hypothesis, our study aimed to use PTN genetically deficient (*Ptn*^{-/-}) mice to determine the role of PTN in neuroinflammation and the crosstalk between CNS and Periphery in a high fat diet (HFD)-induced obesity model. Three months old C57BL/6J wild-type (*Ptn*^{+/+}) and *Ptn*^{-/-} mice were fed with chow (STD, 18 kcal% fat, 58 kcal% carbohydrates, and 24% kcal protein) or HFD (45 kcal% fat, 35 kcal% carbohydrates, and 20% kcal protein) for 80 days. Plasma analyses showed that both HFD and *Ptn* deletion causes alterations in hormones such as insulin, leptin, PAI-1, and resistin. As expected, HFD produced an inflammatory state in the CNS as observed in cerebral quantifications of numerous proinflammatory markers, such as *Iba1*, *CD68*, *Il6*, *Il1β*, *Tnfa*, *Ccl2* and *Ptgs2*. However, the expression of these neuroinflammatory markers were significantly reduced in *Ptn*^{-/-} mice, suggesting that PTN promotes neuroinflammation induced by HFD. Additionally, qPCR quantifications showed that alterations in mitochondrial biogenesis and dynamics induced by HFD are less pronounced in the brain of *Ptn*^{-/-} mice. In summary, using an HFD-induced obesity model, this study provides substantial evidence that *Ptn* deletion protects against neuroinflammation and mitochondrial dysfunction, prominent features in neurodegenerative diseases.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CityU 11103418
CityU 11101019
CityU 11102220

Title: Circadian clock-mediated neuronal gene expression in A β toxicity and excitotoxicity

Authors: X. ZHANG, T. WU, H. HUANG, Y. CHANG, G. LAU, X. WANG, J. KIM;
City Univ. of Hong Kong, Hong Kong, China

Abstract: In recent decades, transcriptome analysis has been widely used to understand human disease pathogenesis and identify therapeutic targets and biomarkers. Accumulated reports in patients with neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, have also provided evidence of dysregulated gene expression related to neuropathogenesis. However, obtaining samples from neurodegenerative patients is more challenging than other human diseases due to low accessibility. Also, brain tissues and cerebrospinal fluid are composed of multiple cell types, so they are unsuitable for obtaining neural cell-type-specific gene expression profiles. Thus, we here report gene expression profiles in primary neuronal cultures exposed to A β toxicity and glutamate excitotoxicity to understand pathological gene expression in neurons. By RNA-sequencing analysis, we compare transcriptomes and find that two groups of genes show similar expression patterns in A β toxicity and excitotoxicity—they are either up- or down-regulated in both conditions. Genes in the two groups are related to synaptic function and cell signaling, which are well-known biological functions altered in A β toxicity and excitotoxicity. Interestingly, the analysis reveals a possibility that circadian clock (molecular oscillator generating daily rhythms)-related genes are dysregulated in both conditions. We confirm the reduced circadian transcription factor *Bmal1* levels in A β toxicity and glutamate excitotoxicity. RNA-sequencing analysis in *Bmal1*-deleted neurons shows potential relationships between BMAL1 and synaptic functions. Thus, this transcriptome study provides evidence of the potential roles of the circadian clock in neuropathogenesis.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Eversight
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Title: Multi-omics analysis of human tissues reveals details of the pathophysiological mechanisms of diabetic retinal diseases

Authors: R. HALIYUR¹, Y. SHAN¹, J. E. ROGER², *P. E. FORT¹;

¹Univ. of Michigan, Univ. of Michigan, Ann Arbor, MI; ²CNRS, CERTO, Saclay, France

Abstract: Diabetic retinopathy (DR), the major ocular complication associated with diabetes, remains the primary cause of vision loss in the working age population. While vascular dysfunction has been the center of attention for decades, recent developments have highlighted the importance of neurodegeneration and neuroinflammation in the onset and early stages of the disease. Non-targeted omics analyses have led to the identification of specific regulatory pathways affected in diabetic rodents. While those animal models have allowed us to gain critical insights on the overall impact of the disease on retinal physiology, translation of these findings has been challenging, due to the inherent anatomical and functional limitations of these models. This led to the current project aiming at using human ocular tissues from donors with and without diabetes and DR to perform a regional analysis of their impact on the retina. Using non-fixed, freshly isolated retinal tissues from human donors, with and without diabetes and with or without retinopathy, we used RNA deep sequencing, lipidomic and quantitative discovery proteomic to assess the multi-omics changes affecting the retina. Subsequently, we paralleled these analyses with an assessment of the proteome of the vitreous fluid of the same donors. In this study, we independently analyzed these changes in a regional manner by dissociating the macular, perimacular and peripheral regions of the retina (n>10 per tissue and per group) in order to identify the impact of diabetes on retinal physiology. Following quality control checks, pathway analysis using integrated software highlighted specific angiogenic, inflammatory, metabolic and neuroglial regulatory pathways. Specific individual regulators such as VEGF-A, complement factors (i.e. C1q, CFB) and cytokines (i.e. IL-1beta) were analyzed by orthogonal targeted methods on larger cohorts for validation of the untargeted analyses. Proteome profiling of the vitreous of the same donors revealed the specific correlation of a particular subset of proteins as a function identifying a short list of biomarker candidates. This study offers the first regional analysis of the pathophysiological mechanisms of diabetic retinopathy with a high potential of identification of specific therapeutic targets and potential biomarkers including specific regulators of the inflammatory response and regulation of the neuroglial tissue homeostasis.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

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Program #/Poster #: 456.27

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 2020R1A2C2010285
I21SS7606036
HU22C0115

Title: Hyperammonemia-derived glutamine storm promoting MAO-A mediated tauopathy in human edema mini-brain

Authors: *M. TRAN, Y. KANG, H. CHO;
Sungkyunkwan Univ., Sungkwunkwan Univ., Suwon, Korea, Republic of

Abstract: Brain edema is a frequent manifestation of hepatic encephalopathy (HE)-induced neuronal disorder exhibits elevated levels of ammonia levels, characterized by liver failure. However, the significance of HE to the etiology of tauopathy remains uncertain due to the absence of models demonstrating the predominance of conversion among cell-cell interactions. Here, we introduce a 3D human mini-brain of hyperammonemia in microfluidic platform, which may recapitulate key features of native edema pathophysiology. First, we stated that ammonia-induced malfunctioning of microglia is accompanied by a decrease in migratory activity and in phagocytic activity, indicating that type II astrocytes play a crucial role in hyperammonemia. Second, we asserted that H₂O₂ and glutamine exert the most extraordinary dominance among the neurotoxic components efflux from astrocytes. The increase of glutamine levels efflux from astrocytes was associated with monoamine oxidase A (MAO-A) overactivation, results in hyperpolarization of the mitochondrial membrane leading to buildup of intracellular reactive oxygen species (ROS) in neurons causing synaptic dysfunction and accumulation of phosphorylated tau. Finally, we confirmed that the MAO-A inhibitor (clorgyline) is a promising therapeutic development option for their ability to restore neuronal function by demonstrating the recovery of mitochondrial membrane potential, reducing intracellular ROS by 2.25-fold, prevention of phosphorylated tau accumulation by 1.6-fold and the enhancement of neuronal cell population by 41.9%. We envision that our findings will provide light on the mechanisms behind tau phosphorylation in brain hypertrophy and, ultimately, on the robust indicators for medication development.

Keywords: *In vitro* model, hepatic encephalopathy, brain edema, glia-neuron interaction, mitochondria dysfunction, oxidative stress, tauopathy

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

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Program #/Poster #: 456.28

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Swedish Research Council 2021-01942
Swedish Diabetes Foundation DIA2020-492

Title: Heat shock proteins in the human posterior interosseous nerve from subjects with type 1 and type 2 diabetes and healthy controls

Authors: *L. B. DAHLIN^{1,2}, E. ISING³, E. ÅHRMAN³, N. THOMSEN³, A. AKESSON^{3,4}, J. MALMSTROM³;

¹Lund Univ., Dept. of Translational Med. - Hand Surgery, Malmö, Sweden; ²Linköping Univ., Linköping, Sweden; ³Lund Univ., Lund, Sweden; ⁴Skåne Univ. Hosp., Lund, Sweden

Abstract: Peripheral neuropathy is common in both type 1- (T1D) and type 2-diabetes (T2D), where both de- and regenerative events, revealed by structural studies in humans, occur simultaneously. Heat shock proteins (HSPs) are crucial molecular chaperones serving to maintain cellular homeostasis and to reduce damage of cellular stress, such as hypo- and hyperglycaemia. Our aim was to analyse presence and patterns of HSPs in human nerve biopsies from subjects with T1D, T2D and healthy controls. Posterior interosseous nerves (PIN) from 56 living subjects with T1D (n = 9), T2D (n = 24) and healthy controls (n = 23) were harvested, in connection with surgery for carpal tunnel syndrome, and prepared for protein quantification using quantitative mass spectrometry analysis. Protein intensities were associated to conventional morphometry of the same nerve, and to electrophysiology data (amplitude and conduction velocity) of nearby uncompressed ulnar nerve. Differences in protein intensities between groups were analysed. A total number of 32 different known, and putative, HSPs were identified and quantified in the nerve biopsies. No statistically significant differences between the groups regarding protein intensities were found. Protein intensities did not correlate with myelinated nerve fibre density of the same PIN or to amplitude or conduction velocity in the ulnar nerve. We conclude that quantitative proteomics can be used to study HSPs in nerve biopsies from humans with and without neuropathy, but, despite clear structural de- and regenerative signs in T1D, no obvious differences in expression of HSPs are seen between groups in the present cohort.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.29

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Clinical involvement of Dickkopf protein-related protein 3 (DKK3) and Prolyloligopeptidase in Glioblastoma multiforme tumor progression and in metastasis.

Authors: *M. CAMPOLO¹, G. CASILI¹, M. LANZA¹, M. CAFFO¹, I. PATERNITI¹, A. CAPRA¹, S. CUZZOCREA¹, E. ESPOSITO²;
²Univ. of Messina, ¹Univ. of Messina, Messina, Italy

Abstract: Glioblastoma multiforme (GBM) is characterized by diffuse infiltration of the brain tissue, associated with chromosomal and genetic mutations that determine the uncontrolled growth of brain cells. Glioblastomas only rarely metastasize to sites outside the central nervous system, for reasons that are poorly understood, however, recent investigations of other tumor types have suggested that particular molecular features may correlate with metastatic potential such as the activation of many angiogenic factors. Aberrant Wnt signaling has been described as a key player in the initiation of and/or maintenance and development of glioma and Dickkopf protein-related protein 3 (Dkk-3), represents the most promising modulators of the Wnt pathway. Moreover, prolyl-oligopeptidase (PREP) is a serine protease involved in the angiogenesis and inflammatory process and has been highlighted the role in the metastasis spread. The aim of the present study was to evaluate the possible correlation between Dkk-3 expression and different GBM patients' parameters in the regulation of tumor growth processes, by investigating the possible role of PREP in GBM and metastasis. Biopsies of GBM and GBM-related metastasis were taken from patients operated at the Neurosurgery of the Polyclinic "G. Martino" of Messina. The study included 20 cases of GBM of different histological grading, taken by female and male patients, aged between 43 to 77 years. The statistical analysis was performed correlating the extent of Dkk-3 expression of the single GBM samples with different patient parameters: gender, age, survival and % Ki-67 labeling index as well the evaluation of PREP modulation in GBM-related metastasis. Clinical data showed that the down-regulation of Dkk-3 was correlated with survival and age; specifically, a low expression of Dkk-3 tends to be higher in male patients compared to female patients, with an age media of 57 years. Instead, the analysis between Dkk-3 expression and survival after the first GBM surgery did not show a statistically significant correlation. Also, increased reactivity for Ki67 and ATRX was found in biopsies of patients with Dkk-3 overexpression, associated with an incidence of the mutation in the gene encoding the isocitrate dehydrogenase IDH132. Moreover, a significant PREP expression was found in both GBM primary tumor and in metastasis. In conclusion, the overexpression of Dkk-3 on GBM samples showed a significant reduction of these markers in biopsies of cancer patients compared to controls, while PREP modulation could slow down metastatic expansion by reducing angiogenesis, suggesting new possible molecular targets for future therapeutic protocols.

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Poster

456. Mechanisms of Neurodegeneration: Molecular, Cellular, and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 456.30

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH BRAIN Initiative UH3NS095553

Title: Detection of movement in the thalamic ventral intermediate nucleus using a commercial deep brain stimulation system

Authors: *D. JOHNSON¹, M. S. OKUN², K. FOOTE³, A. GUNDUZ¹;
¹Biomed. Engin., ²Neurol., ³Neurosurg., Univ. of Florida, Gainesville, FL

Abstract: Essential tremor (ET) is a neurological movement disorder characterized by action tremor or tremor during voluntary movement (Espay et al, 2017). Medically refractory cases of ET are frequently treated with conventional deep brain stimulation (DBS) therapy in which neurostimulators apply continuous electrical stimulation to the relevant subcortical nuclei (Meidahl et al, 2017). Since most patients with ET experience symptoms only during goal-directed movement, there have been recent studies using DBS systems designed for clinical research on the feasibility of adaptive DBS (aDBS) therapy that is active when an electrophysiological biomarker for movement is detected (Opri et al, 2020). The use of aDBS has the potential to reduce DBS-induced side effects and extend the time needed before device replacement (Opri et al, 2020). In this study, we used the Medtronic Percept commercial DBS system with the capability to record local field potential (LFP) signals from implanted electrodes to determine the feasibility of detecting neural correlates of movement in ET patients (n=6). All patients had DBS electrodes implanted bilaterally in the ventral intermediate nucleus (VIM) of the thalamus. We used the Medtronic Percept devices to record LFP signals from the VIM of the patients during movement tasks while accelerometer sensors were attached to various muscle groups. The power spectral density (PSD) of the brain signals was calculated during periods of movement and separately at rest as measured by the accelerometers both when therapeutic stimulation was off and when it was on. The change in log power in the beta band (13-30 Hz) was compared between rest and movement conditions using a one-sided t-test. There was a statistically significant difference in the log power of LFP for movement versus rest for 4 of the 6 patients for movement on the contralateral side from the electrode location in the VIM and 3 of the 6 patients on the ipsilateral side when stimulation was turned off. With stimulation turned on, there was only a statistically significant difference from power in the beta band for two of the patients. These findings indicate that aDBS therapy using existing commercial systems to treat kinetic tremor may be feasible.

References

1. Espay, A. J. et al. Essential pitfalls in “essential” tremor. *Movement Disorders* 32, 325-331 (2017).
2. Meidahl, A. C. et al. Adaptive Deep Brain Stimulation for Movement Disorders: The Long Road to Clinical Therapy. *Movement Disorders* 32, 810-819 (2017).
3. Opri, E. et al. Chronic embedded cortico-thalamic closed-loop deep brain stimulation for the treatment of essential tremor. *Sci Transl Med* 12, eaay7680 (2020).

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Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 457.01

Topic: B.10. Demyelinating Disorders

Support: Trish Foundation,
NHMRC Investigator grant
MSA Fellowship
Bethlehem Griffiths Research Foundation

Title: Hla-dr15 and mertk interact to influence innate immune cell profiling in multiple sclerosis

Authors: T. J. KILPATRICK, E. NWOKE, C. DWYER, V. LI, M. BINDER;
Florey Inst. of Neurosci. and Mental Hlth., Melbourne, Australia

Abstract: We determined how HLA-DRB1*15:01 (DR15) and MERTK, two risk genes for multiple sclerosis (MS), influence innate immune cell profiling during relapse and remission. The MERTK gene variant rs7422195 is an expression quantitative trait locus for the MERTK receptor tyrosine kinase in CD14+ monocytes, circulating cells with both phagocytic and immunomodulatory potential. We aimed to understand how drivers of disease risk and pathogenesis vary with HLA genotype, Mertk genetic variation and disease activity. CD14+ monocytes were isolated from blood collected from people with MS at relapse (n=40) and three months later (n=23). Healthy controls also underwent blood collections, the majority also having samples taken three months apart (n=18 first time-point, n=16 second time-point). Immunophenotypic profiling of CD14+ monocytes from people with MS and controls was performed by flow cytometry. MERTK genotype at rs7422195 and HLA-DR15 status were determined by PCR. HLA-DR15-positive patients had significantly lower proportions of MERTK+ CD14+ monocytes than HLA-DR15-negative patients, independent of rs7422195 genotype ($p < 0.05$, two-tailed Student's t-test). A similar trend was observed in controls. For HLA-DR15-positive patients the proportions of MERTK+ CD14+ monocytes were significantly lower during clinical activity of disease, whereas HLA-DR15-negative patients did not demonstrate such activity-dependent differences ($p < 0.05$, two-tailed Student's t-test). Patients homozygous for the major G allele at rs7422195 exhibited significantly higher proportions of MERTK+ CD14+ monocytes, independent of disease activity, compared to controls ($p < 0.05$, two-tailed Student's t-test). HLA-DR15 and MERTK rs7422195 genotype both influence the proportions of MERTK+ CD14+ monocytes and MS risk. In HLA-DR15-positive patients, the relapse-associated reductions in CD14+ monocytes expressing MERTK, a phagocytic receptor involved in resolving inflammation, provides a potential mechanism by which the two genes interact to influence disease pathogenesis.

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Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 457.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Mayo Graduate School of Biomedical Sciences
NIH Grant 1RF1NS122174-01

Title: Dissecting the role of H-2D^b class I molecule in the development of brain atrophy during Theiler's murine encephalomyelitis virus (TMEV) infection

Authors: *K. WININGER¹, E. GODDERY², K. AYASOUFI³, D. WOLF⁴, M. J. HANSEN⁴, A. J. JOHNSON³;

¹Neurosci., Mayo Grad. Sch. of Biomed. Sci., Rochester, MN; ²Immunol., Mayo Clin. Grad. Sch. of Biomed. Sci., Rochester, MN; ³Immunol., ⁴Mayo Clin., Rochester, MN

Abstract: Dissecting the role of H-2Db class I molecule in the development of brain atrophy during Theiler's murine encephalomyelitis virus (TMEV) infection

Katheryn M. Winger^{1,2}, Emma N. Goddery^{2,3}, Katayoun Ayasoufi², Delaney Wolf³, Michael J. Hansen², Roman H. Khadka^{2,3}, Fang Jin², and Aaron J. Johnson^{1,3,4}

¹ Neuroscience Program, Mayo Clinic, Rochester, MN, ² Mayo Clinic Graduate School of Biomedical Sciences, Mayo Clinic, Rochester, MN, ³ Department of Immunology, Mayo Clinic, Rochester, MN, ⁴ Department of Molecular Medicine, Mayo Clinic, Rochester, MN.

Brain atrophy is a common feature of many neurological diseases as diverse as Alzheimer's disease, cerebral palsy, Huntington's disease, Krabbe disease, multiple sclerosis, Pick's disease, epilepsy, encephalitis, neurosyphilis, neuroAIDS, and Covid-19 infection. We have developed a murine model of brain atrophy using the Theiler's murine encephalomyelitis virus (TMEV) infection mouse model of multiple sclerosis. In this study, we investigated the contribution of the MHC class I molecules, H-2D^b and H-2K^b, in generating an immune response associated with the onset of brain atrophy. To define the roll of the MHC class I molecule in the cellular and molecular mechanisms of brain atrophy, we developed single class I mice with a C57BL/6 background. Using flow cytometry, we assessed both acute (7 dpi.) and sustained (45 dpi) immune response in the brain to TMEV infection. Using T2 weighted MRI, we assessed TMEV induced brain atrophy by change in ventricular volume (day 0-45 dpi.). Although, H-2D^b and H-2K^b mice had significantly different immune responses to TMEV infection (acute and sustained), they both displayed significantly more brain atrophy as compared to their uninfected littermates. We have previously shown that the H-2D^b and CD8 T cell response to viral peptide is necessary for resistance to chronic TMEV infection. However, increases susceptibility to TMEV induced brain atrophy. Thus, we developed a novel bi-transgenic mouse line with tamoxifen induced conditional ablation of either the H-2D^b or H-2K^b class I molecule in Cx3CR1+ brain resident myeloid cells on the C57BL/6 background. Interestingly, conditional ablation of H-2D^b on CNS resident myeloid cells significantly decreased the CNS immune infiltration following TMEV infection and reduced brain atrophy. This change in ventricular volume was sustained at 6 months post infection. These results strongly imply a requirement for antigen presentation of H-2D^b on myeloid cells in mediating CD8 T cell infiltration TMEV infection and provides insight into a potential molecular mechanism of virus induced brain atrophy.

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Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 457.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Pre-clinical characterization of NLRP3 inflammasome inhibitor to modulate chronic neuroinflammation and neurodegenerative pathology

Authors: *J. GONZALEZ MURCIA, A. MOLLARD, D. BEARSS;
Halia Thereapeutics, Salt Lake City, UT

Abstract: Background: Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) are the leading cause of dementia in the elderly. The continuous genetic research on these diseases demonstrates the vast complexity of the genetic architecture. The most recent AD whole-genome sequence analysis (GWAS) included 1.1 million individuals and identified 38 loci associated with disease status with seven novel loci. However, there is no reliable therapeutics to combat the late stages of neurodegeneration as of late. In the last couple of years, neurodegeneration has been tight with the deregulation of neuroinflammation signature. The NLRP3 inflammasome complex is a crucial player in activating the pro-inflammatory cytokine IL-1-beta and IL-18 and leads to cell death via pyroptosis. This work demonstrates that inhibition of the expression of NLRP3 complex yields better outcomes in terms of the neuronal and glial cell viability, modulating the IL-1-beta and IL-18 secretion, and inhibition of ASC specs formation in human iPSC microglia. Method: In-vitro experiments were carried out using the immortalized microglia HMC3 cell line, neuroblastoma N2A, astrogloma U138 and U87. We used the compound reference MCC950 and house compound HT6184 and HT6153 to inhibit the expression of the *NEK7*, the main component of the NLRP3 inflammasome complex. All cells were treated with LPS, TNF-alpha, or IFN-gamma to induce an inflammatory response. We used ELISA protocols to test neurodegenerative cytokine secretion and IL-1-beta, IL-18, and TNF-alpha. Western blots, and immunocytochemistry allowed us to determine the inhibition of the NLRP3 inflammasome complex. We tested single-cell proteomics using IsoPlexis technology. In-vivo behavioral experiments were carried WT and APP/PS1 mice. Hippocampus tissue was collected to run RNA sequence and total proteomics analysis. Results: We determine a compound dose response inhibiting the NLRP3 inflammasome formation with an IC50 less than 1 nM when using the compounds HT6184 and HT6153. IL-1-beta and IL-18 secretions were inhibited using the house compounds at a 3X lower dose than the reference compound. Single-cell proteomics determined that house compound drives inflammatory cytokines back to normal. ASC specs formation was inhibited, showing that the house compound might reverse NLRP3 assembly. In-vivo models showed that HT6184 limits pro-inflammatory cytokine IL-1-beta and TNF-alpha in acute LPS-induced inflammation models. Conclusion: This work provides

insights into the NLRP3 inflammasome complex role in modulating neurodegeneration and neuroinflammation.

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Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 457.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DFG 270949263/GRK2162

Title: Dysregulated homeostasis of astrocytes contributes to the pathogenesis in a MSA mouse model

Authors: *Y. SCHNEIDER¹, A. HOFFMANN³, R. BECKERVORDER SANDFORTH⁴, V. ROTHHAMMER², J. WINKLER¹;

¹Dept. of Mol. Neurol., ²Dept. of Neurol., Univ. Hosp. Erlangen, Erlangen, Germany; ³Ctr. for Discovery Brain Sci., UK Dementia Res. Inst., Edinburgh, United Kingdom; ⁴Inst. of Biochem., Univ. of Erlangen-Nurnberg, Erlangen, Germany

Abstract: Astrocytes are macroglial cells of the central nervous system (CNS) fulfilling a plethora of homeostatic functions. The involvement of astrocytes in neurodegenerative processes urges the need for identifying molecular signatures and defining their contribution to disease onset and progression. Here, we take advantage of a mouse model of multiple system atrophy (MSA) featuring an oligodendrocyte-specific (MBP29) overexpression of human α -synuclein. Using different markers, we have investigated astroglial responses between postnatal day (P) 21 to 28 in the MBP29 mouse model (*mus musculus*, n=12) and further extended astrocyte characterization by analyzing the expression of the glutamate reuptake transporters in the striatum. In addition, we isolated astrocytes expressing ATPase Na⁺/K⁺ Transporting Subunit Beta 2 (ATP1B2) using magnetic activated cell sorting and further developed protocols to increase the purity of sorted ATP1B2 positive astrocytes for mRNA sequencing. Our findings show a 7-fold increased expression of the glial fibrillary acidic protein (GFAP) in the cortical layers IV-VI, but not in layers I-III. Moreover, we observe about a 6-fold higher amount of GFAP expressing astrocytes in the striatum and substantia nigra pars compacta. In contrast, we detect a reduced expression of the glutamate reuptake transporters GLT-1 and GLAST in the striatum of the transgenic mice. More than 1000 genes are differentially regulated in the MSA mice, among others a strong upregulation of GFAP, Vimentin, CD44, Osmr, and Serpina3n. Using a gene set enrichment analysis, transcripts associated with inflammatory processes such as tumor necrosis factor α signaling via NF- κ B, interferon- α , and interferon- γ response were present. In addition, a downregulation of transcripts associated with lipid and fatty acid metabolism as well as cholesterol homeostasis was observed. Our findings provide evidence on

protein and transcriptional level for a region-specific strong upregulation of astrocyte response in the present MBP29 model. Based on the observed decrease of glutamate reuptake transporter expression, we propose a reduced capability of glutamate clearance in the striatum, the most important relay for motoric signals in the CNS. We also provide evidence of inflammatory processes and impaired lipid metabolism, as well as dysfunctional cholesterol homeostasis in the brain of MSA mice. Together, these findings imply a significant involvement of astrocytes in MSA pathogenesis by profound change of homeostatic functions of astrocytes thereby supporting the identification of novel therapeutic targets.

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Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

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Program #/Poster #: 457.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01EY033022
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Title: Modeling the Induction of Reactivity in Human Pluripotent Stem Cell-Derived Astrocytes and Their Contributions to Neurodegeneration

Authors: C. GOMES¹, K.-C. HUANG², S. S. LAVEKAR², Y. PAN³, J. HARKIN⁴, S. G. CANFIELD⁵, T. CUMMINS², *J. S. MEYER⁴;

¹Stark Neurosciences Res. Inst., ²Biol., ³Program in Med. Neuroscience, Paul and Carole Stark Neurosciences Res. In, Indiana Univ. Sch. of Med., Indianapolis, IN; ⁴Stark Neurosciences Res. Inst., Indiana Univ. Sch. of Med., INDIANAPOLIS, IN; ⁵Anatomy, Cell Biology, and Physiol., Indiana Univ. Sch. of Med., Terre Haute, IN

Abstract: Astrocytes are the most abundant cell type in the brain, where they closely associate with neurons to provide support via multiple mechanisms, but can also contribute to their neurodegeneration in a variety of disease states. However, the mechanisms by which astrocytes promote neurotoxicity and contribute to neurodegeneration remain unclear. Human pluripotent stem cells (hPSCs) can serve as powerful tools for the in vitro analysis of human neurodegenerative diseases, including neuron-glia interactions. Using hPSC-derived astrocytes and neurons, we explored how induced reactive astrocytes contribute to neurodegeneration. The induction of a reactive astrocyte phenotype was promoted through incubation with a cocktail of recombinant proteins including C1q, TNF α and IL1 α . Reactive astrocytes displayed profound morphological alterations exhibiting a hypertrophic profile and increased expression of A1-reactive specific markers such as complement C3. Moreover, transcriptional analyses revealed an

upregulation of genes associated with the inflammatory pathway as well as cytokine signaling in reactive astrocytes. Additionally, the secretion of several pro-inflammatory cytokines was increased in reactive astrocytes. Subsequently, the neurotoxic potential of reactive astrocytes was determined through co-cultures with a variety of hPSC-derived neurons, including retinal ganglion cells, cortical neurons, and spinal motor neurons, in which reactive astrocytes promoted marked morphological alterations including neurite retraction and reduced neurite complexity. Furthermore, the ability to more effectively model disease states in astrocytes following induction of reactivity was determined with cell lines from a variety of disease states. Overall, these results demonstrated that hPSC-derived astrocytes can be induced to acquire a reactive profile with a predominant inflammatory and neurotoxic phenotype which profoundly contributes to neurodegeneration. Thus, the modulation of reactive astrocytes could be a novel therapeutic strategy for neurodegenerative diseases.

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Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 457.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Targeting neuroinflammation in X linked dystonia parkinsonism

Authors: *T. PETROZZIELLO¹, S. E. KIM¹, N. JALALI MOTLAG², C. A. VAINE², E. B. PENNEY², R. E. TANZI², C. ZHANG², D. BRAGG², J. W. CHEN², M. C. POZNANSKY², R. SÎRBULESCU², G. SADRI-VAKILI²;

¹Massachusetts Gen. Hosp., ²Massachusetts Gen. Hosp., Boston, MA

Abstract: X-linked Dystonia Parkinsonism (XDP) is a neurodegenerative disease endemic to the Philippines and characterized by generalized dystonia combined with or followed by parkinsonism. Recently, we have demonstrated an increase in astrogliosis and microgliosis in XDP post-mortem prefrontal cortex (PFC), suggesting that increases in neuroinflammatory processes, widely described in neurodegenerative diseases, may also play a role in XDP pathogenesis. Additionally, we reported a concomitant increase in the levels of histone H3 citrullination as well as myeloperoxidase (MPO) in XDP PFC, both linked to inflammatory processes in several pathological conditions, including neurodegenerative diseases. Here, we sought to unravel the molecular mechanisms underlying neuroinflammation in XDP and we hypothesized that mitigating inflammation may provide a potential therapeutic strategy for XDP. First, we set out to assess several markers of inflammation together with central nervous system (CNS) markers by Imaging Mass Cytometry (IMC/ToF) in order to characterize the signature of

neuroinflammation in XDP. Next, we further assessed MPO in post-mortem brain by using a novel MPO-specific fluorescent molecular imaging probe. Our results revealed a marked increase in MPO in XDP PFC compared to controls. Additionally, MPO partially co-localized with the astrocytic marker GFAP and the microglial marker TSPO, suggesting that increases in MPO may be due to activation of CNS resident immune cells in XDP. Lastly, we have begun to assess MPO inhibition as a potential therapeutic strategy for XDP. Our findings reveal that inhibiting MPO with a selective inhibitor led to decreases in MPO and histone H3 citrullination in XDP-derived fibroblasts. Importantly, MPO inhibition reduced the levels of reactive oxygen species (ROS) in XDP-derived fibroblasts as measured by live cell imaging. Together, our results demonstrate that targeting MPO mitigates neuroinflammation in XDP and lay the groundwork for the assessment of other anti-inflammatory compounds as potential therapeutics.

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Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 457.07

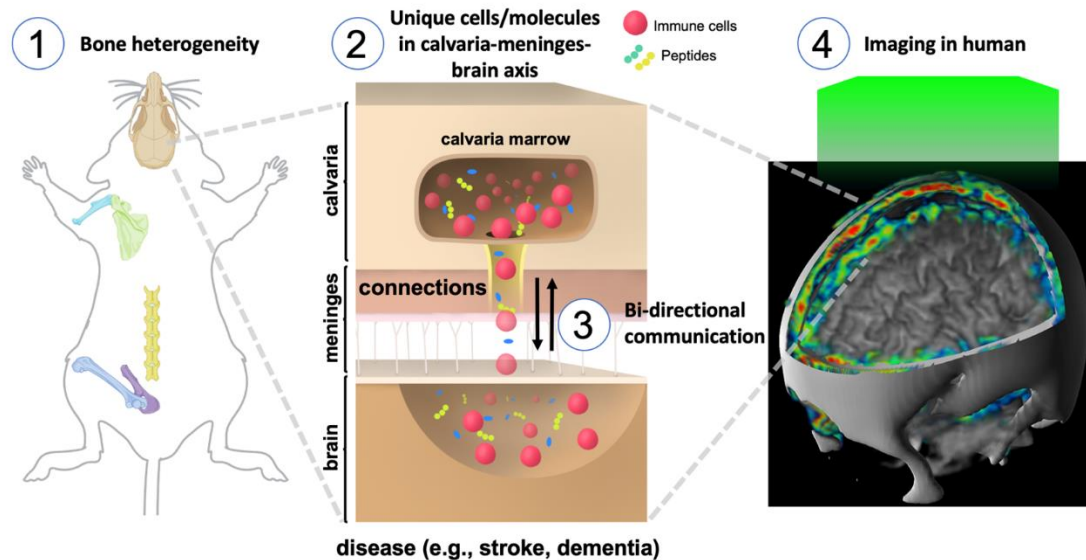
Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Multi-omics and 3D-imaging reveal bone heterogeneity and unique calvaria cells in neuroinflammation

Authors: Z. KOLABAS¹, L. B. KUEMMERLE¹, R. PERNECZKY², M. BRENDDEL², F. J. THEIS¹, *A. ERTURK¹;

¹Helmholtz Munich, Munich, Germany; ²Ludwig Maximilian Univ. Munich, Munich, Germany

Abstract: The meninges of the brain are an important component of neuroinflammatory response. Diverse immune cells move from the calvaria marrow into the dura mater via recently discovered skull-meninges connections (SMCs). However, how the calvaria bone marrow is different from the other bones and whether and how it contributes to human diseases remain unknown. Using multi-omics approaches and whole mouse transparency we reveal that bone marrow cells are highly heterogeneous across the mouse body. The calvaria harbors the most distinct molecular signature with hundreds of differentially expressed genes and proteins. Acute brain injury induces skull-specific alterations including increased calvaria cell numbers. Moreover, TSPO-positron-emission-tomography imaging of stroke, multiple sclerosis and neurodegenerative disease patients demonstrate disease-associated uptake patterns in the human skull, mirroring the underlying brain inflammation. Our study indicates that the calvaria is more than a physical barrier, and its immune cells may present new ways to control brain pathologies. The bioRxiv of the work: <https://www.biorxiv.org/content/10.1101/2021.12.24.473988v1>



Highlights:

- 1) Bone marrow across the mouse body display heterogeneity in their molecular profile
- 2) Calvaria cells have a distinct profile that is relevant to brain pathologies
- 3) Brain native proteins are identified in calvaria in pathological states
- 4) TSPO-PET imaging of the human skull can be a proxy of neuroinflammation in the brain

Disclosures: Z. Kolabas: None. L.B. Kuemmerle: None. R. Pernecky: None. M. Brendel: None. F.J. Theis: None. A. Erturk: None.

Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 457.08

Topic: C.01. Brain Wellness and Aging

Support: RO1NS113969-01

Title: The role and mechanisms of ASC specks in inflammaging

Authors: *B. CYR¹, R. W. KEANE², J. P. DE RIVERO VACCARI³;

¹Univ. of Miami Neurosci. Grad. Program, Miami, FL; ²Univ. of Miami Miller Sch. of Med., Univ. of Miami Miller Sch. of Med., Miami, FL; ³Univ. of Miami, Univ. of Miami, Miami, FL

Abstract: Neurodegenerative diseases are among the top causes of death. Neurodegenerative diseases continue to increase in the expanding elderly population, indicating a critical need for

the development of treatments and preventive strategies. One precursor of neurodegenerative disease is inflammaging. Inflammaging is a chronic, low level of inflammation experienced in old age. A contributor to inflammaging is inflammasome activation. The inflammasome is a multiprotein complex of the innate immune response that processes the cytokines interleukin (IL)-1 β and IL-18. The inflammasome consists of a sensor protein, apoptosis speck-like protein containing a caspase recruitment domain (ASC), and caspase-1. ASC oligomerizes in a prion-like fashion into ASC specks which can be expelled into the extracellular space after cell death and continue to perpetuate the inflammatory response. Here, we show activation of the inflammasome in aged mice that can be decreased with treatment of a monoclonal antibody against ASC (anti-ASC). We also examine by what mechanisms extracellular ASC specks perpetuate inflammatory signaling. We analyzed cortical lysates from young (3 month), aged saline-treated (18 month), and aged anti-ASC-treated (18 month) mice for the expression of inflammasome proteins. My data indicate that protein levels of NLRP1, ASC, caspase-1, and caspase-8 were elevated in the cortex of aged mice, and that anti-ASC decreased the expression of these proteins, consistent with lower levels of IL-1 β . Additionally, these proteins form a novel NLRP1-caspase-8 non-canonical inflammasome comprised of NLRP1, caspase-8, and ASC in neurons and microglia/macrophages in young and aged mice. Moreover, we isolated ASC specks and administered the specks at increasing concentrations to immortalized microglial cells. We measured caspase-1 activity and lactate dehydrogenase (LDH) release in the cell media to determine inflammasome activity and cell death respectively. We found that at high concentrations, ASC specks significantly activate the inflammasome and lead to cell death as measured by caspase-1 activity and LDH release. We then administered ASC specks and measured mitochondrial reactive oxygen species (ROS) and total ROS levels in live cells. Accordingly, ASC specks significantly increased the levels of total ROS, but not mitochondrial ROS. Together, these data show that the inflammasome, specifically ASC specks, play a role in inflammaging in the brain. Thus, anti-ASC may be a potential therapeutic for the amelioration of inflammasome-mediated inflammaging in the central nervous system, which could potentially be used to prevent neurodegeneration.

Disclosures: **B. Cyr:** A. Employment/Salary (full or part-time);; University of Miami. **R.W. Keane:** A. Employment/Salary (full or part-time);; University of Miami. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NINDS. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Zyversa Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inflamacore. F. Consulting Fees (e.g., advisory boards); Zyversa Therapeutics. **J.P. De Rivero Vaccari:** A. Employment/Salary (full or part-time);; University of Miami. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NINDS. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Zyversa Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inflamacore. F. Consulting Fees (e.g., advisory boards); Zyversa Therapeutics.

Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 457.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Microglia and Astrocyte-mediated Neurodegeneration in Parkinson's Disease

Authors: ***T.-I. KAM**¹, V. DAWSON², T. M. DAWSON³;

¹Neurology/Institute for Cell Engin., ²Johns Hopkins Univ., Baltimore, MD; ³Johns Hopkins Univ. Inst. for Cell Engin., Johns Hopkins Univ. Inst. for Cell Engin., Baltimore, MD

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder. During the pathogenesis of PD, monomeric α -synuclein assembles into higher ordered structures that ultimately become pathologic and drive neuronal cell death in a cell autonomous fashion. Pathologic α -synuclein can spread from cell to cell contributing to the progressive pathogenesis of PD, which causes microglia- and astrocyte-mediated neuroinflammation in a non-cell autonomous fashion. However, what drives the abnormal assembly of pathologic α -synuclein and death in neurons as well as the neuroinflammation in non-neuronal cells that are activated by pathologic α -synuclein are not known. Microglia are the resident macrophages and primary immune cells of the central nervous system. We showed that misfolded α -syn activates microglia, which release interleukin 6 (IL-6). IL-6, via its trans-signaling pathway, induces changes in the neuronal iron transcriptome that promote ferrous iron uptake and decrease cellular iron export via cellular iron sequestration response (CISR). Genetic deletion of IL-6, or treatment with the iron chelator deferiprone, reduces pathological α -syn toxicity in a mouse model of sporadic PD. These data suggest that IL-6-induced CISR leads to toxic neuronal iron accumulation, contributing to α -syn-induced neurodegeneration. Astrocytes are the most abundant glial cells in the brain and play a variety of physiological roles. In pathologic conditions, however, reactive astrocytes formed by response to stimulus or injuries in the CNS promote disease pathogenesis. Activated microglia induce neurotoxic reactive astrocyte by secreting interleukin-1 α (IL-1 α), TNF- α and C1q and that reactive astrocytes are found in postmortem brains of human neurodegenerative diseases including PD. We showed that pathological α -synuclein contributes to formation of neurotoxic reactive astrocytes and preventing α -synuclein-induced microglial activation and reactive astrocyte conversion protected against dopaminergic neurodegeneration and behavioral deficits in a mouse model of sporadic PD. More recently, we also found that reactive astrocytes formed by oligomeric amyloid- β (A β) contribute to neurotoxicity and synaptic degeneration in a mouse model of Alzheimer's disease (AD). Taken together, these findings demonstrate that reactive astrocytes contribute to pathogenesis and progression of multiple neurodegeneration.

Disclosures: **T. Kam:** None. **V. Dawson:** None. **T.M. Dawson:** None.

Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 457.10

Topic: B.10. Demyelinating Disorders

Support: NIH Grant P20GM103442
NIH Grant P20GM113123-06

Title: Cortical demyelination and depression-like behavior are associated with histaminergic dysregulation in a mouse model of peripheral inflammation

Authors: ***D. GERMUNDSON-HERMANSON**¹, **K. NAGAMOTO-COMBS**²;
¹Pathology, Univ. of North Dakota SMHS, Grand Forks, ND; ²Biomed. Sci., Univ. of North Dakota Sch. of Med., Grand Forks, ND

Abstract: Demyelinating diseases may manifest as motor, behavioral, and/or cognitive deficits. While autoimmune-based pathophysiology has been extensively investigated, the etiology of demyelinating diseases remains unclear. Recent evidence suggests that histamine and its ability to regulate blood-brain barrier (BBB) permeability and oligodendrocyte differentiation through histamine receptor signaling play a role in demyelination and remyelination. We have previously shown increased histamine and histamine 3 receptor (H3R) levels along with greater BBB permeability and cortical demyelination in the brains of C57BL/6J mice after inducing non-anaphylactic cow's milk allergy. The allergic mice also developed significant depression-like behaviors, leading us to hypothesize that excess histamine produced during the hypersensitivity response influenced behavior through central histaminergic dysregulation, neuroinflammation, and demyelination. To test our hypothesis, we sensitized male and female C57BL/6J mice to a bovine whey protein, β -lactoglobulin, and placed them on an allergen-containing diet for 2 weeks to potentiate the allergic response. During the allergen exposure, mice were given a daily oral dose of a saline vehicle or thioperamide, a selective H3R antagonist permeable to the BBB, to inhibit the action of the excessive histamine through this receptor subtype. Behavioral testing was performed in the second week of the whey diet to characterize the effects of thioperamide on behavior during repeated allergen exposure. Vehicle-treated allergic mice exhibited depression-like behaviors as indicated by greater time immobile with the tail suspension test and less distance traveled at lower speeds during the open field test than sham mice. These mice also performed poorly with the novel object recognition test but not with the cross maze test, presenting with deficits in object recognition but not spatial memory. Grip strength and rotarod testing showed no difference between the treatment groups, confirming that the altered behavior was not due to motor impairment. Importantly, the daily thioperamide treatment improved depression-like behavior and object discrimination performance in allergic mice. Together, our findings strongly suggest that excessive histamine signaling through H3R contributed to the development of allergy-associated depression-like behavior and cognitive decline. Further investigation is warranted to determine whether histaminergic dysregulation directly affects myelination and contributes to the pathogenesis of demyelinating diseases.

Disclosures: **D. Germundson-Hermanson:** None. **K. Nagamoto-Combs:** None.

Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 457.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MOST 110-2320-B-006 -056 -
MOST 107-2320-B-006-058-MY3
MOST 107-2320-B-006-005
NHRI-EX107-10734NI)
DoD W81XWH-08-1-0682

Title: Zfra suppression of epileptic seizure via inhibition of S14-WWOX phosphorylation and expression of inflammatory cytokines and microglial cells, and activating spleen Z cells

Authors: *N.-S. CHANG^{1,2}, K.-Y. WEN¹, T.-Y. LIU¹;
¹Natl. Cheng Kung Univ., Tainan, Taiwan; ²China Med. Univ., Taichung, Taiwan

Abstract: *WWOX* gene is a risk factor for Alzheimer's disease (AD). Functional deficiency of this gene may start to occur in the middle ages, and this may cause activation of a protein aggregation cascade, including TRAPPC6A, TIAF1 and SH3GLB2, which lasts for more than 30 years for AD development. In contrast, *Wwox* knockout mice exhibit brain protein aggregation in less than 3 weeks after birth and the mice survive only for 3 to 4 weeks. In human newborns, *WWOX* deficiency leads to epileptic encephalopathy, intractable seizures, developmental delay and early death. We reported that Zfra protein, a 31-amino-acid peptide zinc finger like protein that regulates apoptosis, blocks AD progression via suppression of *WWOX* phosphorylation at S14 and induction a novel Hyal-2+ CD3- CD19- Z lymphoid cells in triple transgenic AD mice. Compared to wild type mice, *Wwox* heterozygous mice readily suffered pentylentetrazol (PTZ)-induced seizure. Zfra significantly suppressed seizure, and blocked neuronal death via increased *WWOX* expression and importantly suppression of S14 phosphorylation in *WWOX* in auditory cortex and hippocampal regions. Compared to control mice, significantly reduced inflammatory microglia and astrocytes were shown in the Zfra-treated mouse hippocampus upon PTZ challenge. PTZ significantly induced the expression of neuroinflammatory cytokines, such as iNOS, COX2, NLRP3 and IFN- γ by >50-80%, and Zfra significantly decreased the expression in *Wwox*^{+/-} mice. Zfra blocked the expression of an inflammatory protein RE1-silencing transcription factor (REST) but significantly upregulated GRIA2 (Glutamate Ionotropic Receptor AMPA Type Subunit 2) in *Wwox*^{+/-} mice and restored neuronal transmission via upregulating Synapsin-1 and Synaptophysin. By co-immunoprecipitation, *WWOX* and REST physically bound CREB-1, and Zfra blocked the binding. Notably, Zfra activated novel Hyal-2+ CD3- CD19- spleen Z cells to suppress PTZ-induced seizure in recipient mice. Together, Zfra is a potent agent in mitigating seizure via inhibition of inflammatory cytokines and microglial cells and activating Z cells to counter inflammation. Activated Z cells are of great value in cell therapy.

Disclosures: N. Chang: None. K. Wen: None. T. Liu: None.

Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 457.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Cognitive impairment in patients with multiple sclerosis and neuromyelitis optica spectrum disorders: Cross-sectional and longitudinal analyses

Authors: *B. CHOI¹, J. SO², E.-J. LEE², D. SEO², J. KIM², H. KIM², K.-K. KIM², Y.-M. LIM²;

¹Univ. of Ulsan, Col. of Med., SEOUL, Korea, Republic of; ²Asan medical center, Seoul, Korea, Republic of

Abstract: Multiple sclerosis (MS) and aquaporin-4 antibody-positive neuromyelitis optica spectrum disorders (NMOSD) both negatively affect cognitive function of the patients. Cognitive function has been studied lively in MS, but there is limited evidence on the prevalence and prognosis of cognitive impairment in NMOSD. Therefore, we comparatively investigated the prevalence of cognitive impairment of patients with MS and NMOSD, that of cognitive decline over time, and associated factors. Between Oct 2019 and July 2021, consecutive patients with either MS or NMOSD were prospectively enrolled in a tertiary care center. Cognitive function scores, measured by Processing Speed Test (PST), an iPad-based symbol-digit modality test, were collected at enrollment and at follow-up with a 6-12 month interval. During the study period, 190 patients (MS, 143; NMOSD, 47) were included. The mean age and the Expanded Disability Status Scale (EDSS) score were 49.4 years and 2.5, respectively. Cognitive impairment, defined as z score < -1.5, was shown in 30 patients (21.0%) with MS and in 16 (34.0%) in NMOSD (MS vs. NMOSD, p = 0.070) at baseline. Old age, higher education years, and disease duration were contributors to cognitive impairment in both disease groups. Number of clinical attacks was significantly associated with cognitive impairment in MS patients, but not in NMOSD patients. The presence of brain lesions in magnetic resonance imaging was not associated with cognitive impairment. A total of 94 MS and 38 NMOSD patients underwent follow-up PST evaluation after 6-12 months without additional clinical relapses. Of these patients, 13 patients (13.8%) with MS and 9 in NMOSD (23.7%) demonstrated cognitive decline over time (MS vs. NMOSD, p = 0.169). Old age, female sex, higher education years, and lower baseline PST scores (cognitive dysfunction) were predictors for cognitive decline in the MS group, while no risk factors were identified in the NMOSD group. Taken together, both cognitive impairment and decline are prevalent not only in MS but also in NMOSD patients. These findings suggest that degeneration process may also occur in NMOSD patients as well as MS patients, which warrants future confirming studies.

Disclosures: B. Choi: None. J. So: None. E. Lee: None. D. Seo: None. J. Kim: None. H. Kim: None. K. Kim: None. Y. Lim: None.

Poster

457. Mechanisms and Biomarkers of Inflammation in Neurodegeneration

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 457.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R21 NS104560 (JS)
P50HD103538(AF)
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Title: Plasma microRNA and Metabolomics signature and Machine Learning to Predict Disease Severity in Adrenomyeloneuropathy

Authors: B. R. TURK¹, L. M. POISSON⁴, C. L. NEMETH⁶, M. UNBERATH², J. GOODMAN⁷, A. B. MOSER⁷, R. O. JONES⁷, A. FATEMI³, *J. SINGH⁵;

¹Neuroscience, Kennedy Krieger Inst., ³Neurol., ²Johns Hopkins Univ., Baltimore, MD; ⁴Publ. Hlth., ⁵Neurol., Henry Ford Hlth. Syst., Detroit, MI; ⁶Neurogenetics, ⁷Kennedy Krieger Inst., Baltimore, MD

Abstract: Background: Adrenomyeloneuropathy (AMN), the slow progressive phenotype of X-linked adrenoleukodystrophy (X-ALD) has no gold standard clinical biomarker for disease progression. Biomarker discovery for AMN severity would facilitate clinical trial and fulfill an unmet need for determining disease severity in clinical practice. Plasma microRNA (miRNA) and metabolites are associated with phenotype and/or disease severity in several neurodegenerative diseases. No biofluid biomarkers of disease severity/progression are available in X-ALD. Hypothesis: Metabolomic and miRNA analysis combined with machine learning will provide biomarker(s) predictive of AMN severity in X-ALD males. Methods: Plasma samples of healthy control, mild, and severe AMN patients were obtained from Kennedy Krieger Institute, Baltimore. The study was approved by the JHU IRB#86-03-06-01 and HFHS IRB#12159. LC-MS for global metabolomics and miSeq for miRNA sequencing was performed. Metabolites with the p-value for the ANOVA F-test below the threshold for FDR=0.05 were further considered by post-hoc, two-sample, t-test. Assessment of miRNA differential expression used negative binomial modeling (DESeq2 package). Benjamini-Hochberg adjusted $p \leq 0.05$ was used as the threshold for significance. 3374 metabolomic and miRNA variables from 20 subjects were used to train a Classification and Regression Tree (CART), a non-parametric, supervised machine learning technique, using clinical severity score (EDSS) as classes. Results: Comparing control, mild, and severe AMN shows strong metabolic and miRNA clustering. Individual clinical biomarker performance was assessed via regression against patient's EDSS. Significant clinical correlations were shown for: 7-alpha-hydroxy-3-oxo-4-cholestenoate ($p < 0.00001$), dehydroepiandrosterone sulfate ($p < 0.00001$), hypoxanthine

($p < 0.00001$) and miRNA-432-5p ($p < 0.00001$). KEGG pathway identified common downstream systems as a function of disease progression, by comparing mild vs severe disease: GAREM, IGF-1, CALCRL, SMAD2&3, Glutathione peroxidase, LDH and NOS. For machine learning, the Gini criterion was applied to minimize node impurity after split and leave-one-out cross-validation was performed. The metabolomics and miRNA CART demonstrates an 83.2% accuracy in predicting patient EDSS. Conclusions: This is the first report of unique miRNA and metabolic signatures for AMN disease severity. We identify candidate markers with strong correlations with EDSS and developed a machine learning algorithm providing potential biomarkers for clinical use and clinical trial.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.01

Topic: C.09.Stroke

Title: Exploring the mechanisms underlying the restoration of voluntary arm and hand motor control due to spinal cord stimulation of the cervical spinal cord in post-stroke hemiparesis

Authors: ***E. SORENSEN**^{1,2}, **M. P. POWELL**^{3,1}, **N. VERMA**^{7,8}, **E. CARRANZA**^{2,1}, **A. BOOS**^{4,1,10}, **G. F. WITTENBERG**^{4,1,10}, **P. GERSZTEN**⁵, **D. J. WEBER**^{9,8}, **E. PIRONDINI**^{1,2,5,6}, **J. W. KRAKAUER**^{11,12}, **M. CAPOGROSSO**^{5,1,2,6};

¹Rehabil. and Neural Engin. Labs., ²Bioengineering, ³Dept. of Neurolog. Surgery, ⁴Neurol., ⁵Neurolog. Surgery, ⁶Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA; ⁷Mechanical Engin., ⁸NeuroMechatronics Lab., ⁹Mechanical Engin. and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA; ¹⁰GRECC, HERL, VA Pittsburgh Healthcare Syst., Pittsburgh, PA; ¹¹Neurol., Johns Hopkins Univ., Baltimore, MD; ¹²The Santa Fe Inst., Santa Fe, CA

Abstract: In stroke, damage to the corticospinal tract (CST) often leads to severe deficits in volitional motor control, for which there is currently no effective therapy. Importantly though, spinal cord circuits responsible for movement remain intact and lesions to the CST are rarely complete. Here we seek to understand if these residual connections are sufficient to synergistically interact with epidural stimulation of the cervical spinal cord (SCS) to restore volitional upper limb motor control in humans. In three participants with chronic lesions of the corticospinal tract, we implanted epidural leads on the lateral aspect of the cervical spinal cord to selectively target the dorsal roots that provide excitatory inputs to motoneurons controlling the arm and hand. Compared to the no-SCS condition, we observed marked improvements in force production when participants were asked to produce isometric force at the shoulder, elbow, wrist,

or hand with some joints demonstrating over a two-fold increase when volitional effort was paired with SCS. Notably, SCS alone did not induce any measurable force output, suggesting that it is the interaction of stimulation and supraspinal inputs that enables the observed increase in strength. We then asked participants to perform an isometric roadway test in which they were tasked with following a thresholded pathway to gradually increase, sustain, and decrease force between set percentages of their maximum force production capability as established when SCS was off. Participants not only followed the roadway during continuous SCS, but also demonstrated increased smoothness and overall accuracy when compared to no-SCS. This indicates that volitional inputs can regulate continuous SCS excitation to allow for fine force control. Additionally, we investigated the mechanisms underlying this observed improvement in fine motor skills. To this aim, we studied the interaction between residual CST axons and SCS by utilizing transcranial magnetic stimulation (TMS) to artificially induce CST drive. We found that when SCS was conditioned by a pulse of TMS over the motor cortex, EMG-evoked potentials were significantly increased compared to SCS alone. The timing at which this conditioning was optimal suggests that CST axons facilitate transmission of action potentials in the primary afferents recruited by SCS, thereby increasing the excitatory effects of SCS on spinal motoneurons. In summary, we have collected preliminary evidence that SCS interacts with residual CST inputs to improve strength and fine force control in participants with post-stroke hemiparesis. In the future, we aim to further investigate the mechanisms behind this effect.

Disclosures: **E. Sorensen:** None. **M.P. Powell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reach Neuro Inc. **N. Verma:** None. **E. Carranza:** None. **A. Boos:** None. **G.F. Wittenberg:** None. **P. Gerszten:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reach Neuro Inc. **E. Pirondini:** None. **J.W. Krakauer:** None. **M. Capogrosso:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reach Neuro Inc.

Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.02

Topic: C.09.Stroke

Title: Neuromodulation for Stroke: Direct Stimulation of Motor Thalamus Improves Hand Motor Output and Voluntary Control of Force

Authors: ***J. HO**^{1,6}, **E. M. GRIGSBY**^{2,6}, **A. DAMIANI**^{3,6}, **L. LIANG**^{4,6,7}, **J. BALAGUER**^{2,6,7}, **V. KARAPETYAN**^{1,6,7}, **P. GERSZTEN**⁵, **J. BARRIOS-MARTINEZ**¹, **D. J. CRAMMOND**⁸, **M. CAPOGROSSO**^{5,6,7}, **J. A. GONZÁLEZ-MARTÍNEZ**⁵, **E. PIRONDINI**^{3,6,7};

²Bioengineering, ³Physical Med. and Rehabil., ⁴Rehabil. and Neural Engin. Labs., ⁵Neurolog.

Surgery, ¹Univ. of Pittsburgh, Pittsburgh, PA; ⁶Rehabil. and Neural Engin. Labs., Pittsburgh, PA; ⁷Bioengineering, Pittsburgh, PA; ⁸Neurolog. Surgery, Univ. Pittsburgh, Pittsburgh, PA

Abstract: Stroke patients often experience motor paralysis in the limbs. Although physiotherapy is used to rehabilitate lost functions or prevent further progression of symptoms, most patients do not recover to a satisfactory level. Deep brain stimulation (DBS) can target intrinsic pathways and nuclei that project to vital cortical areas, bypassing damaged brain regions. One structure of interest is the ventro-oralis posterior (VOP)/ventro-intermedium (VIM) nucleus of the motor thalamus, which has been utilized to reduce motor symptoms in essential tremor (ET) patients through DBS. The VOP/VIM is a thalamic relay for incoming cerebellar projections with outgoing excitatory connectivity to the motor cortex (M1), and, thus, could be a potential target for DBS to facilitate motor function in patients with a stroke. We hypothesize that VOP/VIM stimulation increases the excitability of M1 and potentially augment hand motor output and voluntary control of hand force output. In acute preparations in macaque fascicularis (N=3), we implanted DBS electrodes into the VOP/VIM nucleus and separate deep brain electrodes in the hand region of the corticospinal tract (CST) within the internal capsule (IC). Intracortical arrays were implanted in the hand area of M1. When stimulating the VOP/VIM, we observed increased peak-to-peak amplitude of evoked local field potentials and single unit spike counts recorded in M1. Additionally, we found that thalamic stimulation at 50 Hz increased the amplitude of motor evoked potentials (MEP) of hand muscles and kinematics of hand movements when paired with IC stimulation of the CST, even after thermo-generated lesions of the CST. We replicated these results in human patients undergoing surgical implantation of DBS electrodes targeting the VOP/VIM for ET. We paired direct cortical stimulation (DCS) of the hand area of M1 and VOP stimulation at 50 Hz to replicate the experiments in primates. When VOP/VIM stimulation was on, we observed amplification of MEPs recorded in the hand compared to with DCS alone. During the awake portion of the DBS surgery, we asked patients to grasp a dynamometer and modulate hand force along a sinusoidal curve. With VOP/VIM stimulation at 50 Hz, we observed a reduction in the root-means-squared error when the subjects' traced the descent portion of the expected curve as compared to force traces without stimulation. These results suggest that VOP/VIM DBS can be used to increase the excitability of M1 which can in turn augment cortical motor output and improve voluntary motor control of the hand. We hope to use these outcomes to implement DBS of VOP/VIM as a potential therapeutic approach to treat post-stroke motor deficits.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.03

Topic: C.09.Stroke

Title: Spinal cord stimulation promotes synergistic activation of upper limb muscles during functional movement in people with post-stroke hemiparesis

Authors: *N. VERMA¹, M. P. POWELL⁴, E. SORENSEN^{5,6}, E. CARRANZA^{5,6}, A. BOOS^{11,12}, L. E. FISHER⁷, G. F. WITTENBERG^{8,12}, P. C. GERSZTEN^{4,12}, E. PIRONDINI^{9,5}, M. CAPOGROSSO^{10,5}, D. J. WEBER^{1,13,2,3},

¹Mechanical Engin., ²Dept. of Biomed. Engin., ³Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA; ⁴Dept. of Neurolog. Surgery, ⁵Dept. of Bioengineering, ⁶Rehab and Neural Engin. Labs, ⁷Physical Med. and Rehabil., ⁸Dept. of Neurol., ⁹Dept. of Physical Med. and Rehabil., ¹⁰Neurolog. Surgery, Univ. of Pittsburgh, Pittsburgh, PA; ¹¹Dept. of Neurol., Univ. of Pittsburgh, Pittsburgh, PA; ¹²VA Pittsburgh Healthcare Syst., Pittsburgh, PA; ¹³Ctr. for Neural Basis of Cognition, Pittsburgh, PA

Abstract: Stroke is the 5th leading cause of death and the leading cause of permanent disability in the United States. There are more than 7 million stroke survivors living in the US, two-third of whom have motor deficits. Even though physical therapy can improve motor function in this population, outcomes are highly variable across patients and a large fraction of the population is left with permanent motor deficits. Here we show that epidural electrical stimulation (EES) of the cervical spinal cord improved motor control of arm and hand in people with chronic hemiparesis post-stroke. Two participants were implanted with 2 x 8-channel percutaneous spinal cord stimulator leads in the lateral epidural space of the cervical spinal cord for a duration of 30 days. EES was delivered using a custom-built stimulation system that allowed stimulation on multiple contacts, either continuously (Tonic EES) or upon movement onset (Phasic EES), detected through EMG signals. The participants performed planar and 3D reaching and grasping movements with their affected limb with and without EES. Stimulation was applied on three monopolar contacts found to facilitate recruitment of muscles at the shoulder, elbow and hand. During tonic EES, the stimulation parameters were fixed and delivered continuously throughout the movement. During phasic EES, an increase in volitional EMG at the shoulder triggered stimulation bursts of fixed duration, linked to the phase of the motion (reach, grasp or retract) while the participant performed the task. Both participants were able to perform the task faster with EES, with smoother hand trajectories and a larger range of motion in shoulder, elbow, and hand, leading to overall better task performance. We hypothesized that the improvements in reaching function were attributable to facilitation of elbow muscle activity and changes in flexion and extension synergies. To test this, EMG from 14 major muscles of the arm were analyzed using nonnegative matrix factorization to identify patterns of correlated muscle activity across subgroups of muscles. Without stimulation, the contribution of the shoulder EMG was the dominant component of the muscle synergy, which contained minimal contribution from the elbow flexor or extensor muscles. However, with stimulation, the biceps and triceps EMG contributed more strongly to the muscle synergies observed during the task. While tonic EES was effective in promoting significant gains in motor function, phasic stimulation produced even stronger effects. These results show that cervical EES improves upper limb motor control in patients with chronic hemiparesis post-stroke.

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Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); REACH NEURO, INC. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BIONIC POWER INC., BLACKFYNN INC., IOTA BIOSCIENCES, INC., NEUROONE, INC., REACH NEURO, INC..

Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.04

Topic: C.09.Stroke

Title: Corticospinal tract evokes afferent depolarization to modulate proprioceptive information

Authors: ***L. LIANG**^{1,2}, J. M. BALAGUER^{1,2}, A. A. MAHROUS⁶, J. C. HO^{1,3}, E. M. GRIGSBY^{1,4}, V. KARAPETYAN^{1,2}, P. C. GERSZTEN⁵, J. A. GONZÁLEZ-MARTÍNEZ⁵, C. HECKMAN⁷, D. J. BENNETT⁸, M. CAPOGROSSO^{1,5,2}, E. PIRONDINI^{1,4,2};

¹Rehab Neural Engin. Labs, ²Bioengineering, ³Sch. of Med., ⁴Physical Med. and Rehabil., ⁵Neurolog. Surgery, Univ. of Pittsburgh, Pittsburgh, PA; ⁶Neurosci., Northwestern Univ., Chicago, IL; ⁷Dept. of Physiol., Northwestern Univ., Oak Park, IL; ⁸Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada

Abstract: The corticospinal tract (CST) is the major efferent pathway for motor control. Damage to this pathway causes sensory and motor deficits in patients. Traditionally considered only in the context of movement generation, the role of CST in sensory regulation remains unclear, limiting the effectiveness of therapies for diseases of the CST such as spinal cord injury and stroke. Anatomical studies showed that most CST axons terminate in the intermediate zone of the spinal gray matter, where GABA interneurons reside. A recent study in rodents revealed that activation of GABA interneurons at axo-axonic synapses generates primary afferent depolarization (PAD) and facilitates sensory spike transmission. This may be a potential pathway of sensory regulation by the CST. Here, we explored the role of CST in modulating upper limb proprioceptive information by observing changes in PAD, motoneuron excitability, and cervical intraspinal neural dynamics in response to afferent and CST stimulations. We used a novel acute experimental setup in anesthetized monkeys (n=3), which have unique CST organization similar to humans. To accurately stimulate the CST of the upper limb, we implanted an electrode in the mid-posterior limb of the internal capsule using a robotic stereotaxic approach. We also placed bipolar nerve cuffs on the radial and median nerves for proprioceptive afferent stimulation. We recorded PAD from a silver hook electrode in a distally severed dorsal rootlet, electromyography (EMG) from arm, wrist, and hand muscles, and intraspinal neuronal activity from a 32-channel linear microelectrode array placed dorsoventrally in the C5/C6 spinal cord. Electrical stimulation of the CST evoked clear PAD signals in the rootlet and across multiple laminae in the grey matter. PAD significantly decreased following the administration of bicuculline, a GABA_A receptor antagonist, indicating that the CST activates GABA interneurons at axo-axonic

synapses. When nerve stimulation was conditioned by subthreshold CST stimulation, afferent evoked PAD increased as compared to nerve stimulation alone. In addition, CST conditioned nerve stimulation also increased sensory-evoked EMG in multiple forelimb muscles. Our results directly demonstrated that 1) the CST generates PAD in the cervical spinal cord of macaque monkeys; 2) PAD is GABA_A-mediated; 3) CST potentiates upper limb proprioceptive input in the spinal cord through activating GABA interneurons. These results suggest that deficits after CST injury may have an origin in impaired sensory regulation. We hope that this understanding will guide us in developing better targeted therapies to relieve central motor syndromes.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.05

Topic: C.09.Stroke

Title: Spinal cord stimulation improves sensorimotor integration in chronic stroke patients

Authors: *E. CARRANZA¹, M. POWELL¹, E. SORENSEN¹, N. VERMA⁷, A. BOSS², J. W. KRAKAUER⁹, L. E. FISHER³, P. C. GERSZTEN², D. J. WEBER⁸, G. F. WITTENBERG⁴, M. CAPOGROSSO⁵, E. PIRONDINI⁶;

¹Univ. of Pittsburgh, ³Physical Med. and Rehabil., ⁴Neurol., ⁵Neurolog. Surgery, ⁶Rehabil. and Neural Engin. Labs., ²Univ. of Pittsburgh, Pittsburgh, PA; ⁷Mechanical Engin., ⁸Mechanical Engin. and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA; ⁹Johns Hopkins Univ., Baltimore, MD

Abstract: Upper limb voluntary movement relies on delivering a motor control signal through the corticospinal tract. This signal is regulated through feedback generated by the integration of sensory afferent pathways and an efferent motor command copy at the level of the cerebellum. After a stroke, damage to subcortical white matter tracts can inhibit the efficacy of this closed loop system. Proprioception, is particularly important for volitional motor control, providing the cerebellum with information about a movement using signals related to muscle and tendon stretch. Loss of proprioception can affect many aspects of motor control including force production, limb positioning, and movement kinematics. Currently, there are no effective clinical therapies to improve sensorimotor integration after stroke, but novel neurotechnologies such as spinal cord stimulation (SCS) show promise for motor function restoration. Here we evaluate the use of SCS to restore sensorimotor integration after a stroke. Three patients (2 moderate, 1 severely impaired) were surgically implanted with two 8-contact percutaneous spinal leads in the epidural space spanning the C4 to T1 dorsal roots. Patients were evaluated during sensorimotor

tasks with stimulation on and off. For this, the KINARM exoskeleton robot was used to determine the patient's ability to detect the position of their limb in space. Tasks were developed to evaluate position sense during passive and active conditions of upper limb movement, in both cases with vision of the arms occluded. In the active condition, subjects were asked to reach targets displayed on the screen using the impaired limb and report when they perceived that the target was reached. In the passive condition, the robot passively moved the impaired limb while the subject actively moved the unimpaired limb trying to mirror the contralateral arm final position. To measure sensory function related to limb position, we measured both the accuracy and variability of final hand position. Interestingly, during passive movement there was no significant change in either metric during SCS. However, during active movement we observed a significant decrease in both accuracy and variability for all three participants. Our preliminary results show that during active voluntary movement, SCS improves sensorimotor integration, specifically in position sense. Future studies will employ novel tasks to further elucidate these improvements and to explore the neural mechanisms and pathways associated with SCS's effect on sensorimotor integration.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.06

Topic: C.09.Stroke

Title: Neural mechanisms underlying the recovery of volitional motor control after paralysis during spinal cord stimulation

Authors: ***J. BALAGUER**¹, G. PRAT-ORTEGA¹, L. LIANG¹, J. HO¹, E. GRIGSBY¹, V. KARAPETYAN¹, A. MAHROUS², J. A. GONZÁLEZ-MARTÍNEZ⁴, P. GERSZTEN⁴, C. HECKMAN³, D. J. BENNETT⁵, E. PIRONDINI¹, M. CAPOGROSSO¹;

¹Univ. of Pittsburgh, Rehab Neural Engin. Labs, Pittsburgh, PA; ²Neurosci., Northwestern Univ., Chicago, IL; ³Dept. of Physiol., Northwestern Univ., Oak Park, IL; ⁴Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA; ⁵Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Mounting evidence shows that epidural spinal cord stimulation (SCS) can restore the ability of people with motor paralysis to recover voluntary motor control. However, the neural

mechanisms underlying the restoration of volitional control are largely unknown. Elucidating these mechanisms is crucial to accelerate the optimization of SCS for clinical use. Here, we utilized computational biophysics to study the integration of residual supraspinal inputs and SCS in the membrane of motoneurons (MNs).

Edgerton and others hypothesized that SCS increases excitability of spinal MNs, thus facilitating the generation of action potentials by residual supraspinal inputs. To test this, we simulated the membrane potential of MNs during concurrent SCS and residual supraspinal inputs. We leveraged previous knowledge showing that SCS directly targets Ia primary afferents in the spinal cord, thereby producing synchronous volleys of excitatory EPSPs in MNs. We then modelled residual supraspinal inputs as Poisson generators, providing excitatory EPSPs to MNs. To simulate a lesion, we reduced the number of supraspinal fibers until remaining inputs did not suffice to produce MNs action potentials. We calculated the impact of SCS parameters (frequency and amplitude) on the ability of residual supraspinal inputs to produce MNs activity during SCS.

In the SCS parameter space, we identified three regimes that we termed sub-threshold, near-threshold and supra-threshold. In the sub-threshold regime, SCS alone did not produce action potentials in the MNs. Even when residual supra-spinal inputs were added, MNs still did not produce action potentials, or firing rates were too low to generate movement (<2 Hz). In the near-threshold regime, SCS produced no or sparse firing. However, when concurrent residual supraspinal input was present, MNs showed sustained firing rates (8 to 20 Hz). In the supra-threshold regime, SCS dominated MNs firing rates irrespective of supraspinal inputs.

Counterintuitively, in the near-threshold regime, MNs firing rates were still dominated by SCS. Indeed, supraspinal inputs modulated MNs membrane potential, turning subthreshold SCS-EPSPs into suprathreshold events.

Our results are consistent with animals and humans experiments showing that force and kinematics are modulated by SCS frequency. We believe that these outcomes can provide a practical guidance for the design of novel stimulation protocols that enhance the efficacy of SCS in patients with motor paralysis.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.07

Topic: C.09.Stroke

Title: Direct electrical stimulation of the motor thalamus augments speech production following stroke

Authors: *E. M. GRIGSBY^{1,2}, A. DAMIANI^{1,2}, J. C. HO^{1,3}, J. BELKHIR^{3,6,7}, J. BARRIOS-MARTINEZ⁴, B. Z. MAHON^{4,6,7}, D. J. CRAMMOND⁴, M. CAPOGROSSO^{1,4,5}, J. A. GONZALEZ-MARTINEZ⁴, E. PIRONDINI^{1,2};

¹Rehab Neural Engin. Labs, ²Physical Med. & Rehabil., ³Sch. of Med., ⁴Neurolog. Surgery, ⁵Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; ⁶Neurosci. Inst., ⁷Psychology, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Stroke is a leading cause of permanent motor disability in the United States, often resulting in the loss or impairment of speech production. The inability to effectively communicate affects about half of acute stage and a third of chronic stage stroke patients and can result in greater isolation and accelerated deterioration of patients' health. While there have been promising developments in neurostimulation for limb motor deficits, speech therapy remains the gold standard for language deficits despite its limited impact on more severe cases. Interestingly, previous studies noted an array of secondary effects on speech when applying deep brain stimulation (DBS) to the motor thalamus to improve essential tremor. We posit that thalamic stimulation improves speech by facilitating primary motor cortex. We present our preliminary results using targeted DBS of motor thalamus to improve speech and vocalization. We performed acute stimulation studies in participants (n=4) implanted with either temporary (n=3) or permanent (n=1) multichannel depth/DBS electrodes in the ventralis intermediate nucleus (VIM) and ventro-oralis posterior (VOP) nuclei of the motor thalamus. Two subjects had histories of chronic seizures, but no impairment of speech or stroke. Two subjects had chronic motor symptoms from stroke, resulting in mild and severe speech impairments, respectively. The participants completed speech-therapy exercises to measure their face muscle strength and articulation. To quantify subjects' performance, we collected video and audio recordings, and surface EMG recordings from jaw, cheek, and neck muscles. In the facial motor task, subjects rapidly moved between instructed and neutral facial expressions. In the speech tasks, subjects recited two-word "tongue-twister" phrases or single words composed of commonly impaired phonemes. All tasks were repeated with and without thalamic stimulation. In all subjects, we observed larger amplitude and more consistent facial movements during VIM/VOP stimulation. In the speech tasks, 50 Hz stimulation produced increased duration of the speech envelope plateau, decreased pitch variability, and increased frequency resonance. This resulted in improved sustained sound, clearer speech, and cleaner phoneme separation, or more refined articulation. When stimulating the stroke participants, we observed a reduction in consonant slurring and, in the mildly impaired subject, we observed a significant decrease in the number of errors in the "tongue-twister" task. These preliminary results provide promising evidence that some aspects of speech production may be improved with stimulation of the motor thalamus.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.08

Topic: C.09.Stroke

Support: R01NS105318
Hamot Foundation

Title: Unveiling Thalamo-Cortical Interactions in Focal Epilepsy: Neuromodulation of the Motor Thalamus for Seizure Suppression

Authors: *J. A. GONZÁLEZ-MARTÍNEZ¹, A. DAMIANI^{2,3}, E. M. GRIGSBY^{2,3}, J. C. HO^{2,4}, L. LIANG^{2,5,6}, S. BORGOGNON^{5,6}, E. R. OBY^{5,6}, A. P. BATISTA^{5,6}, M. CAPOGROSSO^{1,2,5}, E. PIRONDINI^{2,3,5};

¹Neurolog. Surgery, ²Rehab Neural Engin. Labs, ³Physical Med. & Rehabil., ⁴Sch. of Med., ⁵Bioengineering, ⁶Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The current standard of care for medically refractory epilepsy (MRE) relies on the focal resection of the epileptogenic zone. However, resection of non-associative cortical areas can lead to devastating neurological consequences as motor deficits. Here, neuromodulation is an alternative. Previous studies have provided evidence of the involvement of subcortical structures in the organization of epileptiform activity. However, our understanding of specific changes in thalamocortical communication during focal motor seizures is currently inadequate. Here, we aim to characterize the dynamic relation between thalamic nuclei and seizure activity in the motor cortex. We posit that deep brain stimulation (DBS) of the motor thalamus will result in cortical activity desynchronization and decrement of epileptiform activity in rolandic cortical areas. In acute experiments, we implanted a multichannel depth electrode in the ventro-oralis posterior (Vop) nucleus of the thalamus in two anesthetized nonhuman primates. We simultaneously stimulated Vop at varying frequencies (from 1 to 200 Hz) and recorded neural activity from the ipsilateral motor cortex (M1). Importantly, one animal expressed chronic spontaneous epilepsy characterized by focal clonic motor seizures in the right forelimb. The intraoperative electrocorticography demonstrated the presence of repetitive polyspikes located in the left motor and pre-motor cortex. Following low-frequency Vop stimulation, we identified clear evoked potentials in ipsilateral M1, thought to reflect orthodromic monosynaptic neurotransmission. Interestingly, in the epileptic animal, we observed synchronous thalamocortical connectivity enhanced during the ictal phase. The thalamic recording showed a slow electrical potential drift accompanied by an enhancement of high frequency activity power simultaneous with the occurrence of epileptiform cortical activity. Additionally, we observed a frequency-dependent suppression of high-frequency cortical discharges with Vop stimulation. Attenuation of the increased epileptic M1 activity started after 50 Hz stimulation, reaching an almost complete suppression at 200 Hz. These results suggest a potential neuromodulatory effect of high-frequency thalamic DBS in seizure suppression. Overall, results provide evidence of the electrophysiological interaction among cortical and subcortical areas. Moreover, we demonstrated the suppression of epileptiform activity with high-frequency stimulation of the motor thalamus. Future studies in animal models of focal epilepsy will provide further data to validate our hypothesis.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

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NSF CAREER Award (Grant number: 2145321)

Title: Effects of myoelectric signal-guided exercises on cortico-muscular and inter-muscular coherence of synergistic upper-limb muscles

Authors: M. HOUSTON¹, G. SEO¹, J. PARK², F. FANG¹, H. PARK², J. ROH¹, *Y. ZHANG¹;
¹Univ. of Houston, Houston, TX; ²Korea Advanced Inst. of Sci. & Technol., Daejeon, Korea, Republic of

Abstract: Diseases negatively impacting cortico-spinal integrity such as stroke ultimately precede abnormal inter-muscular coordination, requiring motor rehabilitation to hopefully regain some level of motor control. We have previously shown the feasibility of inducing new inter-muscular coordination patterns in neurologically healthy persons (Seo et al., 2021) and that stroke patients exhibit reduced levels of inter-muscular connectivity compared to neurologically healthy individuals (Houston et al., 2021). However, the effects of these newly induced inter-muscular coordination patterns in cortico-spinal connectivity are unknown. This study aims to determine for the first time the feasibility of producing exercise-induced changes to cortico-muscular and inter-muscular connectivity of synergistic upper-limb muscles via myoelectric signal-guided exercises. Young, neurologically healthy participants (N=6) underwent a 6-week novel motor-training protocol designed to de-couple two previously identified synergist muscles (Roh et al., 2013): brachioradialis (BRD) and biceps brachii (BI). Participants were asked to perform 2D isometric force target matching in the cardinal leftwards and upwards directions using newly trained muscle recruitment strategies to assess the training effects. Concurrent whole-head electroencephalographic and surface electromyographic signals from BRD and BI muscles were analyzed via cortico-muscular (CMC) and inter-muscular coherence (IMC) to assess the exercise-induced changes in connectivity. Average CMC and IMC values from alpha (8-15 Hz) and beta (15-30 Hz) frequencies were compared pre- vs. post-training via paired-sample t-tests. Statistical group analyses indicated a significant reduction in alpha-band IMC between BRD-BI ($p < 0.05$) and a significant reduction in alpha-band CMC between P3-BRD ($p < 0.05$) post-training for the up-target condition. Results suggest that de-coupling two upper-limb synergist muscles during isometric conditions is associated with training-induced changes in alpha-band connectivity involving the contralateral somatosensory cortex and the synergist

muscles. Further studies can expand upon these findings by testing on neurologically impaired persons suffering from cortico-spinal damage such as stroke patients and spinal cord injury patients with the purpose of assessing changes in connectivity associated with motor rehabilitation designed specifically to mitigate aberrant inter-muscular coordination.

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Poster

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Support: AHA Scientist Development Grant (Grant number: 17SDG33670561)
NSF CAREER Award (Grant number: 2145321)

Title: Developing new intermuscular coordination patterns in the stroke-affected upper extremity through an electromyographic signal-guided training: a pilot study

Authors: G. SEO¹, J.-H. PARK², S. LI^{3,4}, H.-S. PARK⁵, **J. ROH**¹;

¹Biomed. Engin., Univ. of Houston, Houston, TX; ²Mechanical Engin., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of; ³Physical Med. and Rehabil., Univ. of Texas Hlth. Sci. Ctr. - Houston, Houston, TX; ⁴TIRR Mem. Hermann Res. Ctr., TIRR Mem. Hermann Hosp., Houston, TX; ⁵Mechanical engineering, Korea Advanced Inst. in Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Stroke induces major functional motor impairments including disrupted intermuscular coordination, decreasing the quality of activities of daily living (ADLs) in the upper extremity (UE). Previous studies have quantified characteristic activation patterns of a group of muscles (i.e., muscle synergies) to identify impaired UE intermuscular coordination after stroke. Also, our previous study validated the modifiability of naturally expressed intermuscular coordination patterns of young able-bodied adults in UE. However, it is still unclear whether developing newly emerging intermuscular coordination patterns is feasible via a noble exercise and may improve motor function after stroke. In this study, as a continuation of our previous work, we tested the feasibility of inducing new intermuscular coordination in stroke and its resultant changes in UE motor function. Two chronic stroke survivors with mild-to-moderate motor impairment and two age-matched, able-bodied individuals participated in a six-week isometric electromyographic (EMG) signal-guided training to activate two major synergists, biceps and brachioradialis, independently. At weeks zero, one, two, four, and six of the training, the participants performed an isometric reaching in a virtual three-dimensional force space to assess

any potential changes in muscle synergies in the trained arm. EMGs of 12 UE muscles and end-point forces generated at hand were recorded simultaneously during the assessment and the training. A non-negative matrix factorization (NMF) algorithm was applied to the recorded EMGs to identify muscle synergies. Our preliminary results showed that both stroke and able-bodied groups could activate the targeted elbow flexor muscles, which were naturally co-activated, in isolation from each other and develop new intermuscular coordination patterns through the training. These newly emerged synergies were expressed if the participants intended to use the newly learned motor skill. The participants still could use their habitual intermuscular coordination patterns throughout and after the exercise regardless of their acquisition of the new motor skill. After the training, both groups showed improvement in the accuracy and efficiency of the motor control of the trained arm. Specifically, enhancement of the functional movement of the impaired arm, such as wrist rotation and arm lifting, was observed in the stroke group. These findings suggest that our isometric exercise protocol has the potential to benefit stroke survivors' performance in ADLs and, eventually, their quality of life by increasing their repertoire of intermuscular coordination.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

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Topic: C.09.Stroke

Support: NIH # P2CHD086844
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VA

Title: Magnetic and electrical stimulation to assess descending motor pathway connectivity in severe upper limb hemiparesis

Authors: M. E. STOYKOV^{1,2}, C. BUTLER^{1,2,3}, G. F. WITTENBERG⁴, C. J. WINSTEIN⁵, E. ROTH^{1,2}, M. A. PEREZ^{1,2,6};

¹Shirley Ryan AbilityLab, Chicago, IL; ²Dept. of Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; ³Dept. of Biomed. Engin., Northwestern Univ., Evanston, IL; ⁴Neurol., Univ. of Pittsburgh, Pittsburgh, PA; ⁵Div. Biokinesiol/Physical Ther, USC, Los Angeles, CA; ⁶Edward Hines Jr., VA Hosp., Hines, IL

Abstract: Background: A critical clinical question in stroke rehabilitation is which individuals have recovery potential. There is evidence that individuals post-stroke with motor evoked potentials (MEP+) in upper limb muscles, elicited by transcranial magnetic stimulation (TMS) over the primary motor cortex, are more likely to benefit from neuroplasticity interventions compared with individuals without MEPs (MEP-). However, this assumption has been

challenged by studies showing that individuals with stroke who are MEP+ and MEP- experience clinically meaningful changes after a neuroplasticity intervention. In our study, we tested the presence or absence of MEPs elicited by TMS as well as cervicomedullary electrical stimulation. Due to differences in the temporal dispersion of descending volleys elicited by TMS and electrical stimulation of the corticospinal pathway, we hypothesized that a greater number of motor evoked potentials would be observed in upper limb muscles of individuals with stroke during electrical stimulation compared with magnetic stimulation. **Methods:** Ten individuals in the chronic post-stroke phase previously categorized as MEP(-) during TMS were included. Active MEP status of the affected biceps brachii (BB), extensor carpi radialis (ECR), and first dorsal interosseous (FDI) muscles were determined using single pulse TMS (Magstim, 200, UK) and the protocol documented by Stinear et al (2017). Motor evoked potentials at the cervicomedullary junction (CMEPs) were collected during voluntary movement using high-voltage electrical stimulation (Digitimer DS7R) passed between two small gold-cup electrodes placed behind the mastoid process at the cervicomedullary junction. Background EMG in both conditions were compared using paired t-tests. **Results:** We found that 9 of the 10 individuals were categorized as CMEP+ in all muscles tested when responses were elicited by electrical stimulation. With TMS, only 2 individuals were categorized as MEP+ and only in one of the three muscles tested. Note that mean background electromyographic activity was maintained at a similar level when MEPs were tested using magnetic and electrical stimulation in all muscles tested. **Conclusions:** Our results suggest that CMEPs elicited by electrical stimulation of corticospinal axons represent a more sensitive tool to detect residual descending motor pathway connectivity compared with TMS in humans with chronic stroke.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

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National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NRF-2018R1D1A1A02086038)

Title: Could cerebellar rTMS enhance the effects of high-frequency rTMS over M1 in stroke patients?

Authors: *W. CHANG¹, S.-M. BAIK², H.-J. NA², H. KIM¹;
¹Samsung Med. Ctr., ²Samsung Med. Ctr., Seoul, Korea, Republic of

Abstract: Introduction: The facilitating affected primary motor cortex (M1) with repetitive transcranial magnetic stimulation (rTMS) could improve motor function in stroke patients. However, it has a lack of clinical routine application because there was a relative small effect size to improve motor function. One of important principle in stroke motor rehabilitation is based on the motor learning theory. Recently, many articles have reported that that plasticity at synapses in the cerebellum (Cbll) mediates motor learning, especially, motor learning consolidation. Therefore, Cbll might be one of target of non-invasive brain stimulation for motor rehabilitation in stroke patients. The objectives of this study was to investigate the additional effects of Cbll rTMS on motor recovery of facilitatory rTMS in subacute stroke patients. Methods: Twenty-two subacute hemiplegic stroke patients (mean age 68.4 yrs) were recruited in this single-blind randomized controlled study. All participants showed the response in transcranial magnetic stimulation-induced motor evoked potentials (TMS-induced MEPs) of affected M1. Each participant in the Cr-Cbll group was received the Cr-Cbll rTMS stimulation consisted with the high-frequency rTMS over the ipsilesional M1 (10 min), motor task with fine motor training (10 min), and high-frequency rTMS over the contralesional Cbll (10 min). Each participant in the Cr-sham group was received the Cr-sham rTMS stimulation consisted with sham rTMS over Cbll instead of high-frequency rTMS over Cbll. Ten daily sessions were conducted for 2 weeks in all participants. The total, upper, and lower scores of Fugl-Meyer Assessment (FMA) were measured before (T0), and immediately after the intervention for 2 weeks (T1). Results: There was no significant adverse effects in all participants. Total 16 participants (8 in Cr-Cbll group and 8 in Cr-sham group) performed the Cr-Cbll rTMS intervention for 2 weeks. There was no significant difference in the general and clinical characteristics at T0 between the two groups. The scores of total, upper and lower FMA were significantly improved after the intervention in each Cr-Cbll and Cr-sham group ($p < 0.05$). At T1, the improvements of each score of FMA in the Cr-Cbll group tended to be higher than in the Cr-sham group without statistical significance. Conclusion: These results revealed the potentials of the Cbll rTMS for enhancing facilitatory rTMS for improving motor function in subacute stroke patients. However, further study with a large number of participants will be needed to clarify this hypothesis.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

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Topic: C.09.Stroke

Support: DAAD scholarship

Title: Measuring the effect of rTMS therapy on wrist spasticity and motor function in chronic stroke subjects using an objective device

Authors: *W. MAHMOUD¹, A. RAMOS MURGUIALDAY², H. R. HULTBORN³, U. ZIEMANN⁴;

¹Univ. of Tuebingen, Tuebingen, Germany; ²Univ. of Tubingen, Tubingen, Germany; ³Dept of Neuroscience, Copenhagen Uni, Dept. of Neurosci., Copenhagen, Denmark; ⁴Eberhard Karls Univ., Eberhard Karls Univ., Tübingen, Germany

Abstract: Introduction: Beside paresis, stroke leads to the development of abnormal responses to passive muscle stretch (Burke, 1988) which includes both neurologic (spasticity and spastic dystonia) and non-neurologic (muscle and soft-tissue contracture) components (Lorentzen et al., 2010). The use of repeated transcranial magnetic stimulation (rTMS) for stroke rehabilitation has a positive effect both on motor function and spasticity (Lefaucheur et al., 2020; McIntyre et al., 2018). Establishing therapeutic effects of rTMS on spasticity requires the quantification of the neurologic components of response to passive stretch, but the clinically used modified Ashworth Scale (MAS) is unable to do that. A hand-held dynamometer (Portable Spasticity Assessment Device (PSAD)) has been developed, which integrates electromyography (EMG) with joint movement and applied torques (Lorentzen et al., 2012; Yamaguchi et al., 2018) to enable the objective quantification and discrimination of neural and non-neural aspects. In this study, we investigated the validity and reliability of the PSAD device to measure spasticity in the wrist joint in stroke patients. Then, we investigated the therapeutic effects of rTMS therapy on motor function and spasticity in the upper limb in these subjects. **Methods:** 57 subjects (57±11y, 12 females) with a haemorrhagic or an ischaemic stroke for at least six months (46±53 months) were included. All Subjects received (~15 sessions, 3 session/week) of rTMS and were assessed before and after the end of the intervention both the affected and the non-affected sides, twice. Clinical measures included the PSAD, MAS and Fugl-Meyer Assessment scale (FMA). **Results: Reliability:** Intraclass correlation coefficient of absolute agreements between two measures of the same experimenter showed a high reliability ($r > 0.92$) for all the PSAD extracted parameters (0.92-0.99). **Validity:** The paired samples T test between the affected and non-affected sides showed a significant difference on all measured parameters. Spearman correlation analysis showed a significant correlation between each of the PSAD parameters and the MAS $r = (0.43$ and $r = 0.68)$. **Therapeutic effect:** the repeated measures ANOVA showed a significant effect of the rTMS intervention on the motor function measured by the FMA ($F_{1,76} = 117$, $p < .001$), and the MAS ($F_{1,71} = 19.2$, $p < .001$). The reflex-mediated stiffness measured with the PSAD was significantly reduced after the intervention ($F = 8$, $p = .007$).

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Poster

458. Neuromodulation Strategies for Stroke Recovery

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Topic: C.09.Stroke

Support: NRF Grant 2020R1A2C3010304
NRF Grant 2017M3A9G5083690

Title: Effect of high-definition transcranial direct current stimulation simultaneously applied with robotic-assisted gait training in chronic stroke patients

Authors: *E. KIM^{1,2}, S.-H. LEE¹, J. KIM¹, G. LEE¹, S. SHIN¹, Y.-H. KIM^{1,2,3};
¹Samsung Med. Ctr., Seoul, Korea, Republic of; ²Dept. of Med. Device Mgmt. and Res., ³Dept. of Hlth. Sci. and Technology, Dept. of Digital Hlth. Sungkyunkwan Univ., Sungkyunkwan Univ. (SAIHST), Seoul, Korea, Republic of

Abstract: Gait disorder induced by one of the most common aftereffects after stroke persists through the chronic phase and significantly impacts on quality of life after stroke. Improving the gait ability is one of the primary goals of stroke rehabilitation. The purpose of this study was to investigate whether the training effect of robotic-assisted gait training (RAGT) for chronic stroke patients can be further enhanced by simultaneously applied high-definition transcranial direct current stimulation (HD-tDCS). Twenty-four chronic hemiplegic stroke patients (15 males; mean age 60.54 ± 13.90 years) participated. The subjects were randomly allocated to either the RAGT with real HD-tDCS group or RAGT with sham HD-tDCS group. Each group completed ten intervention sessions (for 45 minutes) during the consecutive 4 weeks. Gait and physical functions were measured by the 10-Meter Walk Test (10MWT), Timed Up and Go (TUG), Functional Reach Test (FRT), Berg Balance Scale (BBS), Dynamic Gait Index (DGI), Fugl-Meyer Assessment (FMA), and Korean Version of Modified Barthel Index (K-MBI). All assessments were performed before intervention (Pre), immediately after intervention (Post), and 1 month follow-up after intervention (F/U). The RAGT with real HD-tDCS group showed significant improvement at Post and F/U in the 10MWT, TUG, FRT, and BBS ($P < 0.05$, $P < 0.01$) compared to Pre, but not in the RAGT with sham HD-tDCS group. There were significant improvements in DGI and FMA at Post compared to Pre only in the RAGT with real HD-tDCS group ($P < 0.05$, $P < 0.01$). The repeated measures ANOVA revealed significant time \times group interactions in K-MBI and FMA ($P < 0.05$), indicating that the RAGT with real HD-tDCS group had greater improvement than the RAGT with sham HD-tDCS group. These results demonstrated that the simultaneous application of RAGT and HD-tDCS had a positive effect on improving gait and physical functions of chronic stroke patients. In addition, HD-tDCS ensured long-term training effects maintained for up to 1 month. We conclude that HD-tDCS can be suggested as a complementary tool for enhancing robotic gait rehabilitation treatment in chronic stroke patients after confirming these effects by a larger confirmatory study.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

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Topic: C.09.Stroke

Support: KAIST-funded Global Singularity Research Program for 2022 (N11220050)

Title: Development and validation of virtual reality-based hand rehabilitation system using video game and vibration feedback

Authors: *S. BAE, H.-S. PARK;
Korea Advanced Inst. in Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Grasping and manipulating objects using hands is one of the unique features that allow human-being to use various tools compared to other species. However, neurological disorders such as stroke contribute significantly to the loss of hand function, which limits the ability to participate in activities of daily living and reduces the quality of life. Although high-intensity, repetitive, goal-directed rehabilitation training has been reported as an effective way to recover from the loss of hand function, it is hard to be achieved without the high engagement of participants. Since the degree of engagement and attention are closely associated with functional recovery after rehabilitation, it is required to find a way to effectively induce and maintain engagement. The application of virtual reality and video game in the field of rehabilitation has been proven to be effective in motor recovery by inducing the attention of stroke survivors. Also, the inclusion of vibration feedback within the virtual reality environment affects immersion and induces much attention. In this study, we developed a virtual reality-based hand rehabilitation system using a video game and vibration feedback. After wearing the virtual reality head-mounted display, participants had to make the same gestures as the approaching gesture target which is customized based on their hand condition. If the participant hit the target with an appropriate gesture at a precise time, the target explodes and they receive the vibration feedback on their wrist with visuoauditory feedback. For validation of the hand rehabilitation system, 11 healthy subjects were recruited and cortical activation was measured by functional near-infrared spectroscopy during the experiment. The experiment consisted of 12 tasks and 13 rests session based on the block design paradigm. The duration of rest and task blocks is 20 seconds each. As a result of group analysis, significant activation was observed in the hand area of the primary motor and somatosensory cortex of both hemispheres. In addition, we observed activation in the prefrontal cortex which plays a key role in the switching attentional control and cognitive process, and the premotor cortex, a representative area of motor planning. Also, the effect of multisensory feedback consisting of visual, auditory, and vibrotactile feedback was confirmed by activation of the somatosensory association cortex, a central area of multisensory integration. In the future, the effectiveness of our virtual reality-based hand rehabilitation system will be further investigated through clinical trials for people with neurological diseases.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

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Topic: C.09.Stroke

Support: VA RR&D Merit Review I01RX-003056-01

Title: A hybrid exoskeleton for stroke survivors improves joint kinematics and gait speeds

Authors: ***M. N. FITZPATRICK**^{1,2}, **H. V. HEIDECKER**^{1,2}, **M. L. AUDU**^{1,2}, **R. D. QUINN**¹, **R. J. TRIOLO**^{1,2}, **N. S. MAKOWSKI**^{1,2,3};

¹Case Western Reserve Univ., Cleveland, OH; ²Louis Stokes Cleveland Veterans Affairs Med. Ctr., Cleveland, OH; ³MetroHealth Med. Ctr., Cleveland, OH

Abstract: Poststroke motor control impairments result in kinematic gait deviations at the hip, knee and ankle. Impairments can result in slower walking speeds, an increased risk of falls, and limited balance. We have developed a unilateral, hybrid exoskeleton combining non-invasive neural stimulation and a motorized knee orthosis to correct these deficits and enhance mobility and independence after stroke. This study evaluates the feasibility of prioritizing muscle activation and having the motor serve as a backup to assist as needed. The device was evaluated on an ambulatory stroke survivor to determine the impact on walking. Surface stimulation was applied to the quadriceps, hamstrings, gastrocnemius, and tibialis anterior in coordination with the gait cycle while the motor remained inactive. This approach enables assessing whether stimulation can generate the desired motions even in the presence of passive resistance from the exoskeletal motor. The participant completed a series of 10m walks at baseline and then again during the training and controller development process with the hybrid neuroprosthesis. Walking speed and step length were compared to determine the overall impact on walking while peak knee flexion during swing and knee extension at heel strike were measured by onboard sensors to assess the impact of multi-joint surface stimulation on gait kinematics. The participant's walking speed increased from 0.94m/s without the device to 1.1m/s with the device. Average step length increased from 0.53m to 0.67m. Without assistance, he had limited knee flexion during swing and had difficulty maintaining knee extension in terminal swing. Stimulation enabled knee flexion of 61.6 (\pm 4.9) degrees during swing and generated -3.4 (\pm 1.2) degrees of flexion at heel strike. The participant reported that walking with the device felt more natural than walking without it. These results suggest that the hybrid neuroprosthesis can improve stroke survivors walking ability and that it is feasible to primarily drive movements through muscle contraction so that the motor can assist as needed. Ongoing assessments include evaluating the device's potential as a therapeutic tool and as an assistive device.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.17

Topic: C.09.Stroke

Support: Research Fellowship Program (RFP) 19002, Singapore

Title: Robot-assisted Active Somatosensory Retraining of Upper Limb Stroke - a Preliminary Finding

Authors: *A. SIDARTA¹, Y. C. LIM¹, C. ZENG², W. K. KUAH CHRISTOPHER³, Y. J. LOH³, W. T. ANG¹;

¹Nanyang Technological Univ., Singapore, Singapore; ²St Luke's Hosp., Singapore, Singapore;

³Tan Tock Seng Hosp., Singapore, Singapore

Abstract: Prior studies have established that the limb position and movement senses in space are important for motor control and learning. In stroke rehabilitation, it has been estimated that half of the stroke patients demonstrate impaired ability to sense their movements. Despite the high prevalence of such impairment, somatosensory retraining focusing on proprioceptive and kinaesthetic senses is often overlooked. Tasks that simultaneously target motor and somatosensory aspects are thought to be beneficial for relearning somatosensory functions and increasing mobility of the affected limb concurrently. Recently, robotic technology has been incorporated into stroke rehabilitation for a more controlled therapy in terms of intensity, duration, and frequency. The current work presents an evidence-based program that aims to retrain upper limb somatosensory and motor functions of stroke survivors using a compact table-top robotic device. Fifteen robotic training sessions were conducted over a 5-week period, with each session lasting approximately an hour. The training involved reaching tasks in the form of an interactive game. Participants performed a point-to-point planar movement while holding on to a robotic handle with the view of the forearm blocked. Once the handle reached the target, the robot would bring the system back to the start position. Robot-generated haptic guidance was provided along the movement path as somatosensory cues while participants actively moved towards the target location. Audio-visual feedback was given following every successful movement as a reward. Baseline, post-day 1, and post-day 30 assessments were conducted, where the last two sessions were done after the last training day. Robotic-based performance indices and clinical assessments of upper limb functions after stroke were used to acquire outcome measures respectively. Data from N = 6 participants who have fully completed the program were analysed and reported herewith as preliminary results. Throughout the training sessions, all participants showed a gradual improvement in accuracy. When assessed, movement accuracy and joint position matching performances were also enhanced as compared to the baseline. In contrast, clinical scores showed inconclusive outcomes, suggesting that the robotic-based assessment was able to capture improvements at a higher resolution. We observed the presence of an endpoint drift that was almost consistently found in all participants. The recruitment is still happening at local rehabilitation centres. The outcomes of this study will provide preliminary evidence and help inform the translational aspect of the proposed exercise.

Disclosures: A. Sidarta: None. Y.C. Lim: None. C. Zeng: None. W.K. Kuah Christopher: None. Y.J. Loh: None. W.T. Ang: None.

Poster

458. Neuromodulation Strategies for Stroke Recovery

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Program #/Poster #: 458.18

Topic: C.09.Stroke

Support: National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NRF-2018R1D1A1A02086038)
Grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) by the Ministry of Health Welfare, Republic of Korea (grant number : HR21C0885)

Title: Additional effects of cerebellar rTMS on inhibitory rTMS over M1 in stroke patients

Authors: *S.-M. BAIK¹, W. CHANG², H.-J. NA¹, H. KIM³;
¹Samsung Med. Ctr., ²Physical and Rehabil. Med., ³Samsung Med. Ctr., Seoul, Korea, Republic of

Abstract: Introduction: The most common strategy with repetitive transcranial magnetic stimulation (rTMS) to improve motor function in stroke patients is inhibiting the unaffected primary motor cortex (M1). Although this rTMS strategy has been known as a promising therapeutic tool, there was a relatively small effect size to improve motor function in stroke patients. Recently, many articles have reported that plasticity at synapses in the cerebellum (Cbll) mediates motor learning, especially, motor learning consolidation. According to these researches, Cbll might be one of targets of rTMS for motor recovery in stroke patients. In this study, we aimed to investigate the enhancing effects of Cbll rTMS on inhibitory rTMS for motor recovery in subacute stroke patients. **Methods:** Twenty-three subacute hemiplegic stroke patients who showed no response in transcranial magnetic stimulation-induced motor evoked potentials (TMS-induced MEPs) of affected M1 were recruited in this single-blind randomized controlled study. Each participant in the Cr-Cbll group received the Cr-Cbll rTMS stimulation consisting of the continuous theta burst stimulation (cTBS) over the contralesional M1 (40 secs), a motor task with shoulder mobilization exercise (10 min), and high-frequency rTMS over the contralesional Cbll (10 min). In addition, each participant in the Cr-sham group was received the Cr-sham rTMS stimulation consisting of sham rTMS over Cbll instead of high-frequency rTMS over Cbll. Ten daily sessions were conducted for 2 weeks on all participants. The total, upper, and lower scores of Fugl-Meyer Assessment (FMA) were measured before (T0), immediately after (T1), and 2 months after the intervention (T2). **Results:** Total of 21 participants (9 in Cr-Cbll group and 12 in Cr-sham group) performed the Cr-Cbll rTMS intervention for 2 weeks and were assessed at T2. There was no significant difference in the general and clinical characteristics at T0 between the two groups. The scores of the total, upper and lower FMA were

significantly improved after the intervention in each Cr-Cbll and Cr-sham group ($p < 0.05$). At T1, each score of FMA in the Cr-Cbll group tended to be higher than in the Cr-sham group without statistical significance. However, there was a significant difference in both total and upper FMA at T2 between the two groups ($p < 0.05$). **Conclusion:** These results demonstrated that the Cbll rTMS might have additional effects on inhibitory rTMS over contralesional M1 for improving motor function in subacute stroke patients.

Disclosures: **S. Baik:** None. **W. Chang:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NRF-2018R1D1A1A02086038), Grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) by the Ministry of Health Welfare, Republic of Korea (grant number : HR21C0885). **H. Na:** None. **H. Kim:** None.

Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.19

Topic: C.09.Stroke

Title: A closed-loop electromyography-controlled functional electrical stimulation system for stroke rehabilitation.

Authors: M. DARROW, D. GABRIELI, L. WENGERD, I. BAUMGART, D. FRIEDENBERG, *E. MEYERS;
Battelle Mem. Inst., Columbus, OH

Abstract: In the United States, approximately 5 million people are living with hemiparesis that limits their functional independence. Functional electrical stimulation (FES) devices have been recognized for decades as a promising means to restore hand function after stroke. A growing body of literature has shown that synchronized activation of descending motor commands and ascending sensory feedback from FES is critical for effectiveness. However, current FES devices are externally controlled by a therapist and do not guarantee coupling between motor intention and FES, and suffer from a range of other limitations including a limited number of controllable movements and extensive setup time. Battelle has recently developed the NeuroLife Sleeve: an experimental wearable garment like a compression sleeve worn on the forearm, with 150 embedded electrodes for bidirectional neural interfacing. The sleeve operates in the following way: 1) muscle activity is recorded through high-density surface electromyography (EMG), 2) advanced machine learning algorithms decode muscle activity to decipher motor intention, and 3) FES is delivered to facilitate the intended movement. Our system tightly couples motor intention with evoked movement to enhance neuroplastic change and improve responsiveness to

therapy. We have shown that three participants can effectively use the sleeve to volitionally control their paretic arm simply by attempting the desired movement (e.g., hand open or hand close) during functional rehabilitation. The sleeve can evoke movements critical for functional task practice that are central to activity-based therapy used in clinical practice. Using the system, the participants could successfully perform tasks by manipulating object position and orientation with their paretic arm, and these tasks were challenging or impossible to complete without the system. During a recently completed rehabilitation study, two participants with moderate hemiparesis due to stroke underwent 8 weeks of FES therapy with the NeuroLife Sleeve 3 times per week. Following therapy, both patients demonstrated clinically meaningful improvements in both the Action Research Arm Test (ARAT) (9 ± 3 pts) and Fugl-Meyer Assessment - Upper Extremity (FMA-UE) assessments (10 ± 1 pts). Improvements lasted for 10 weeks after the cessation of therapy (ARAT: 13 ± 1 ; FMA-UE: 8.5 ± 0.5), suggesting that the functional benefits are driven by long-lasting neuroplastic change. These results position the NeuroLife Sleeve as a promising stroke therapeutic, and future studies will investigate these therapeutic effects in a larger patient population.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.20

Topic: C.09.Stroke

Support: NIH R01NS099210
NIH R01NS112942

Title: Improving arm function in chronic stroke using home-based training with a wearable myoelectric interface for neurorehabilitation

Authors: A. KHORASANI¹, V. PAUL¹, J. HULSIZER¹, N. HUNG¹, P. PRAKASH¹, *M. W. SLUTZKY²;

²Northwestern Univ., ¹Northwestern Univ., Chicago, IL

Abstract: Abnormal muscle co-activation (a.k.a. abnormal synergies) is a significant cause of arm impairment after stroke. Previously, we showed that in-lab training with a myoelectric interface for neurorehabilitation (MINT) can reduce this co-activation and also arm impairment in chronic stroke survivors. We are now testing a wearable version of MINT conditioning in a randomized, sham-controlled trial in severely impaired, chronic patients. MINT maps EMGs to orthogonal components of cursor movement, which participants use to control customized computer games that operantly condition them to reduce co-activation. Here, participants were randomized to one of four groups, based on how they trained: 2 muscles at a time without (2D)

or with (Reach) prompts to reach in the direction of the muscle in each trial, 3 muscles at a time (3D), or one muscle at a time (sham control). Participants trained 5 d/wk at home and 1 d/wk in lab for 6 weeks. To date, 42 participants have completed training and achieved 299 ± 8 repetitions/d, testifying to high enjoyment and motivation. Muscle co-activation was reduced by participants (2D and Reach) during MINT conditioning by $86 \pm 23\%$. At 6 weeks, our primary clinical outcome, the timed Wolf Motor Function Test (WMFT), showed a trend toward more improvement in all experimental groups combined than in sham controls (means -4.1 vs -2.0 s, $p=0.2$, t-test), and the 3D group showed a stronger trend (-7.3 s, $p=0.10$). At 4 weeks post-training, significant improvement compared to sham was seen (-12.9 s in 3D, $p=0.01$; -8.5 s in combined experimental vs. -1.9 s sham, $p=0.02$). Importantly, WMFT improved significantly in severely impaired participants at 4 weeks post-training in combined experimental groups compared to sham ($p=0.006$). There was a trend toward increased active range of motion of the wrist relative to the shoulder after training during a separate reaching task, with increases compared to baseline of 6.5 cm in 3D ($p=0.06$), 3.8 cm in combined experimental groups ($p=0.2$) but a decrease of 5.9 cm in the sham group ($p=0.3$). Finally, muscle synergy analysis was applied to the multi-channel EMGs during the reaching task. The number of synergies did not change from baseline to week 6. However, MINT conditioning did increase the disparity between within-synergy weights of the muscle set with highest co-activation, suggesting that the CNS can make highly fractionated changes to muscle control even after stroke. Thus, MINT conditioning may improve arm movement, even in severely impaired stroke survivors, by reducing abnormal muscle co-activation.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.21

Topic: C.08. Ischemia

Support: The Tiny Blue Dot Foundation
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Title: The effect of non-invasive transcutaneous auricular vagus nerve stimulation (taVNS) on hypoxic-ischemic injury in newborn rats

Authors: *M. G. WILEY¹, C. S. ROBINSON², H. A. BOGER¹, D. D. JENKINS³, M. S. GEORGE^{4,5};

¹Neurosciences, ²Neurol., ³Pediatrics, ⁴Psychiatry and Behavioral Sci., Med. Univ. of South Carolina, Charleston, SC; ⁵Ralph H. Johnson VA Med. Ctr., Charleston, SC

Abstract: Background: Neonatal hypoxic-ischemic brain injury (HI) is an acute neurologic syndrome where decreased blood flow and oxygen to the brain causes acute and chronic brain dysfunction. The only neuroprotective intervention for HI is therapeutic hypothermia (TH), started within 6 hours of birth. 50% of survivors have long-term deficits. Preclinical adult ischemic brain injury models demonstrate that vagus nerve stimulation (VNS) has anti-inflammatory effects and attenuates brain damage. A non-invasive VNS, transcutaneous auricular VNS (taVNS), is safe and feasible in infants and may improve the motor skill of bottle feeding. We hypothesize that a combined TH-taVNS treatment shortly after HI birth will have neuroprotective effects, improve motor function, and attenuate infarct area compared to TH alone.

Methods: The +HI model includes ligation of the right common carotid artery (CCA) in postnatal day 7 (P7) rats, 2.5 h hypoxia (8% oxygen), and 2 h TH at 31°C. The sham surgery (-HI) groups included exploring the CCA without ligation, 2.5 h on room air, and 2 h at 36°C. We administered +taVNS per clinical protocol, 0.1mA below the perceptual threshold (PT) in awake rats. We used a bipolar electrode placed on the auricular concha region for 30min [30sec trains, 0.5msec duration, 20Hz frequency, followed by 4.5min breaks]. Sham taVNS (-taVNS) was administered at 0mA. Experimental groups included +HI/+taVNS, +HI/-taVNS, -HI/-taVNS, and -HI/+taVNS. We assessed reflexes and strength at P7 before surgery through 72 h post-surgery. The infarct area was evaluated at 72 h after HI-injury by staining coronal sections with 2,3,5-triphenyl tetrazolium chloride (TTC) staining.

Results: Sixty P7 rat pups (45 +HI and 15 -HI) underwent surgery with a 2.2% mortality rate at 72 h post-HI. taVNS was well tolerated by awake P7 rats, with an average PT of 0.59mA, ranging from 0.4 to 1.0 mA. There were no animals with infarcts in -HI groups. We analyzed the interaction of sex and taVNS on infarct size among the +HI groups with a mixed-effects model. Tukey posthoc revealed taVNS-treated +HI males had significantly smaller average infarct area compared to taVNS treated (p=0.039) and untreated (p=0.038) +HI females. All groups had similar neonatal reflexes and increasing strength over the 72hrs post-HI.

Conclusions: This study supports that taVNS is safe and feasible in awake neonatal rats and may reduce the risk of infarct in males after HI. While there is robust prior research on VNS in adult ischemic brain injury models, these first preclinical studies of a non-invasive brain stimulation technique in neonatal HI will provide important information for further development of taVNS in this population.

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Poster

458. Neuromodulation Strategies for Stroke Recovery

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 458.22

Topic: C.09.Stroke

Support: 1R01NS105 646-01A1
RC1MH090912-01
N660 01-12-C-4025
N66001-11-1-4013
1T32EB011434-01A1
T32GM008692
UL1TR000427

Title: Bci-fes intervention is associated with cognitive improvements in stroke survivors

Authors: *A. REMSIK¹, T. HOSSEINI², P. VAN KAN³, N. ADLURU⁴, V. A. NAIR⁵, J. C. WILLIAMS⁶, V. PRABHAKARAN⁷;

¹Univ. of Wisconsin-Madison, Mequon, WI; ²Radiology, ³Kinesiology, ⁴UW-Madison, Madison, WI; ⁵UW-MADISON, Madison, WI; ⁶Arizona State Univ., Tempe, AZ; ⁷Dept Neurosci, Univ. of Wisconsin, Madison, WI

Abstract: Objective Brain damage resulting from stroke can cause impairments in thinking and mood. Brain-Computer Interfaces (BCI) are used to treat survivors. Reinforced training of voluntary brain modulation requires meta-cognition. We sought to determine if BCI-FES intervention poststroke has a beneficial effect on stroke survivors' cognition and affect compared to a time matched control. Methods Participants (waitlist control n = 21, 9 female, mean age 59.4 years, \pm 14.2 years, and intervention n = 35, 16 female, mean age 62 years, \pm 12.8 years) completed <30 hours of multimodal BCI-FES training, were assessed with a battery of neuropsychological tests at baseline (M1), the mid-time-point of intervention (M2), and completion (M3). Group mean differences at completion of each phase (i.e., M3) were calculated and compared between treatment conditions using independent samples *t*-tests. Results Participants in either condition realized increases in cognition from baseline to completion (MMSE waitlist control mean difference = 0.67, SD = \pm 0.65, intervention mean difference = 0.15, SD = \pm 0.57), and Stroke Impact Scale (SIS) Communication domain (waitlist control mean difference = 1, SD = \pm 2.2, intervention mean difference = 0.8, SD = \pm 0.41) while in intervention there was a group mean difference in SIS-thinking subdomain (intervention mean difference = 0.08, SD = \pm 0.09). In CES-D scores, both conditions decreased (waitlist control mean difference = -4.0, SD = \pm 3.90, intervention mean difference = -0.68, SD = \pm 2.22), the difference in intervention was not significant. Group mean differences between intervention and waitlist control groups (i.e., treatment conditions) at M3 using MMSE (group mean difference at M3 = -0.30, SD = \pm 0.24), the SIS-thinking domain (group mean difference at M3 = -0.28, SD = \pm 0.42), and SIS Communication domain (group mean difference at M3 = -1.2, SD = \pm 1.28). Significant differences were not observed. Group mean differences between treatment conditions at M3 in CES-D scores (group mean difference = 2.49, SD = \pm 3.0) showed a positive effect for the intervention group, though not significant ($p = 0.070$, $t = -1.074$, $df = 53$). Discussion Adaptive changes in cognitive brain function may be a beneficial side effect of participation in multimodal BCI-FES intervention for upper extremity motor rehabilitation in stroke survivors though these data do not support the hypothesis that participation in a BCI-FES intervention has a strong effect on cognition, when compared to controls. Conclusion BCI-FES designs may have adaptive plastic effects beyond the sensorimotor system which can positively affect cognition and affect.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.01

Topic: C.10. Brain Injury and Trauma

Support: Stanford Behavioral Functional Neuroscience Laboratory
Stanford University - Wu Tsai Neuroscience Institute

Title: Death-associated protein kinase (DAPK1) and its role in a 6-OHDA mouse model of Parkinson's disease

Authors: *M. SANTORO¹, R. K. LAM¹, P. CIARI¹, C. E. WOODS¹, M. SHAMLOO²;
¹Stanford Univ., Palo Alto, CA; ²Neurosurg., Stanford Univ. Sch. Of Med., Palo Alto, CA

Abstract: Death-associated protein kinase (DAPK1) belongs to a large family of Ca²⁺/calmoduline regulated serine/threonine kinases. Its cellular functions entail regulation of the cell cycle, autophagy, phagocytosis, and apoptosis. Mounting evidence suggests the involvement of DAPK1 in neurodegenerative diseases due to its dysregulated activity linked to Ca²⁺/glutamate neurotoxicity and the initiation of pro-apoptotic cell pathways. In addition, a strong link between variants of DAPK1 and the incidence risk of Alzheimer's disease has been reported in several studies. To date, the role of DAPK1 in Parkinson's disease (PD) is poorly understood. In light of the pivotal roles of DAPK1 on cell survival and cell death, we hypothesize that dysregulation of DAPK1 activity in the aged brain contributes to the apoptotic cell demise of nigrostriatal dopaminergic neurons observed in PD patients. To answer our questions, we identified a lead molecule with selective inhibitory activity against DAPK1. Three analog compounds were synthesized and selected based on their IC₅₀ for *in-vitro* screening. *In-vivo* experiments relied on male C57BL/6J mice aged 4 and 19 months. In addition, male mice C57BL/6J mice 12 weeks old were stereotaxically infused in the striatum with 2 µl of a 0.05 % solution of ascorbic acid containing 10 µgr of 6-OHDA per µl. We observed DAPK1 protein levels significantly down-regulated in the hippocampus of aged mice (55.6 % ± 24.9). Oppositely, protein levels of the inactive form of DAPK1 (phosphorylated on the serine 308) are increased against total DAPK1 (n=10 per group, p < 0.05). A similar trend was observed in the 6-OHDA treated mice with total protein levels of DAPK1 decreased. *In-vitro* assessment of toxicology and neuroprotective effects of our three novel DAPK1 inhibitors showed high tolerability of our analogs in human microglial cells and rat cortical neurons with a survival rate above 95 %. On the other hand, pretreatment of rat cortical neurons did not protect them against NMDA neurotoxicity. Furthermore, incubation of human microglia with our novel DAPK1 inhibitors does not impair phagocytosis. Our findings provide robust evidence for DAPK1 involvement in PD. Elucidation of autophagy and phagocytosis pathways using knocked-out

DAPK1 mice will be conducted in α -synuclein mouse models to elucidate the role of DAPK1 in amyloidosis and dopaminergic neuron vulnerability.

Disclosures: M. Santoro: None. R.K. Lam: None. P. Ciari: None. C.E. Woods: None. M. Shamloo: None.

Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.02

Topic: C.10. Brain Injury and Trauma

Support: Dell Medical School

Title: Neutrophils and the injured developing brain: Validation of specific immunologic depletion and genetic-based strategies to assess their pathogenicity

Authors: *K. SMITH, M. H. DONOVAN, R. E. VON LEDEN, L. J. NOBLE;
Univ. of Texas, Austin, Univ. of Texas, Austin, Austin, TX

Abstract: Injury to the developing brain results in a prolonged recruitment of neutrophils. As neutrophils are capable of transcriptional changes in response to the local environment, they may exhibit distinct roles in the injured brain, as it shifts from an early toxic environment to wound healing. To address this hypothesis, there is a need to selectively deplete these leukocytes at various times post injury. Early studies using immunologic depletion, yielded varying results, which were likely due to antibodies that were not specific to neutrophils. Thus, we have first tested a new method, developed by others, to immunologic deplete neutrophils using anti-Ly6G. Traumatic brain injury (TBI), performed in male mice at postnatal day 21, were randomly assigned to receive either Ly6G or IgG control. Temporal depletion was confirmed by blood smears; percent neutrophils per total white blood cell count was determined the day before TBI (baseline), day of TBI and days 5, 7 and 14 post-injury (PI). For acute depletion of neutrophils, mice received either Ly6G or IgG one day prior to injury and immediately after TBI (N=8/group). Neutrophils were elevated at 1 day PI in the IgG group (25.55% vs baseline 17.65%, $p=0.0002$), while the Ly6G group showed a significant decrease (9.19% vs. baseline 15.21%, $p=0.0002$). By day 5 post injury, both groups returned to baseline values. For delayed depletion, mice received similar treatments at days 4 and 5 post TBI (N=4/group). Both groups showed a significant increase from baseline to day 1 PI. The IgG group then remained elevated while the Ly6G group showed a decrease in neutrophils by day 7 PI (9.33%; $p=0.0026$) and by day 14 both groups returned to baseline. As an alternative approach, reported to generate greater depletion, we also evaluated the following groups: Ly6G, Ly6G in combination with secondary, IgG and saline control (N= 12/group). There was a significantly greater percentage of neutrophils in the control groups compared to Ly6G treated groups at baseline ($p < .001$ and $p < .001$) and day 1 PI ($p < .001$ and $p < .001$). However, no differences were found between the Ly6G treated

groups. Complimentary to the depletion strategies, we have defined the distribution of neutrophils in the injured young brain using genetically engineered Catchup mice which, through modulation of the Ly6G locus, allows for fluorescent imaging-based tracking of neutrophils. Contrary to prior studies, our preliminary findings reveal recruitment of neutrophils into the cortical mantle with very limited evidence in the external capsule and hippocampus. Collectively, these findings establish the foundation for exploring the role of neutrophils in the injured brain.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.03

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant F31HD104491

Title: A second hit wonder: Early life stress prior to traumatic injury to the developing brain results in regional differences in microglial density within the hippocampus

Authors: ***K. PARKER**^{1,2}, **M. DONOVAN**², **L. J. NOBLE-HAEUSSLEIN**^{2,1};
¹Psychology, ²Dell Med. Sch., Univ. of Texas at Austin, Austin, TX

Abstract: Little attention has been directed towards the impact of early life stress (ELS) on recovery in children who have sustained traumatic brain injuries (TBIs). Recent studies have examined ELS, using a model of maternal separation, from birth up to postnatal (P) day 21, the day of injury. Findings, at P35 and P42, revealed increased activation of microglia and pro-inflammatory cytokines in the hippocampus. Here we build upon these findings, using a different model whereby a nursing dam and pups are placed in a cage with a metal mesh bottom and a partial nestlet square from P2-9. We hypothesized that early, short-term exposure to maternal stress will amplify the microglial response in the hippocampus to an early age TBI. Continuous video recordings confirmed fragmented maternal care in the ELS group (N=3-4 litters/group; unpaired t-test, $p < 0.05$) that corresponded to lower pup body weights (N=30/group; mixed-effect model, $p < 0.05$). Physiological stress, confirmed using qPCR on homogenates of the hypothalamus, revealed an activated hypothalamic-pituitary-adrenal axis response at P9, evidenced by increased vasopressin and decreased corticotropin-releasing hormone (N=4-5/group; unpaired t-test, $p < 0.05$). On P21, pups from ELS and control cages were randomly assigned to sham surgery or TBI and euthanized 1 day later. Iba-1 and the CA2 marker, PCP4, were used to separately quantify microglia in CA1, CA2, and CA3 and the dentate gyrus. ELS + TBI showed the highest microglia density in CA3 compared to all other groups (N=6/group; ANOVA; $p < 0.05$). Microglial density was increased in ELS + TBI compared to control groups

and ELS + Sham resulted in increased microglia density compared to control + sham (N=6/group; ANOVA; $p < 0.05$) in CA2. ELS + TBI had increased microglial density compared to sham groups in CA1 (N=6/group; ANOVA; $p < 0.05$). Microglial density was increased in ELS + Sham animals compared to ELS + TBI and Control + Sham in the dentate gyrus (N=6/group; ANOVA; $p < 0.05$). This is the first study to show that TBI in combination with short-term exposure to early maternal stress results in a heightened microglial response within distinct CA subfields and the dentate gyrus. Such findings across different models reinforce the increased vulnerability of the young brain when ELS precedes an early age TBI.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.04

Topic: C.10. Brain Injury and Trauma

Support: Department of Veterans Affairs RR&D Grant 5I01RX002931
Advancing A Healthier Wisconsin Seed Grant

Title: Head impact exposure causes dose dependent axonal damage that is correlated to behavioral disruptions

Authors: J. SEIFERT¹, A. SHAH², R. CHIARIELLO³, M. D. BUDDE³, C. M. OLSEN⁴, M. MCCREA³, *B. STEMPER^{5,6};

²Dept. of Neurosurg., ³Neurosurg., ⁴Neurosci. Res. Ctr., ¹Med. Col. of Wisconsin, Milwaukee, WI; ⁵Marquette Univ. & Med. Col. of Wisconsin, Milwaukee, WI; ⁶Neurosci. Res., Zablocki Veterans Affairs Med. Ctr., Milwaukee, WI

Abstract: Contact sport athletes experience repetitive subconcussive head impact exposure (HIE), which can lead to incremental physiologic and behavioral changes, and reduced concussion tolerance. Identification of objective markers associated with HIE can help reduce concussion risk and limit progressive changes in the brain. Our objective was to demonstrate that accumulating brain injury can be quantified using blood serum biomarkers that are correlated to behavioral disruptions. Sprague Dawley rats were separated into four groups: high-HIE (HE), moderate-HIE (ME), single injury (SI), and sham. HE rats received 30 and ME rats 8 low level head accelerations per day, 5 days per week, for 4 weeks. The SI group received 1 head acceleration with magnitude sufficient to cause mild traumatic brain injury (mTBI). Number and magnitude of head accelerations were based on our human studies of contact sport athletes. Blood serum concentrations of neurofilament light protein (NF-L) were measured at the end of each week and emotional changes were assessed at the end of the 4 weeks using the elevated plus maze (EPM). Compared to baseline, HE rats had elevated NF-L concentrations ($p < 0.05$) after 1 week that persisted for all 4 weeks, whereas ME rats had elevated NF-L concentrations

only after 4 weeks of HIE. NF-L concentrations immediately following HE/ME and SI protocols were similar between the 3 injury groups ($p>0.05$) and were greater than shams ($p<0.05$). This may indicate a plateau of axonal injury for HE and ME groups that was consistent with mTBI. HE/ME and SI EPM open area and closed arm entries were different than shams ($p<0.05$). NF-L concentrations showed a positive correlation with open area entries ($p=0.05$) and closed arm entries ($p=0.06$); rats with increased open area and closed arm entries had greater NF-L concentrations. Our analysis showed dose-dependent changes, with elevated NF-L concentrations immediately after one week in the HE group and delayed elevation of NF-L concentrations in the ME group. However, NF-L concentrations plateaued after four weeks in both exposure groups and were not different from the SI group. EPM trials indicated anxiety-like behavior, as both open area and closed arm frequencies positively correlated with increasing NF-L concentrations. Understanding these effects of HIE and the underlying mechanisms can help in the development of a HIE threshold to protect athletes from unnecessary damage and possible long-term effects.

Disclosures: **J. Seifert:** A. Employment/Salary (full or part-time);; Medical College of Wisconsin. **A. Shah:** A. Employment/Salary (full or part-time);; Medical College of Wisconsin. **R. Chiariello:** A. Employment/Salary (full or part-time);; Medical College of Wisconsin. **M.D. Budde:** A. Employment/Salary (full or part-time);; Medical College of Wisconsin. **C.M. Olsen:** A. Employment/Salary (full or part-time);; Medical College of Wisconsin. **M. McCrea:** A. Employment/Salary (full or part-time);; Medical College of Wisconsin. **B. Stemper:** A. Employment/Salary (full or part-time);; Medical College of Wisconsin, Zablocki Veterans Affairs Medical Center.

Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.05

Topic: C.10. Brain Injury and Trauma

Title: Treating mild repetitive head injury with a vasopressin V1a receptor antagonist.

Authors: P. KULKARNI¹, N. BENS¹, J. LEASTON³, A. SHINDE¹, M. POMPILUS¹, M. FEBO¹, E. E. EBONG⁴, N. SIMON^{5,6}, C. GHARAGOUZLOO³, C. F. FERRIS^{1,2};

¹Ctr. for Translational Neuroimaging, ²Dept. of Psychology, Northeastern Univ., Boston, MA;

³Imaginostics, Inc., Cambridge, MA; ⁴Departments of Bioengineering and Chem. Engin., Northeastern university, Boston, MA; ⁵Biol. Sci., Lehigh Univ. Biol. Sci., Bethlehem, PA;

⁶Azevan Pharmaceuticals, Bethlehem, PA

Abstract: There are presently no clinically approved treatments for mild head injuries that commonly occur in organized sports, motor accidents, falls, or in combat. To address this need, the present study tested a highly selective arginine vasopressin V1a receptor antagonist SRX251 in a closed-head, momentum exchange model of repetitive mild head injury in rats. MRI revealed there was no brain damage or contusion attesting to the mild nature of the head impacts

in this model. It was hypothesized that drug treatment would reduce edema, stabilize blood brain barrier (BBB) permeability, and reduce brain neuroinflammation. Female rats maintained on a reverse light-dark cycle were head impacted three times while fully awake with and without drug treatment. The impacts separated by 24 hrs each, were delivered under red light illumination. Within 1-2 hrs of the last impact, rats were assessed for changes in water diffusivity and resting state connectivity. On the following day rats were imaged for changes in BBB permeability using QUTE-CE and then tested for motor and cognitive function. The data for each imaging modality was registered to a 3D MRI rat atlas with over 170 segmented brain areas providing site specific information on altered brain structure and function. Postmortem histology was performed two weeks post head injury. There were no differences in cognitive or motor function between experimental groups. Rats treated with SRX251 showed a significant reduction in both apparent diffusion coefficient and fractional anisotropy as compared to sham controls that were not hit and vehicle treated females hit three times. The reduction was most notable in the hippocampus, cerebellum, basal ganglia, and thalamus. There were a modest number of brain areas that showed increased BBB permeability in head impacted, vehicle treated rats but not with SRX251 treatment. Rats treated with SRX251 presented with a significant decrease in cerebral blood volume along the prefrontal cortex, hippocampus, olfactory system, and midbrain. The drug-induced decrease in blood volume to these areas after repetitive head injury may reflect a period of hypometabolism to help in recovery.

Disclosures: P. Kulkarni: None. N. Bens: None. J. Leaston: None. A. Shinde: None. M. Pompilus: None. M. Febo: None. E.E. Ebong: None. N. Simon: None. C. Gharagouzloo: None. C.F. Ferris: None.

Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

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Program #/Poster #: 459.06

Topic: C.10. Brain Injury and Trauma

Support: NHMRC Grant APP1141643

Title: The effect of BDNF Val68Met polymorphism on spatial memory and anxiety-like behaviour after repeated mild TBI in rats

Authors: *L. P. GIESLER¹, W. T. O'BRIEN¹, J. W. P. BAIN¹, E. J. JAEHNE², M. VAN DEN BUUSE², R. MYCHASIUK¹, S. R. SHULTZ¹, S. J. MCDONALD¹;

¹Monash Univ., Melbourne, Australia; ²La Trobe Univ., Melbourne, Australia

Abstract: Repeated mild traumatic brain injury (rmTBI) has been associated with neurobehavioural deficits such as cognitive impairment and mood-related changes such as anxiety. Brain-derived neurotrophic factor (BDNF) is particularly interesting in the context of rmTBI due to its crucial role in the development and maintenance of the central nervous system.

The Val68Met is a functional single nucleotide polymorphism that results in a valine to methionine amino acid substitution at codon 68 (i.e. the rat equivalent of the human codon 66) in the BDNF prodomain, resulting in reduced activity-dependent secretion of BDNF. The current study evaluated the effects of five rmTBIs in adolescent male and female genetically modified Val68Met rats using the awake closed head injury model, with the primary outcomes to date being spatial learning and memory and anxiety-like behaviour. Rats were allocated between six groups (preliminary analysis: n=16) based on injury (i.e., rmTBI or Sham) and genotype (i.e., Val/Val, Val/Met, or Met/Met). The Morris water maze was used to measure spatial memory and the elevated plus maze (EPM) was used to assess anxiety-like behaviour. Rats were tested on the elevated plus maze at 24 hrs post-final injury. Water maze testing occurred in two sessions, one acquisition session at 48 hrs post-final injury and one reversal session at 72 hrs post-final injury. Preliminary results show that rmTBI rats had increased latency to locate the hidden platform in both the acquisition and reversal protocols of the water maze when compared to sham rats, indicating impaired spatial memory. Furthermore, rmTBI rats spent less time in the open arm of the EPM, indicating increased anxiety-like behaviour. Data collection from additional cohorts and further analysis is ongoing; however, preliminary analysis indicates that BDNF genotype may not influence spatial memory and anxiety-like behaviour in the tasks administered in the acute stages after rmTBI in rats.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

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Program #/Poster #: 459.07

Topic: C.10. Brain Injury and Trauma

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Brain & Behavior Research Foundation (NARSAD)
8G12MD007600
UPR MSC Chancellor's Office and Medicine Deanship
NSF PRCEN undergraduate and graduate Fellowships
NEUROD-ID undergraduate fellowship

Title: Effects of closed head injury on ethologically-relevant behaviors related to anxiety on platform-mediated avoidance

Authors: *L. VICENTE-RODRÍGUEZ, T. JIMÉNEZ-RIVERA, N. M. JIMÉNEZ-RIVERA, O. MARTÍNEZ-GUZMAN, M. GONZÁLEZ-PEDRAZA, M. CÁCERES-CHACÓN, M.

RIVERA-LÓPEZ, D. SIERRA-MERCADO;
Anat. & Neurobio., Univ. of Puerto Rico, Sch. of Med., San Juan, Puerto Rico

Abstract: Converging lines of evidence suggest that concussion may impair emotionally-relevant behaviors, such as fear. One type of fear-related behavior, avoidance, occurs when the need to escape from difficult situations such as an aversive stimulus (i.e. footshock) is presented. However, the behavioral effects of concussion on avoidance remains largely unknown. Concussion can be modeled in rodents with a closed head injury (CHI). Here, a guide tube is placed above the head of anesthetized rats, and a weight is dropped through the tube. In platform-mediated avoidance, rats are conditioned in an operant chamber to auditory tones co-terminating with a mild footshock. An acrylic platform in the opposite corner of a sucrose-delivering bar allowed rats to avoid the shocks. Our preliminary results suggest that CHI (n=12) versus sham-controls (n=12) increases the amount of time spent on the avoidance platform (p=0.0127). Of note, the ethologically-relevant behaviors related to anxiety that the animal displays on the platform is unclear. To address this issue, we hypothesize that CHI impairs freezing, grooming, and rearing, each of which is utilized as an indicator of emotional stress. The translational relevance of this work suggests that brain injury may contribute to mental health disorders, since excess avoidance is characteristic of patients with fear and anxiety disorders.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.08

Topic: C.10. Brain Injury and Trauma

Title: Multimodal parametric mapping of function, microstructure and relaxometry MRI in two traumatic brain injury models: characterization with serum-based biomarkers

Authors: *R. KOMMIREDDY, S. MEHRA;
Univ. of Florida, Gainesville, FL

Abstract: Multimodal parametric mapping of function, microstructure and relaxometry MRI in two traumatic brain injury models: characterization with serum-based biomarkers Shray Mehra¹, Rohan Srinivas Kommireddy¹, Rawad Daniel Arja¹, Tian Zhu¹, Zhihui Yang^{1,3}, Yueqiang Fu¹, Jiepei Zhu¹, Marjory Pompilus², Marcelo Febo², Firas Kobaiassy¹, Kevin K.W. Wang¹⁻³ Human traumatic brain injury (TBI) is heterogeneous in terms of severity, cause, and also in terms of the underlying pathophysiological mechanism involved. These can include axonal injury, white matter integrity, microvascular injury and neuroinflammation. The failure of numerous Phase 3 clinical trials in TBI has prompted a re-thinking of our animal models and TBI

study design to improve translation of preclinical results to be applied in the clinical. A primary objective of this study was to analyze data obtained from two rat TBI models, the lateral fluid percussion injury model (L-FPI) and controlled cortical impact (CCI). We investigated a panel of biofluid-based time-varying biomarkers and MRI-based neuroimaging sequences *in vivo* with the objective of addressing a range of clinically relevant TBI pathological mechanistic subphenotypes. Serum based biomarkers were assessed at 1-2d, 7d and 4 weeks post-injury, MRI imaging was conducted at day 2 and 4 weeks. Candidate blood-based biomarkers included phosphorylated neurofilament heavy (pNF-H), neurofilament light (NF-L), Tau for axonal injury, neuron specific enolase (NSE) and glial fibrillary acidic protein (GFAP) for contusion/necrosis. In parallel, *in vivo* MRI scanning sequences were conducted on carried out at 11.1 Tesla (Bruker) and 4.7 Tesla (Agilent), included T2* and T2 relaxometry, diffusion-weighted imaging (DWI), susceptibility weighted imaging (SWI) and resting state fMRI. Based on the results so far, all three TBI models showed a different temporal serum Tau profile - peaking at day 1 in CCI, while peaking at day 7-30 for FPI and WD. Serum GFAP signals peak at d1 across models. Serum pNF-H signals are much higher in FPI models (median 280 pg/mL) in FPI at d1 when compared to the CCI counterpart (median 50 pg/mL). Consistent with the biofluid markers, our imaging results showed distinct time varying changes, which included tissue signal changes in T2 and T2*, lower FA for white matter (WM) relative to naïve controls at d2 and this change persists to a lesser extent by d30. In contrast, mean diffusivity (MD) is high in WM at d2 and remains high in the lesion site. Our data suggest the feasibility of using blood-based temporal biomarkers and MRI neuroimaging precision biomarkers in assessing TBI subphenotypes in several rat models.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

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Program #/Poster #: 459.09

Topic: C.10. Brain Injury and Trauma

Support: TriService Nursing Research Program
Center for the Study of Traumatic Stress

Title: Latency to Righting Reflex and its Associations with Outcomes after Mild Traumatic Brain Injury in Rats

Authors: *H. SPENCER¹, R. BERMAN², M. BOESE³, J. T. MCCABE⁴, K. RADFORD³, K. CHOI²;

¹Program in Neurosci., ²Psychiatry, ³Grad. Sch. of Nursing, Uniformed Services Univ., Bethesda, MD; ⁴Dept. of Anatomy, Physiol. & Genetics, Uniformed Services Univ., Bethesda, MD

Abstract: Despite the fact it has no standard approved therapeutics, traumatic brain injury (TBI) affects millions of people each year and constitutes a critical public health problem. Lasting functional and behavioral deficits have detrimental effects on the quality of life for TBI patients. Though 80-90% of TBI cases can be classified as mild, many widely used preclinical TBI models produce more severe TBI phenotypes and include the use of craniotomies. These models have limited clinical relevance to closed head injuries with free head movement, which comprise the majority of human TBIs. Additionally, though clinical TBI is classified in severity based on factors including loss of consciousness (LOC), few preclinical studies have assessed the rodent analog to LOC, the latency to righting reflex, for correlations with severity of injury. For the current study we used the Closed Head Impact Model of Engineered Rotational Acceleration (CHIMERA), a recently developed technique that replicates the biomechanical mechanism of how humans sustain a closed-head impact TBI with resulting free head movement. Adult male Sprague-Dawley rats with indwelling jugular venous catheters sustained repetitive CHIMERA impacts (sham or 3x, 1.5 J) in a single session and the latency to righting reflex immediately following the final injury or sham procedure was measured. Catheter blood samples were collected at baseline, 2 and 4 hours, and 1, 2, and 3 days after CHIMERA. Behavioral metrics included rotarod, acoustic startle reflex, and pre-pulse inhibition (ASR/PPI) at 1- and 3-days post-injury. Brain tissue was collected at 8- or 15-days post-CHIMERA to determine effects on microglial and astrocyte activation. CHIMERA injured animals showed impaired rotarod ability on Day 1 post-injury and impaired PPI on Day 2 post-injury compared to sham animals. Additionally, animals with longer latency to righting reflex (High RR) exhibited a phenotype of greater injury severity compared to those with shorter latency to righting reflex (Low RR). Impaired rotarod, PPI, and disruptions in ASR were associated with the High RR animals. Further analysis is in progress to determine the relationship between righting reflex, inflammatory cytokine levels, and brain pathology. A single-session repeated CHIMERA paradigm produced a mild TBI phenotype which resulted in sensory gating deficits. The latency to recovery of the righting reflex in animals, a preclinical measure of LOC after TBI, may serve as a useful predictor of symptom development after injury.

The views represented are those of the authors and do not reflect policy of the US Government, Department of Defense, US Navy, or USU.

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Poster

459. Brain Injury: Biomarkers

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Topic: C.10. Brain Injury and Trauma

Support: UCLA: NIH/NINDS-UG3/UH3 Cooperative Agreement 5UG3/UH3NS106945-02
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UCSF: NIH/NINDS-UH3 Cooperative Agreement UH3NS106899
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Title: Classifying acute traumatic brain injury phenotypes via biomarker profiles and quantitative neuroimaging using machine learning strategies. - A TOP-NT consortium project.

Authors: ***I. B. WANNER**¹, H. RADABAUGH³, A. TOBAR¹, A. PAYDAR⁵, J. LOPEZ¹, T. E. VAN METER⁶, G. SHAW⁷, A. CHOU³, N. G. HARRIS², R. HUIE⁸, A. R. FERGUSON⁴, T. H. E. TOP-NT INVESTIGATORS¹;

¹Semel Institute, IDDR, BIRC, ²Dept. of Neurosurgery, BIRC, UCLA, Los Angeles, CA;

³Dept. of Neurolog. Surgery, ⁴Neurolog. Surgery, UCSF, San Francisco, CA; ⁵Dept. of Neurosurgery, BIRC, UCLA, Dept. of Neurosurgery, BIRC, Los Angeles, CA; ⁶BrainBox Solutions, Inc., Richmond, VA; ⁷EnCor Biotechnology, Inc, Gainesville, FL; ⁸Dept. of Neurosurgery, BIRC, UCSF, San Francisco, CA

Abstract: Evaluating traumatic brain injury (TBI) is hampered by poor injury classification and limited understanding of early injury events. Translational Outcomes Project in Neurotrauma (TOP-NT) Consortium aims to address these gaps by advancing reproducible biomarkers from animal models improving translation of pre-clinical research. Male and female rats (n=51) were given cortical contusion followed by 34 hour measures of fluid biomarkers, MRI and histopathology. Aldolase C (ALDOC) and glial fibrillary acidic protein (GFAP) with breakdown products (BDPs) were profiled in cerebrospinal fluid (CSF) and serum. Brain volumes and MRI intensities of T2 and diffusion weighted imaging were quantified by deviation from controls (n=29). Analysis of histopathology measured astrocyte density, structural integrity, neuronal fiber damage, and blood extravasation in contusion and pericontusional cortex. A new analytical pipeline characterized domain-specific phenotypes. Principal component analysis (PCA) described the whole dataset variance. K-means clustering identified 3 clusters in each domain and linear discriminant analysis (LDA) provided cluster interpretation. LDA analysis and PCA loadings were integrated to arrive at acute TBI phenotypes. LDA successfully described 3 k-means clusters of whole brain MRI burden. Clusters 1 and 2 reflected axial and radial diffusivity changes, while mean diffusivity (MD) dominated cluster 3. The first MRI principal component (PC1) captured reduced directional diffusivity accompanied by increased MD, suggesting that acute edema from cellular disruption was a dominant pattern (65% variance in PC1). Astrocytic injury biomarker profiles showed CSF GFAP and BDPs dominated PC1 (25%). Serum GFAP and ALDOC contributed substantially to PC2 (20%). LDA interpretation of k-means revealed temporal serum GFAP profile dictated cluster identity. Histopathology aided in understanding these phenotypes. PC1 (49%) was strongly driven by early interstitial bleeding, more pronounced in females. PC1 was associated with reduced pericontusional glutamine synthetase (GS). Time correlated with acellular deposition of ALDOC, neurofilament light, and GS in the injured cortex. PC2 (25%) captured an overall decline in intact and ALDOC-positive astrocyte densities. Thus, histopathology defined TBI classes, validated temporal biomarker profiles and acute MRI diffusivity changes were consistent with cytotoxic edema. In conclusion, we present a multivariate syndromic representation of key TBI phenotypes. These support the use of biomarker trajectories and neuroimaging signatures to facilitate classification of TBI patients.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

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Program #/Poster #: 459.11

Topic: C.10. Brain Injury and Trauma

Support: NIH R61/R33 NS115089
Minnesota SCI-TBI fund (Grant Contracts: 143722 and 191546)
University of Minnesota's MnDRIVE (Minnesota's Discovery, Research, and Innovation Economy) initiative

Title: Wide-field calcium imaging reveals widespread changes in cortical connectivity following repetitive, mild traumatic brain injury in the mouse

Authors: *S. W. CRAMER¹, S. P. HALEY², L. S. POPA², R. E. CARTER², E. P. SCOTT², E. B. FLAHERTY², J. DOMINGUEZ³, J. D. ARONSON², L. SABAL², D. SURINACH³, C. C. CHEN¹, S. B. KODANDARAMAIAH³, T. J. EBNER²;

¹Dept. of Neurosurg., ²Dept. of Neurosci., ³Dept. of Mechanical Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: More than 2.5 million individuals in the United States suffer minor traumatic brain injuries annually. The physiologic basis underlying the long-term consequences of repetitive, mild traumatic brain injury (mTBI) remains poorly understood. Mild traumatic brain injury often results in brief loss of consciousness, impaired attention and concentration, memory problems, impulsivity, and headache, without objective findings on clinical imaging or examination. The effects of mTBI can persist and become cumulative with repetitive injury, suggesting global alterations in cortical networks. Using transparent polymer skulls, we performed mesoscopic Ca²⁺ imaging in mice to evaluate how mTBI alters patterns of neuronal interactions across the dorsal cerebral cortex. Spatial Independent Component Analysis (sICA) and Localized semi-Nonnegative Matrix Factorization (LocaNMF) were used to quantify changes in cerebral functional connectivity (FC). Repetitive, mild, controlled cortical impacts induce temporary neuroinflammatory responses, characterized by increased density of microglia exhibiting de-ramified morphology. These temporary neuro-inflammatory changes were not associated with compromised cognitive performance in the Barnes maze or motor function as assessed by rotarod. However, long-term alterations in FC were observed. Widespread, bilateral changes in FC occurred immediately following impact and persisted for up to 7 weeks, the duration of the experiment. Network alterations include decreases in global efficiency, clustering coefficient, and nodal strength, thereby disrupting functional interactions and information flow throughout the dorsal cerebral cortex. A subnetwork analysis shows the largest disruptions in FC were concentrated near the impact site. Therefore, repetitive mTBI induces a transient neuroinflammation, without alterations in cognitive or motor behavior, and a reorganized cortical network evidenced by the widespread, chronic alterations in cortical FC.

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Poster

459. Brain Injury: Biomarkers

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Topic: C.10. Brain Injury and Trauma

Support: NINDS U54NS100064
NIH R01NS111744

Title: Characterization of traumatic brain injury in rodents using diffusion weighted imaging and machine learning classifiers

Authors: *A. SHARMA¹, R. CABEEN¹, A. BENNETT¹, R. GARNER¹, C. ALBA¹, X. E. NDODE-EKANE², P. ANDRADE², T. PAANANEN², R. CISZEK², R. IMMONEN², E. MANNINEN², N. PUHAKKA², M. HEISKANEN², C. E. SANTANA-GOMEZ³, G. D. SMITH³, D. WRIGHT⁴, I. ALI⁴, S. SHULTZ⁴, N. JONES⁴, N. G. HARRIS³, J. TOHKA², O. GRÖHN², T. O'BRIEN⁴, R. J. STABA³, A. PITKÄNEN², D. DUNCAN¹;

¹USC, Los Angeles, CA; ²Univ. of Eastern Finland, Kuopio, Finland; ³Univ. of California Los Angeles, Los Angeles, CA; ⁴Monash Univ., Melbourne, Australia

Abstract: Traumatic brain injury (TBI) may trigger epileptogenesis, leading to post-traumatic epilepsy (PTE) in up to 53% of subjects. Currently, there are no prognostic biomarkers to diagnose epileptogenesis and predict PTE risk with high sensitivity and specificity. Diffusion-weighted imaging (DWI) in conjunction with machine learning (ML) can help identify white matter regions affected by TBI. The goal of this study was to utilize ML tools with DWI to identify a candidate ML classifier for the detection of TBI pathology and microstructural changes across time points. We used DWI to measure and analyze fractional anisotropy (FA) obtained from tract-based spatial statistic analysis. These measurements were used to train four ML models to differentiate TBI rodents from shams.

A subset of data for 128 rodents (36 shams, 92 TBI) from the Epilepsy Bioinformatics Study for Antiepileptogenic Therapy collected at the University of Eastern Finland in Kuopio, University of California, Los Angeles, and Monash University, was used. TBI was induced with lateral fluid-percussion injury. Rats were imaged in vivo at four post-TBI time points: day (D)2, D9, D30, D150. Structural and microstructural data were captured using magnetization-prepared multi-echo-gradient sequence and DTI. Tractography-based analysis was performed using Quantitative Imaging Toolkit to extract mean FA values from 48 white matter bundles. Classification of sham vs. TBI animals was performed using four ML models: Random Forest (RF), Support Vector Machine (SVM), ADA Boost, and XGB DART. Mean FA values from

each white matter tract were used as predictor variables. The evaluation was done with a 5-fold cross-validation repeated over 100 rounds using the mean area under the receiver operating characteristic curve (AUC), mean sensitivity, and specificity. Weighting was performed to reduce class imbalance.

The SVM classifier outperformed other classifiers with an average AUC of 0.9 (sensitivity: 0.89; specificity: 0.87) for FA features on D2, 0.73 (sensitivity: 0.84; specificity: 0.6) on D9, 0.8 (sensitivity: 1; specificity: 0.7) on D30, and 0.95 (sensitivity: 1; specificity: 0.9) on D150 post-TBI. This preliminary work demonstrates that a linear SVM trained on FA values derived from DWI performs best compared to RF, ADA Boost, and XGB DART across all post-TBI time points, with high sensitivity and specificity. As longitudinal seizure outcomes and additional neuroimaging features become available, we speculate that this model has the potential to detect PTE among TBI rodents with high accuracy and sensitivity.

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Poster

459. Brain Injury: Biomarkers

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.13

Topic: C.10. Brain Injury and Trauma

Title: Stochastic resonance phenomenon reduces repolarization latency in the mice injured brain

Authors: *P. TRUJILLO GARIBAY¹, J. MARTINEZ CABRAL¹, L. P. OSUNA CARRASCO¹, C. TORO-CASTILLO¹, N. HUIDOBRO², B. DE LA TORRE-VALDOVINOS*¹;

¹Bioingeniería Traslacional, Univ. de Guadalajara - CUCEI, Guadalajara, Mexico; ²Decanato de Ciencias Biológicas, Univ. Popular Autónoma del Estado de Puebla, PUEBLA, Mexico

Abstract: Mice whiskers are sensorial communication structures which transduce environmental information to electrical impulses converging at the Barrel Cortex (BC). It has been well described the neural pathways between the whiskers and the BC and its electrophysiological response. A symmetrical penetrating injury (0.5mm diameter) was performed in the BC (-1 mm anteroposterior, +3 mm mediolateral (Paxinos, Franklin, 2004)) of the C57BL Black (24±4 grams) mice which produces a decrease in the amplitude (80% ± 8%) of the Local Evoked Field Potentials (LEFP). We added 8 levels of white gaussian noise to evaluate the stochastic resonance effect in tamoxifen treated (n = 10) and untreated (n = 10) injured mice which was applied 3 continues days after injury (1mg/kg). (Franco Rodríguez et. al., 2013, Trujillo Garibay

P. E et. Al. 2021). The response of the LEFP was recorded to assess functional recovery before and after injury. Two electrodes were implanted on the surface of the primary somatosensory cortex for the recording of LEFP 3, 7, 14 and 30 days after injury of both groups. We examine the amplitude versus repolarization latency in both treated and untreated mice previous to the injury and 3, 7, 14 and 30 days after injury within the 8 gaussian noise levels. We observed three levels of noise which express the typical stochastic resonance U-inverted form which we define as Zero Noise (ZN), Optimal noise (ON) and High noise (HN). A statistical significance differences of the amplitudes was observed between ZN and ON ($p < 0.05$) and between ON and HN ($p < 0.05$) in both treated and untreated mice. Additionally, when comparing both groups we observed that tamoxifen treated mice had a significant increased amplitude in the ON condition ($p < 0.05$). Also, we observed a decrease in the repolarization latency at the ON condition which compared with ZN and HN was statistical different ($p < 0.05$) and matches with the maximum amplitude in all mice (treated and untreated at ON condition). These results are according to the previous observations made by Remedios et. al., 2019. In conclusion, the stochastic resonance phenomena (in the ON condition) could shorten the repolarization time of the LEFP in both treated and untreated mice. Furthermore, ON produces a significant change in the amplitude in both groups but higher in the tamoxifen treated group. Additionally stochastic resonance it could be part of injured brain therapy.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.14

Topic: C.10. Brain Injury and Trauma

Support: NINDS Grant U54 NS100064

Title: Increased rates of high-frequency oscillations during the first week after lateral fluid percussion injury: UCLA data for the EpiBioS4Rx Project 1 study

Authors: *C. E. SANTANA-GOMEZ¹, G. OZAWA¹, A. PAVADE¹, M. SHAMAS¹, G. SMITH², M. HUDSON³, I. ALÍ³, P. M. CASILLAS-ESPINOSA³, S. SHULTZ³, N. JONES³, T. J. O'BRIEN³, N. HARRIS², J. ENGEL¹, R. STABA¹;

¹Neurol., David Geffen Sch. of Med. At UCLA, Los Angeles, CA; ²Neurosurg., David Geffen Sch. of Med. at UCLA, Los Angeles, CA; ³Neurosci., Central Clin. School, Monash Univ., Melbourne, Australia

Abstract: Rationale: Traumatic brain injury (TBI) is a serious health problem associated with acute and chronic neurological sequela and in some cases, seizures. The mechanisms underlying the dysfunction are complex and not well known, but likely involve neuronal hyperexcitability.

However, pathological high-frequency oscillations (HFO; 80-500 Hz) correspond with abnormal synchronous neuronal burst firing and can be recorded in patients and chronic models of epilepsy as well as the rat lateral fluid percussion injury (LFPI) model of human TBI. Thus, the current study quantitatively evaluated HFO in LFPI rats to begin to understand the neuronal disturbances during the first week after a TBI. **Methods:** LFPI was induced in adult male Sprague-Dawley rats (n=21) and during the same surgery, rats were implanted with electrodes for EEG recording. Electrodes consisted of bilateral frontal (C₃, C₄) and occipital (O₁, O₂) epidural screws, plus three paired microelectrodes located in the ipsilateral prefrontal (Y₁₋₂) and occipital (X₁₋₂) perilesional cortex and hippocampus (H₁₋₂). EEG recordings were designed as follows: continuously for the first 7 days, then for 2 consecutive days at the end of each month for 6 months, and concluding with continuous 30-day recording at month 7. Sham injured rats (n=7) underwent the same surgical procedures and recording as the TBI rats, but LFPI was not induced. RippleLab was used to detect fast ripples (FR, 200-500Hz) and ripples (R, 80-200 Hz) in 40 min EEG epochs during the light period each day of the first week. A linear mixed model was used to assess the effects of injury (TBI vs. sham), time (day after injury), and location of electrode on the rate of FR. A separate model was generated for R. **Results:** Rates of FR were variable and higher in TBI than in sham rats during each day of the first week after injury and significantly higher on day 6 (p=.04). Rates of FR were higher in TBI than sham rats in 8 of the 10 recording sites, particularly in the hippocampus (H₂, p=.005) and in the prefrontal cortex (Y₂, p=0.06). Like FR, rates of R were higher in TBI than in sham rats on each day of the first week, especially on days 1 and 3 (both p=.02) and day 7 (p=.06). Rates of R were higher in TBI than sham rats in 9 of the 10 recording sites and chiefly in ipsilateral frontal (C₃, p=.03) and prefrontal cortex (Y₁, p<.001; Y₂, p=.07). **Conclusions:** Results support the hypothesis that FR and R in the perilesional cortex correspond with neuronal disturbances that could contribute to acute and possibly chronic neurological dysfunction. Ongoing analysis in the EpiBioS4Rx project continues to investigate the short- and long-term HFO in rats with early and late seizures.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.15

Topic: C.10. Brain Injury and Trauma

Title: Determination of oxidative stress biomarkers in moderately traumatic brain injured rats treated with GCEE: A strategy to lower lipid peroxidation in TBI

Authors: *T. REED¹, M. QUIJAS²;

¹Eastern Kentucky Univ., Richmond, KY; ²Neurosci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Traumatic brain injury (TBI) is one of the leading causes of death and disability with an estimated 69 million cases annually worldwide and 2.8 million cases in the United States alone. As there are no current treatment strategies for this neurological disorder, immediate medical intervention is imperative to combat secondary injuries such as oxidative damage and lipid peroxidation. Oxidative damage occurs through the accrual of reactive oxygen species (ROS) and reactive nitrogen species (RNS). With an increase in both ROS and RNS in brain tissue, an imbalance between oxidants and antioxidants is observed leading to oxidative stress. Lipid peroxidation releases toxic products including 4-hydroxynonal (4-HNE) and 4-hydroxyhexenal (4-HHE) which increase injury in the brain. Reactive oxygen and reactive nitrogen species, 4-HNE and 4-HHE can modify proteins and DNA making them unstable. This modification can lead to a reduction in ATP production, increase in apoptosis, and inhibit cellular communication which are exhibited in neurodegeneration disorders. Glutathione stimulation can counteract the increase in ROS and RNS species leading to an overall reduction in oxidative damage and lipid peroxidation markers. Gamma-glutamylcysteine ethyl ester, GCEE, is a naturally occurring glutathione mimetic shown to decrease oxidative stress levels. This work was performed to determine the ability of gamma-glutamylcysteine ethyl ester to lower multiple biomarker of TBI levels including protein carbonyls, 4-hydroxynonal, and 4-hydroxyhexenal in traumatically brain injured rats. Rats in all groups (except sham) were subjected to a craniotomy and a moderate TBI via cortical contusion. Post-TBI rats treated with saline or GCEE groups received 150 mg/kg of saline and GCEE, respectively. Results demonstrate that GCEE administration at 30 and 60 minutes post injury significantly lowers these aforementioned biomarkers compared to comparable saline treatment. These results provide evidence that GCEE could potentially be therapeutic strategy for moderate traumatic brain injury and other neurodegenerative disorders.

Disclosures: T. Reed: None. M. Quijas: None.

Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.16

Topic: C.10. Brain Injury and Trauma

Support: NIH grant U54NS100064

Title: Discovery and validation of differentially expressed circulating microRNAs in a rat model of post-traumatic epilepsy: EpiBioS4Rx multicenter study

Authors: *M. HEISKANEN¹, S. DAS GUPTA¹, X. EKOLLE NDODE-EKANE¹, P. ANDRADE¹, T. PAANANEN¹, R. CISZEK¹, N. PUHAKKA¹, I. ALI², S. SCHULTZ², P. CASILLAS-ESPINOSA², G. YAMAKAWA², N. JONES², M. HUDSON², J. SILVA², E. BRAINE², R. BRADY², D. WRIGHT², C. SANTANA-GOMEZ³, G. SMITH³, N. HARRIS³, R. STABA³, T. O'BRIEN², A. PITKÄNEN¹;

¹A.I. Virtanen Inst. for Mol. Sci., Univ. of Eastern Finland, Kuopio, Finland; ²Dept. of Neurosci., Monash Univ., Melbourne, Australia; ³David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Annually, about 70 million people worldwide suffer traumatic brain injury (TBI), and 16-20% of patients with severe TBI develop post-traumatic epilepsy (PTE). So far, no prognostic biomarkers are available to predict epileptogenesis or severity of epilepsy after TBI. Circulating microRNAs (miRNAs) are a potential source for biomarkers as brain-enriched miRNAs can enter blood circulation after brain injury, allowing minimally invasive testing. Objective: To identify circulating miRNA biomarkers to predict epileptogenesis after TBI and assess reproducibility of results in a multicenter study. We hypothesized that TBI-induced changes in plasma miRNA levels at acute time point after TBI predict development and severity of PTE. The EpiBioS4Rx study was conducted at three sites: UEF, Monash and UCLA. TBI was induced to male Sprague-Dawley rats by lateral fluid-percussion injury. Sham-operated controls underwent surgery without TBI induction. One-month long video-EEG was performed on the 6th post-TBI month to assess occurrence of spontaneous seizures. Plasma collected on day (D) 2 after TBI was small RNA-sequenced to identify differentially expressed (DE) miRNAs (10 sham, 20 TBI rats [10 with epilepsy (TBI+), 10 without epilepsy (TBI-)]. Thereafter, miRNAs selected for validation were analyzed in a total of 235 samples available from the 3 study sites, including 26 baseline (naïve), 45 sham and 164 TBI (32 TBI+, 132 TBI-) using droplet digital PCR (ddPCR). We found 23 DE miRNAs TBI vs. sham, but no DE miRNAs TBI+ vs. TBI- group. The 7 ddPCR-validated miRNAs (miR-434-3p, miR-183-5p, miR-323-3p, miR-9a-3p, miR-124-3p, miR-132-3p and miR-212-3p) were elevated on D2 after TBI compared to naïve or sham ($p < 0.001$). All except miR-212 were also higher in the craniotomized sham-operated than naïve animals ($p < 0.05$). ROC analysis indicated that 6 of the 7 miRNAs differentiated TBI rats from naïve with $AUC \geq 0.80$. In addition, miR-323 and miR-9a differentiated sham from naïve with $AUC \geq 0.80$. No differences were detected in any of the miRNAs between TBI+ and TBI-. However, miR-212 levels were lower in the TBI+ rats with seizure clusters ($\geq 3sz/24h$) than in rats without clusters ($p < 0.01$). Interestingly, miR-212 distinguished TBI+ rats with seizure clusters from other TBI+ rats ($AUC 0.81$) and from the rest of the TBI rats ($AUC 0.74$, $p < 0.01$ for both). In general, the data were similar in samples collected at different sites. Conclusions: Several plasma miRNAs differentiate rats with craniotomy or severe TBI from naïve animals, however, none of the 7 miRNAs predicted epileptogenesis.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.17

Title: WITHDRAWN

Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.18

Topic: C.10. Brain Injury and Trauma

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Brain & Behavior Research Foundation (NARSAD)
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NSF PRCEN undergraduate and graduate Fellowships
PRCTRC Pilot Project Program

Title: Effects of Concussive-like Brain Injury on Fear Behaviors in Rats

Authors: ***H. G. HADDOCK-MARTÍNEZ**, O. MARTÍNEZ-GUZMÁN, M. CÁCERES-CHACÓN, M. RIVERA-LÓPEZ, D. SIERRA-MERCADO;
Anat. & Neurobio., Univ. of Puerto Rico Sch. of Med., San Juan, PR

Abstract: Each year 40 million people worldwide suffer from traumatic brain injury, mainly in the form of concussions. Human studies have linked concussions with the development of fear-related disorders such as post-traumatic stress disorder (PTSD). However, the relationship between concussions and fear behaviors remain unclear and animal studies show conflicting results. To evaluate the potential link between concussions and fear behaviors, a biological link must be examined using reliable injury models and behavioral tests. Given that failure to extinguish fear is a hallmark of PTSD, we hypothesized that a concussive brain injury will impair fear extinction using Pavlovian conditioning in rats. To address this gap, rats underwent fear conditioning where they learned that a tone predicts a shock, and freezing behavior is measured as an index of fear. Freezing behavior is characterized by lack of movement save those necessary for breathing. Afterwards, rats received a concussive-like or sham injury using a weight drop. Following recovery from concussive-like injury, rats underwent three sessions of extinction where they learn that the tone no longer predicts a shock. Results showed no significant difference ($p > 0.05$) between concussive-like ($n=9$) and sham ($n=8$) injured groups suggesting that concussive brain injury does not affect the ability to extinguish fear. Future studies will aim at examining changes in neuronal activity in brain regions relevant to fear behaviors such as the amygdala using cFos immunohistochemistry.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.19

Topic: C.10. Brain Injury and Trauma

Title: Behavioral changes and fMRI biomarkers in a Yucatan minipig model of pediatric concussion

Authors: *L. KLEYN¹, A. H. NETZLEY², K. A. MUNOZ³, A. C. COLBATH³, J. HUANG⁴, A. BADACHHAPE⁶, A. MEODED⁶, G. PELLERD^{1,4,5};

¹Dept. of Neurosci., ²Biomed. Engin., ³Col. of Vet. Med., ⁴Dept. of Radiology, ⁵Mechanical Engin., Michigan State Univ., East Lansing, MI; ⁶Dept. of Radiology, Baylor Col. of Med., Houston, TX

Abstract: It is estimated that 3 million individuals in the United States suffer a mild Traumatic Brain Injury (mTBI), also known as concussion, every single year (CDC). Concussions remain difficult to diagnose, and often leads to long-term impairments and disorders that includes sleep disturbances, mood and behavioral changes, attention disorders, sensory hypersensitivity, and learning disabilities. Concussion in kids could be particularly devastating, with no current treatment. Developing biomarkers that could inform on a concussion are of major clinical interest. Pigs are becoming a popular translational model in the field of neurotrauma due to the large size of their brains, their intelligence and their physiology. We established a pediatric model of closed-head injury in Yucatan minipigs. 16-week old pigs received an impact of 2J over the frontal cortex, mimicking a sports-related mTBI in youth (n=5 mTBI; n=5 sham). A battery of behavioral tests were used to measure memory, behavior, mood, anxiety, and social hierarchy. Wearable devices (Fitbits[®]) and video analysis were also used to measure motor activity and sleep patterns. We also obtained functional MRI measurements at clinically-relevant time points after the injury (24h, 30, and 90 days). **Results:** In the weeks following injury, mTBI pigs showed an impairment in memory that was reflected by the time it took the injured pigs to solve a learned task (58.5% \pm 0.394 s increase for mTBI pigs; 1.1% \pm 0.419 s increase for sham pigs). mTBI pigs also showed a greater long-term increase in aggressiveness following injury, which was demonstrated in a food dominance task. When comparing group performance in the dominance task, mTBI pigs increased their aggressiveness by 20.3% following injury, while sham pigs decreased by 3.58%. Additionally, when comparing recorded Fitbit[®] steps, mTBI pigs showed a 28% increase in steps taken following injury, whereas sham pigs had a reduction in activity of 24%. Lastly, the fMRI analyses following tactile stimulation showed increased, non-specific activation in the mTBI pigs compared to sham. **Discussion:** Through the use of Yucatan minipigs, we are able to replicate the presentation of various symptoms commonly displayed following pediatric concussion. In several of our behavioral tests and fMRI analyses, clear differences have been found between healthy and injured pigs. Therefore, our model of mTBI is extremely clinically relevant as it allows us to track recovery following injury and will allow for later testing of possible therapeutics and biomarkers.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.20

Topic: C.10. Brain Injury and Trauma

Title: Biomarkers of the opioid-induced neuronal degeneration in mouse brain and plasma

Authors: *N. KORNEEVA¹, R. FAN², J. CUTLER², T. ARNOLD²;

¹Emergency Medicine; Pharmacology, Toxicology and Neurosci., Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, LA; ²Emergency Med., LSUHSC, Shreveport, LA

Abstract: Opioids are the most effective drugs commonly prescribed to treat pain. Despite widespread abuse of opioids, we know little about the long-term consequences of chronic use. Recently, concern regarding the effect of chronic opioid exposure on neuronal degeneration has emerged. Toxic effect of opioids has been documented for patients with a history of long-term use of prescription opioids. Currently, there is no inexpensive, minimally invasive method to monitor neuronal degeneration. To investigate the effect of chronic opioid use we treated mice with either water or morphine (15 mg/kg) for 30-60 days. We monitored biomarkers of neuronal degeneration in brain tissues using immunohistochemical and western blot analyses. We demonstrated that chronic morphine administration is associated with activation of pro-apoptotic signaling in mouse brain. In addition, in plasma of animals chronically treated with morphine, we observed increased levels of neuronal proteins such as myelin basic protein and tau as well as accumulation of pro-inflammatory cytokines. The findings of this project support the hypothesis that chronic opioid use causes a negative impact on neuronal health. Our data also suggest that measuring the level of neuronal and pro-inflammatory biomarkers in blood samples may serve as a diagnostic tool to monitor drug-induced neuronal degeneration in research and clinical settings.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.21

Topic: C.10. Brain Injury and Trauma

Title: The Effects of Time Duration Between Hits in Repeated Traumatic Brain Injury

Authors: *E. GIANG¹, T. A. TOGASHI², A. D. TROFIMOVA³, M. MENDOZA⁴, W. HARDEMAN⁵, R. E. HARTMAN⁶;

¹Loma Linda Univ., Yucaipa, CA; ²Loma Linda Univ., Redlands, CA; ³Psychology, Loma Linda Univ., Loma Linda, CA; ⁴Psychology, Loma Linda Univ., LOMA LINDA, CA; ⁵Loma Linda Univ., Loma Linda, CA; ⁶Psychology, Loma Linda Univ., Temecula, CA

Abstract: Elissa M. Giang, Tori A. Togashi, Alexandra D. Trofimova, Madeleine M. Mendoza, Willie Hardeman, and Richard Hartman

The Effects of Pomegranate Juice on Behavioral Deficits Induced by Repeated Traumatic Brain Injury in *Drosophila melanogaster*

Traumatic brain injuries (TBIs) are when an outside force, usually blunt and violent, contacts the head resulting in brain impairment. When the brain experiences repeated blows, this is known as repeated traumatic brain injury (rTBI), which can result in severe consequences, such as cognitive disruption, behavioral impairment, and development of neurodegenerative diseases like Alzheimer's Disease (AD) and chronic traumatic encephalopathy (CTE). This study identifies the consequences of injury timing in a *Drosophila melanogaster* model of rTBI. Briefly, each fly was subjected to four, closed-head strikes with a modified high impact trauma (HIT) device. The strikes were separated by 5 minutes, 2 hours, 4 hours, or 36 hours. These intervals provided an opportunity to study outcomes of repeated brain injury during times of unresolved rTBI mechanisms. Climbing ability, locomotor activity, and age at death were measured. Additionally, dietary supplementation of pomegranate juice was given to the flies to determine whether it modulated behavioral deficits. Results indicated that rTBI reduced lifespan, climbing ability, and pomegranate juice supplementation was an effective treatment in increasing lifespan.

Specifically, flies separated by the 36-hour interval showed the worst performance on climbing ability. Flies that were given pomegranate juice were 72.2% less likely to die 24 hours post HIT trial (OR = .278, 95% CI [.106, .733]). Although there was no significant effect of rTBI on movement ability, rTBI flies demonstrated a trend of restricted activity levels across four days when compared to control flies.

These results suggest that the time between rTBI occurs may reduce lifespan and induce behavioral deficits, causing a decrease in quality of life. Furthermore, our findings suggest that dietary supplementation with pomegranate juice may increase the nervous system's resiliency to rTBI, regardless of time in between strikes. These findings are significant for populations at risk for rTBI, such the elderly, athletes, construction workers, and occupations that involve risk of injury. Furthermore, these results warrant further investigation into the use of pomegranate juice as a treatment for rTBI and related brain injuries.

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Poster

459. Brain Injury: Biomarkers

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Program #/Poster #: 459.22

Topic: C.10. Brain Injury and Trauma

Support: NS094655

Title: Arousal units, cortical signatures that track motor and autonomic restoration during emergence from diverse comatose states

Authors: *S. GAO^{1,2}, Y. BIBINEYSHVILI¹, D. CALDERON¹;

¹Weill Cornell Med., Weill Cornell Med., New York, NY; ²Cornell Univ., Ithaca, NY

Abstract: Accurate assessment of arousal levels is crucial for diagnosing and prognosis of patients with disorders of consciousness. However, current approaches are subjective, sporadic, and rely on multiple measurements such as electroencephalographic (EEG) traces and behavior scale evaluations, adding challenges to monitoring and interpretation. Therefore, a more reliable, objective, and quantitative method to track how an individual regains wakefulness is in great demand. Here, we propose a novel automated method that combines spectral analysis and statistical learning to detect and classify cortical patterns associated with autonomic and motor recovery. Moreover, extracted features of these arousal patterns defined arousal levels in different coma-induced conditions. We previously determined that cortical changes and re-establishment of movements while subjects awaken are consistent across animals and independent of the induced-coma circumstances. This suggests that cortical and motor restoration follow a defined progression of discrete states. Thus, we hypothesized that cortical neural network interactions resulting in cortical signatures could establish accurate motor progression during awakening. To test this hypothesis, we developed an algorithm to extract cortical patterns associated with increased breathing frequency and body-part movements from animals exposed to gradually decreased anesthetic levels. We defined these cortical patterns as Arousal Units. The algorithm yielded a precision and sensitivity of approximately 80% while detecting arousal units. We examined the oscillatory dynamics within arousal units and identified gamma-theta patterns as the main drivers of cortical change linked with the motor recovery. Moreover, by determining arousal-unit-centroids per cortical periods in the gamma-theta space and measuring the distance of these centroids to the centroid belonging to fully awake arousal units, we transformed the gamma-theta distances into a quantitative approach to determine the levels of arousal through a logistic curve. We applied this methodology on a blast injury and a hypoglycemic coma model. While emergence from anesthesia rendered a linear trajectory when we establish arousal levels as a function of the arousal units, recovery from blast injury and hypoglycemic coma showed a nonlinear restoration. Our results reveal that detecting arousal units can define movement initiation, classify the movement, and quantitatively establish the level of arousal in multiple induced-coma conditions. These results provide a novel cortical biomarker that traces overall restoration and could apply to the clinic.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.23

Topic: C.10. Brain Injury and Trauma

Title: Effects of Acute Administration of Hypotonic versus Isotonic Solutions on Juvenile Traumatic Brain Injury

Authors: *S. E. SHONKA, M. J. HYLIN;
Southern Illinois Univ., Carbondale, IL

Abstract: Vehicles should be carefully considered as they can exacerbate secondary injury following TBI. Normal saline solution is an isotonic solution, whereas Ringer's solution is a hypotonic solution. Hypotonic solutions cause edema, and are associated with greater neuronal damage following TBI (Bumberger et al., 2022). Therefore, isotonic saline solutions are recommended in patients following TBI (Colegrave et al., 2016). The primary goal of this study was to determine the behavioral and histological effects caused by an isotonic versus hypotonic solution administration following juvenile TBI. It was hypothesized that a hypotonic solution would increase the lesion size leading to more impairment on behavioral tasks. In this study, animals (n=54) received a bilateral frontal cortical contusion injury at post-natal day (PND) 28 and animals completed behavior tasks (n=24) including a foot fault task, open field task, Morris water maze, social preference task, and tube task. The groups included TBI animals with no treatment, 20 μ l Ringer's, and 20 μ l normal saline who were sacrificed on PND 30. The remaining animals were sacrificed on post-injury days 1, or 14 (n=30). The brains were then processed using cresyl violet procedures to measure lesion size. Results showed that animals administered Ringer's showed more errors on the foot fault task than untreated animals ($p < 0.01$). In the open field task, there were no significant differences. In the Morris water maze, the normal saline group demonstrated a faster latency to the platform on day 1 of training than untreated animals ($p < 0.05$). Furthermore, in the social preference task, untreated animals demonstrated more interactions with the novel rat compared to Ringer's-treated ($p < 0.05$) and saline-treated animals ($p < 0.01$). Finally, there were no significant differences between groups on the social dominance task. Additional histological analyses are ongoing. Our hypothesis regarding the effects of Ringer's solution on behavior was partially supported. Since Ringer's solution is known to increase edema following injury, this could be increasing diaschisis during recovery. Diaschisis represents the process of functional disruption at the lesion site followed by some recovery of function via compensatory mechanisms (Feeney & Baron, 1986). Therefore, saline- and Ringer's-treated animals demonstrated more functional disruption following injury. But as time went on, there were few differences observed between the groups, suggesting that functional recovery from diaschisis occurred. Future analysis of the lesion volume will help to illustrate whether these solutions create the observed behavioral effects.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

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Program #/Poster #: 459.24

Topic: C.10. Brain Injury and Trauma

Support: T32 AG071474

Title: The role of tau acetylation in mediating primary cilia collapse in neurodegeneration

Authors: *E. MILLER¹, P. S. SRIDHARAN¹, Y. KOH¹, S. BARKER¹, C. J. CINTRÓN-PÉREZ², K. Z. FRANKE², E. VÁSQUEZ-ROSA², T. E. TRACY³, L. GAN⁴, M.-K. SHIN², A. A. PIEPER^{5,6,7,8};

¹Neurosciences, Case Western Reserve University, Sch. of Med., Cleveland, OH; ²Psychiatry, Univ. Hosp. Cleveland Med. Ctr., Cleveland, OH; ³Buck Inst., Buck Inst., Novato, CA; ⁴Weill Cornell Med., Weill Cornell Med., New York, NY; ⁵Univ. Hosp., Univ. Hosp. of Cleveland, Cleveland, OH; ⁶Neurosciences, Case Western Reserve Univ. Sch. of Medicine, Cleveland, OH, Cleveland, OH; ⁷Harrington Discovery Institute, Univ. Hosp. Cleveland Med. Ctr., Cleveland, OH; ⁸Geriatric Res. Educ. and Clin. Ctr. (GRECC), Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH

Abstract: There is a tremendous unmet need for neuroprotective strategies, and emerging evidence has linked structural loss of neuronal primary cilia to different neurodegenerative conditions. An early event that occurs in neurodegeneration is pathological acetylation of microtubule stabilizing protein tau. The structural backbone of primary cilia is composed of microtubules, which bind tau protein. Currently, there is a significant gap in our understanding of what drives primary cilia degeneration, and whether protection of primary cilia can prevent or stop neurodegeneration. Tau also binds microtubules in neuronal axons, where its acetylation drives axonal degeneration. Thus, we hypothesize that pathological tau acetylation similarly drives primary cilia collapse. Here, we have modeled neurodegeneration in mice by inducing traumatic brain injury (TBI). Our multimodal model of TBI produces a complex yet rigorously reproducible brain injury with neurodegeneration and neurobehavioral impairment, beginning with acute axonal degeneration and persisting chronically with blood-brain barrier degradation and nerve cell death. This multimodal TBI also produces the same systemic metabolic alterations that have been reported in TBI patients. Adult male and female C57/bl6 mice were subjected to either TBI or sham injury at 2 months of age. Six hours after injury, we observed that tau is pathologically acetylated in hippocampus, and 24 hours after injury we observed that the length of primary cilia collapses in the same region. To investigate whether pathological tau acetylation could be driving neuronal primary cilia collapse, we visualized the structure of this organelle in a genetic mouse model (TauKQhigh) that mimics pathological tau acetylation after TBI. We found that neuronal primary cilia length was also shortened in these mice in the same region of the brain as neuronal primary cilia after injury. This positions the primary cilia as a previously unrecognized locus of injury that may drive neurodegeneration. Future direction will focus on investigating the mechanism by which pathological acetylation of tau disrupts primary cilia microtubules, as well as how this phenomenon may contribute to neurodegeneration.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.25

Topic: C.10. Brain Injury and Trauma

Support: NIH-NINDS R01NS113950

Title: Time-course changes in serum levels of NfL, GFAP, and UCH-L1 throughout a high school football season

Authors: *T. R. ZUIDEMA, D. J. RETTKE, I. L. ALEXANDER, N. CHENCHAIAH, R. Z. BONDAY, K. A. KERCHER, J. A. STEINFELDT, K. KAWATA;
Indiana Univ., Bloomington, IN

Abstract: This study investigated the time-course trend of serum neurofilament-light (NfL), glial fibrillary acidic protein (GFAP), and ubiquitin c-terminal hydrolase L1 (UCH-L1) over the course of a high school football season. A total of 99 healthy high school American football players participated in the study and attended data collection sessions at preseason, three mid-season time points (August, September, and October), and a post-season follow up. At each time point, blood was collected from the upper arm using Tasso-SST devices. Blood samples clotted for 30 minutes at room temperature before centrifugation. Serum was aliquoted and stored at -80°C until analysis. NfL, GFAP, and UCH-L1 were measured using Simoa® assay kits and a Quanterix SR-X™ Biomarker Detection System. Changes in biomarker expression across the season were assessed using mixed-effect regression models. Serum GFAP was significantly elevated at all mid-season time points (August: B=9.29, SE=2.84, p=0.001; September: B=21.43, SE=2.82, p<0.001; October: B=24.40, SE=2.84, p<0.001) and at post-season follow up (B=13.43, SE=2.83, p<0.001), compared to preseason baseline. Serum UCH-L1 was also significantly elevated at all mid-season time points (August: B=122.43, SE=14.31, p<0.001; September: B= 179.71, SE=14.27, p<0.001; October: B=188.51, SE=14.32, p<0.001) and at post-season follow up (B=183.60, SE=14.27, p<0.001). Serum NfL was significantly elevated at the October mid-season time point (B=1.49 [SE=0.39], p<0.001) compared to preseason baseline. However, NfL concentrations at the August and September mid-season time points, in addition to the post-season follow up, did not significantly differ from baseline. These findings suggest that football players may experience astrocyte activation and an increase in cellular proliferation, as indicated by sustained elevation of GFAP and UCH-L1 levels, which persisted past the end of the season. Additional investigation is needed to understand the late season increase in serum NfL and, more broadly, the relationship between blood biomarker expression and contact sports participation.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.26

Topic: C.10. Brain Injury and Trauma

Support: Department of Veterans Affairs Offices of Research & Development (VA ORD) BLR&D Director Service program (UFR-002-18F)
Open-Field Blast (OFB) Core
Collaborative Merit Review for TBI Research Program (I01 BX004313-01A1)
DoD Congressionally Directed Medical Research Programs (CDMRP) for the Peer Reviewed Alzheimer's Research Program Convergence Science Research Award (PRARP-CSRA; AZ180043)
Research funds of the University of Missouri

Title: Ultrastructural Abnormalities of the Neurovascular Unit Induced by Low-Intensity Blast Exposure in Mice

Authors: *H. R. SIEDHOFF^{1,2}, C. LI^{1,3}, S. CHEN^{1,2}, D. GRANT⁴, P. LIU⁵, A. BALDERRAMA^{1,2}, C. GREENLIEF⁵, R. G. DEPALMA^{6,7}, I. CERNAK⁸, J. CUI^{1,2}, Z. GU^{1,2}; ¹Dept. of Pathology and Anatom. Sci., Univ. of Missouri Sch. of Med., Columbia, MO; ²Truman VA Hosp. Res. Service, Columbia, MO; ³Dept. of Rehabil. Med., The Third Affiliated Hospital, Sun Yat-sen Univ., Guangzhou, Guangdong, China; ⁴Electron Microscopy Core Facility, ⁵Charles W. Gehrke Proteomic Ctr., Univ. of Missouri, Columbia, MO; ⁶Office of Res. and Develop., DVA, Washington, DC; ⁷Dept. of Surgery, Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; ⁸Dept. of Biomed. Sci., Mercer Univ. Sch. of Med., Columbus, GA

Abstract: Mild traumatic brain injury (mTBI) caused by low-intensity blast (LIB) exposure causes numerous health impairments, including a broad range of neurological deficits. The neurovascular unit (NVU), comprised of multiple components, plays a vital role in regulating cerebral blood flow and cellular communications, and is commonly impaired in neurological diseases. Previously, we reported LIB-induced ultrastructural impairments of myelin sheaths, axons, mitochondria, and synapses with no gross neuropathology in mice. In the present study, we provide an extended assessment of the LIB-induced ultrastructural damages involving the NVU. Anesthetized C57BL/6J mice in prone position were exposed to open-field LIB. The animals were positioned 1-meter above ground and 3-meters from a 350g C4-explosion, which generated a 46.6 kPa blast peak-overpressure with a maximal impulse of 60 kPa × ms. No mouse head- or body-movements were observed. Transmission electron microscopy (TEM) with quantitative and qualitative analyses revealed ultrastructural abnormalities of luminal

irregularities, indicative of vasoconstriction at 7 days post injury (DPI) and vasodilation at 30 DPI. Pericyte degeneration was identified at 30 DPI. Quantitative proteomics assays revealed alterations of vasomotor-related proteins at 1 DPI. Ultrastructural evidence of endothelial cell, basement membrane, and astrocyte end-foot swellings, as well as vacuole formations suggested cellular edema in LIB-exposed mice. Moreover, LIB exposure resulted in tight junction abnormalities and astrocyte end-foot detachment from the basement membrane, indicating impaired integrity of the NVU. These findings provide further insight into the neurovascular effects of LIB exposure, and offer a platform that could aid in the development of future therapeutic targets.

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Poster

459. Brain Injury: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 459.27

Topic: C.10. Brain Injury and Trauma

Support: RGPIN202006590
PJT376309

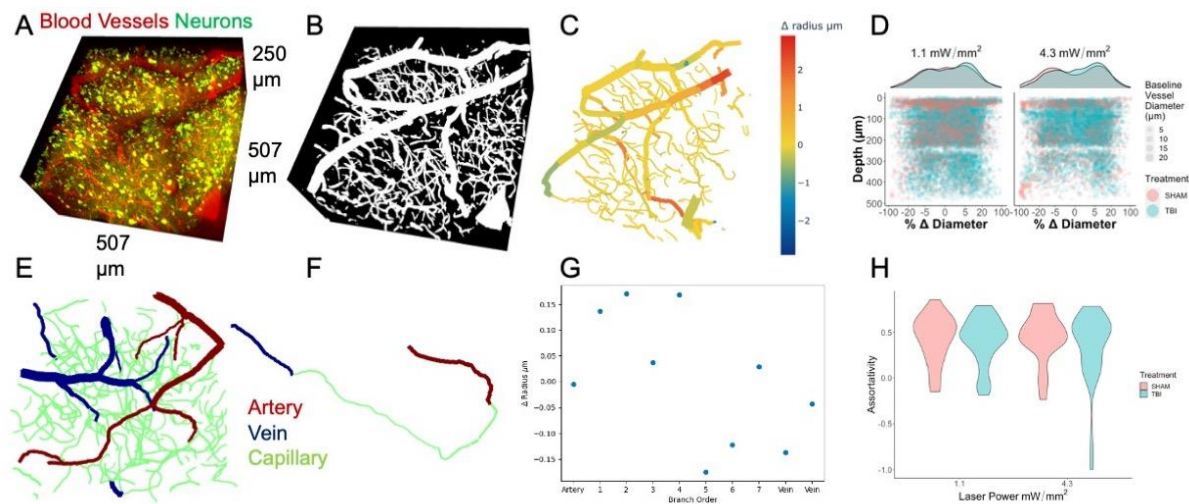
Title: Deep-learning insights into cerebrovascular networks coordination in moderate traumatic brain injury

Authors: *M. W. ROZAK^{1,2}, J. R. MESTER^{1,2}, A. DORR², M. GOUBRAN^{1,2}, B. STEFANOVIC¹;

¹Med. Biophysics, Univ. of Toronto, York, ON, Canada; ²Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: Traumatic brain injury (TBI) often elicits sustained injury to brain vessels. While cerebrovascular damage is thought to limit long term recovery, little is known about the cellular level changes in the brain microvascular networks. Two photon fluorescence microscopy (2PFM) allows for *in vivo* characterization of the microvascular geometry. We developed a deep learning platform for high throughput analysis of cerebrovascular networks, based on *in vivo* 2PFM imaging in murine somatosensory cortex. We applied this novel pipeline in a model of repeated moderate TBI (mTBI), involving three impacts (or sham) with a three day inter-impact interval in Thy1-ChR2-YFP mice (11 TBI, 7 sham). Two weeks following the final impact, mice were implanted with cranial windows centred over the impact location, and the underlying cortex was imaged on 2PFM during baseline periods alternated with blue light photostimulation. We developed deep learning segmentation models with a 3D UNETR architecture for segmentation and vessel categorization. The segmentation model was trained with data from 15 mice,

validated on 4 mice, and tested on 6 mice. The model achieved an F1 score of 0.77 on validation images. To enable detailed morphological analysis, the segmented cerebrovascular networks were rendered as graphs (C), and vessel type and branch order were mapped onto the graphs. The pipeline produced a wealth of structural information on cerebrovasculature and vascular network reactivity, permitting detailed analysis of different vessel types' morphology changes following stimulation (C). Following optogenetic stimulus, the bimodal vascular radii change distribution shifted from a balance of contractions and dilations in sham mice towards dilations in TBI mice shifting the average change $+0.16 \mu\text{m}$ (D). We identified numerous vessel paths from penetrating arteries to penetrating veins and mapped the vessel reactivity along each path (E-G). Our pipeline's application in the subacute phase of mTBI revealed spatial patterns of altered cerebrovascular reactivity and coordination in the concussed cortex.



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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.01

Topic: C.10. Brain Injury and Trauma

Support: Funded By Biosplice Therapeutics, Inc

Title: Dyrk1a inhibition reduced tau hyperphosphorylation, neuroinflammation and improved locomotor function, in a mouse model of repeat mild traumatic brain injury

Authors: B. MELCHIOR¹, M. BROWNING², R. MCCARTAN², C. LAI¹, M. GRIFMAN¹, C. HAHN-TOWNSEND², A. GRATKOWSKI², A. MORIN², F. C. CRAWFORD², *B.

MOUZON^{2,3};

¹Biosplice Therapeutics, Inc, San Diego, CA; ²The Roskamp Institute, Inc., Sarasota, FL; ³James A. Haley Veterans' Hosp., Tampa, FL

Abstract: Dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) is a serine/threonine protein kinase which contributes to the phosphorylation of Tau (pTau) and regulates several immune response mediators. Chronic neuroinflammation and pTau deposits are a feature of several neurodegenerative pathologies and can be observed in material from survivors of mild and severe traumatic brain injury (TBI). This pathology is characterized by the accumulation of abnormal, hyperphosphorylated Tau in neurons, glia, or both. Here, we explore DYRK1A inhibition as a potential treatment to limit the neurodegenerative consequences of repetitive mild TBI (r-mTBI) in a transgenic mouse model. Transgenic mice expressing human tau (hTau) were subjected to r-mTBI weekly over a period of 3 months and treated with either vehicle or the potent and selective brain-penetrant DYRK1A inhibitor SM07883 (3 mg/kg by daily gavage for 5 months). A total of 64 male hTau mice (n=16 per group; 3 months old) were randomly assigned to one of 4 groups: r-sham/vehicle; r-sham/SM07883; r-mTBI /vehicle; r-mTBI/SM07883. Neuropathological assessments were blindly conducted 6 months after the first injury. pTau was analyzed by western blot and neuroinflammation was assessed by immunohistochemistry and ELISA. Effects on behavior were measured by Rotarod, Barnes and Elevated Plus Maze tests. Mice treated with SM07883 exhibited significant reductions in pTau in the midbrain and brain stem compared to vehicle. Reduced TBI-dependent gliosis in the corpus callosum, brain stem and in the deeper cortical layers beneath the injury site was also a physiological consequence of DYRK1A inhibition. Associated with these neuropathological changes, SM07883 treatment restored the locomotor deficit of the injured group to that of the sham vehicle performance. Motor deficit caused by natural aging was also improved in the r-sham/SM07883 mice when compared to r-sham/vehicle. However, no treatment effect on learning or spatial memory was reported using the Barnes or Elevated Plus Maze neurobehavioral tests. These data demonstrated that DYRK1A inhibition reduced chronic neuroinflammation, pTau accumulation and restored the locomotor deficits related to repetitive mild traumatic brain injury in this context.

Disclosures: **B. Melchior:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biosplice Therapeutics, Inc. **M. Browning:** None. **R. McCartan:** None. **C. Lai:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biosplice Therapeutics, Inc. **M. Grifman:** A. Employment/Salary (full or part-time);; Biosplice Therapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biosplice Therapeutics, Inc. **C. Hahn-Townsend:** None. **A. Gratkowski:** None. **A. Morin:** None. **F.C. Crawford:** None. **B. mouzon:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Biosplice Therapeutics, Inc.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.02

Topic: C.10. Brain Injury and Trauma

Support: the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D), Merit Review Award # 1 I01 RX003123-01A1
Brain Rehabilitation Research Center pilot grant

Title: Therapeutic impact of noradrenergic upregulation following closed head traumatic brain injury (CH-TBI)

Authors: *F. J. THOMPSON^{1,2}, J. HOU^{1,3}, S. TSUDA^{3,1}, D. PLANT¹, S. E. NADEAU^{1,4}, P. BOSE^{1,3,4};

¹North Florida/South Georgia Veterans Hlth. Syst., Gainesville, FL; ²Neurosci., ³Anesthesiol., ⁴Neurol., Univ. of Florida, Gainesville, FL

Abstract: TBI induces significant and enduring cognitive, anxiety, and motor disabilities. Our previous and current studies demonstrate significant loss of the locus coeruleus neurons, projection fibers, and noradrenergic (NA) innervation to cognitive, anxiety, and motor neural substrates following acceleration/deceleration CH-TBI; these losses correlated with alterations in cognitive, anxiety, and motor functions. NA projections are known to be critical for regulation of neuronal excitability, BDNF production, inflammatory signaling, and blood brain barrier (BBB). Accordingly, use of agents that target and upregulate NA expression, correlated with measures of TBI-induced cognitive, anxiety, and motor disabilities, could provide additional insights into the role of NA injury and NA therapy in TBI pathobiology. We used Methylphenidate, which prolongs the re-uptake of NE increasing the effectiveness of intrinsically distributed NE. In addition, we tested therapy with phenylephrine and ephedrine (direct adrenergic agonists) to evaluate the more selective contribution of adrenergic alpha-1 vs alpha & beta receptor activation, respectively, for treatment efficacy. These studies were carried out in normal, CH-TBI, and CH-TBI/treated animals. Mild/moderate TBIs were produced by a 450-gram weight drop (1.25 meter) impact on the helmeted head. Methylphenidate (0.5 mg/kg; oral), phenylephrine (0.6 mg/kg; SQ), and ephedrine (3.8 mg/kg; SQ) treatments were initiated one week following injury, and continued daily for three weeks. Behavioral measures were tested during the fourth post-injury week. Collectively, significantly decreased disability scores were observed in the treated animals compared to saline-treated TBI animals. However, the therapeutic impact of methylphenidate was most robust and consistent across all three behavioral measures. For example, when tested at 4 weeks post injury, cognitive scores (escape latency, Morris Water Maze) were increased by 98.0% in TBI/saline treated animals compared to escape latency scores in normal animals. By contrast, escape latencies in Methylphenidate treated animals were increased by 35% compared to scores in normal animals, reflecting a 63% reduction in disability score. Similar ratios of improved scores were observed for elevated plus maze (anxiety) and velocity dependent ankle torque (spasticity) in methylphenidate treated animals compared to TBI/saline treated controls.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.03

Topic: C.10. Brain Injury and Trauma

Support: the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D), Merit Review Award # 1 I01 RX003123-01A1

Title: Iron Chelator therapy attenuated hemorrhagic iron toxicity in neural substrates for motor, cognitive and anxiety disorders in a rodent model of traumatic brain injury (TBI)

Authors: *P. K. BOSE^{1,2,3}, J. HOU^{1,2}, S. TSUDA^{2,1}, D. PLANT¹, B. W. RENGERT¹, K. ZHANG¹, H. C. SHROCK¹, G. M. DOOLEY¹, R. J. BERGERON, Jr.⁴, F. J. THOMPSON^{1,5}; ¹North Florida/South Georgia Veterans Hlth. Syst., Gainesville, FL; ²Anesthesiol., ³Neurol., ⁴Medicinal Chem., ⁵Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Traumatic brain injury induces damage of micro-vessels which results in endothelial shear injury, blood brain barrier (BBB) dysfunction, and micro-hemorrhage. Microhemorrhage-derived iron can provide enduring oxidative stress and inflammation, further breakdown of the BBB tight junctions, and cell death through multiple inflammatory pathways. The current studies tested the therapeutic impact of an iron chelator on hallmark chronic disabilities in a rodent model of closed head (CH-) acceleration deceleration TBI. Mild/moderate CH-TBI was produced using our previously reported protocol (450 g/1.25m). An hexadentate iron chelator, NaHBED treatment was initiated at PO Day-0 and continued for 2 weeks (50 mg/kg/day, SQ; n=12). The control animals received an equal volume of saline (SQ; n=10). Tests for motor, anxiety, and cognitive functions were conducted at multiple time points during 9 months of post-injury study. Clinically relevant MRI (SWI/QSM, T2* map, and DTI), immunohistochemistry (IHC), and histology were performed to chart the time course for iron deposition and inflammation. Our data to date revealed long-term enduring disabilities in motor/vestibulomotor, anxiety, and cognitive behaviors following CH-TBI, and significant reductions in these disabilities in the NaHBED treated animals. IHC and histology studies of TBI tissues showed patterns of a) iron deposition and disruption of BBB, b) increased expression of markers for inflammation (activation of NF-kB via TLR4 pathway, proinflammatory cytokines, proIL-1 β , TNF α , etc.), c) pyroptosis of neuronal cells in specific regions essential for the studied motor, cognitive, and anxiety behaviors, and d) loss of regulatory noradrenergic and trophic supports in these regions. Tissue from the NaHBED-treated animals exhibited robust normalization of each of these markers. Collectively, our studies demonstrate that: a) TBI-induced microhemorrhage contributes to the development and persistence of multiple chronic disabilities, b) iron deposited

via TBI induces BBB disruption, and accelerates neuroinflammation and neuronal cell death, and c) this collective trauma portfolio of chronic disability and inflammation were attenuated by an iron chelator therapy. Taken together, iron chelator treatment offers the potential for a mechanism-based therapy that addresses a significant contributor of long-term TBI disabilities, contributes to trophic support for neuronal and vascular healing, and enhances neuroplasticity for adaptive compensation.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.04

Topic: C.10. Brain Injury and Trauma

Support: DoD CDMRP
Owens Foundation of San Antonio

Title: Pre-clinical Data and Mechanistic Information Confirm Potent Prevention of Both Short- and Long-Term Brain Damage and Dysfunction, After Two Types of Traumatic Brain Injury, by Acute Administration of Pharmacological "Openers" of Kv7 (KCNQ, "M-type") K⁺ Ion Channels

Authors: F. A. VIGIL¹, I. BAZALDUA¹, D. CORTES⁴, H. A. BELCHIOR⁴, S. S. SHYAM², A. STOJA¹, S. CHUN³, V. BUGAY³, D. HOLSTEIN³, M. PAUKERT¹, J. D. LECHLEITER³, R. BRENNER¹, *M. S. SHAPIRO¹;

¹Cell & Integrative Physiol., ²Sch. of Med., ³Cell Systems & Anat., Univ. Texas Hlth. Sci. Ctr., San Antonio, TX; ⁴Brain Inst., Natal, Brazil

Abstract: Traumatic brain injury (TBI) often results in post-traumatic seizures (PTS), long-term post-traumatic epilepsy (PTE) and chronic traumatic encephalopathy (CTE). Kv7 ("M-type," KCNQ) voltage-gated K⁺ channels underlie the neuronal "M current," which plays a dominant role in control over neuronal excitability throughout the nervous system. We have previously shown in a blunt TBI mouse model that acute pharmacological increase of Kv7 K⁺ currents impairs various short-term deleterious effects of TBI. We here tested if the same treatment could reduce or block long-term effects of multiple air shock-wave blast TBIs. Additionally, we tested possible mechanistic hypotheses for these effects of acute post-TBI treatment. In our continued line of inquiry involving a single-blunt TBI mouse model, we determined the optimal dose and therapeutic window for this acute treatment, testing these parameters for the prototype Kv7.2-7.5 "opener," retigabine, and of the newer, more potent, and more selective Kv7.2/7.3 opener, RL648_81. We also evaluated the effects of the treatment on blood-brain barrier (BBB)

permeability and neuronal excitability. We also used a repetitive blast-TBI mouse model to test if this same approach occludes the development of PTE, CTE and hypersomnia. Electroencephalogram (EEG) and video recording revealed 1 mg/kg RTG (*i.p.*) to be the optimum dose. Interestingly, RTG was more effective than RL648_81 in preventing acute post-TBI seizures, reduction in BBB breakdown acutely (2h) after TBI. RTG injection up to 1 h after injury was most effective. *In vivo* two-photon microscopy using a glass “window” over the cortex of transgenic mice expressing the genetically-encoded Ca²⁺ sensor, GCAMP6f, showed RTG to prevent acute TBI-induced neuronal hyperexcitability, disruption of network firing, and expression of a [glutamate] “sniffer” via AAV-packaged Cre-driven stereotaxic expression showed RTG to prevent TBI-induced elevations of glutamatergic excitation. RTG treatment also occluded the repetitive mild TBI-induced development of hypersomnia. From 9-12 months post-TBI, acute post-TBI treatment with RTG sharply reduced PTE development, hyper-expression of the CTE marker, TDP-43, and impairment of the EEG gamma frequency necessary for proper cognition during aging. Thus, acute pharmacological enhancement of Kv7 currents may be the first treatment available for preventing short-term and long-term deleterious effects that follow one or multiple TBIs, thus also preventing the development of long-term chronic brain diseases and dysfunctions, such as PTE and CTE. Supported by the Owens Foundation of San Antonio and the Department of Defense CDMRP.

Disclosures: **F.A. Vigil:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-Inventor of Patent Application by Advanced Neuroresearch Therapeutics, L.L.C.. **I. Bazaldua:** None. **D. Cortes:** None. **H.A. Belchior:** None. **S.S. Shyam:** None. **A. Stoja:** None. **S. Chun:** None. **V. Bugay:** None. **D. Holstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Support from Astrocyte Pharmaceuticals. **M. Paukert:** None. **J.D. Lechleiter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Chief Scientific Officer and Patent Holder, Astrocyte Pharmaceuticals. **R. Brenner:** None. **M.S. Shapiro:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CEO of Advanced Neuroresearch Therapeutics, L.L.C., Patent Pending.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.05

Topic: C.10. Brain Injury and Trauma

Support: US Department of ACCCRP award number W81XWH-20-C-0114

Title: Point of injury treatment with hydrogel containing dexamethasone improves cognitive function in a rat moderate controlled cortical impact TBI model

Authors: *B. ELLIOTT¹, C. JONES¹, F. MA¹, Z. JOHNSON¹, Z. LIAO¹, K. WEBB¹, Z. S. BAILEY², J. S. GILSDORF², A. H. SCULTETUS², D. A. SHEAR², J. LEE¹;
¹Bioengineering, Clemson Univ., Clemson, SC; ²Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Point of injury treatment with hydrogel containing dexamethasone improves cognitive function in a rat moderate controlled cortical impact TBI model Functional recovery after traumatic brain injury (TBI) is hindered by progressive neurodegeneration resulting from neuroinflammation and other secondary injury responses. In our published work, we developed PEG-bis-AA/HA-DXM hydrogels composed of polyethylene glycol-bis-(acryloyloxy acetate) (PEG-bis-AA) and dexamethasone-conjugated hyaluronic acid (HA-DXM). We observed that PEG-bis-AA/HA-DXM hydrogel treated animals exhibited significantly improved motor function by rotarod test and cognitive function by Morris water maze test compared to untreated TBI animals and reduced inflammatory response, apoptosis, and lesion volume compared to untreated TBI animals at 14 DPI in a rat mild controlled cortical impact (CCI) TBI model. In this study, we evaluated the effect of PEG-bis-AA/HA-DXM hydrogel on motor function and cognitive function in a rat moderate CCI TBI model. The moderate CCI TBI model was generated using a CCI device armed with a 5 mm blunt tip to deliver an injury at a velocity of 4 m/sec and a depth of 2.5 mm after the craniectomy (performed using a 6 mm diameter Trephine bur tip over the right cortex at 1mm posterior and 3 mm lateral of bregma). The rats were randomly assigned to three groups: 1) Normal group: no surgery, 2) Untreated TBI group, and 3) PEG-bis-AA/HA-DXM gel treated group (3µg of DX/hydrogel). After injury, PEG-bis-AA/HA-DXM gels were placed on the top of the injured brain and the skin was sutured. Motor function recovery after TBI was evaluated by open field test at 3, 5, and 7 DPI. Cognitive function recovery after TBI was evaluated with the Morris Water Maze (MWM) test starting on 8 DPI and continuing for 5 training days and a final probe test at 14 DPI. After the functional studies, rats were sacrificed via cardiac perfusion with saline followed by 4% PFA under deep anesthesia. For motor function, hydrogel treated rats traveled longer total distance than untreated rats in open field test at all time points. For cognitive function, we observed that hydrogel treated rats demonstrated a decreased time to find hidden platform (target), decreased distance to swim to hidden platform, and decreased percent time to swim in border when compared to TBI untreated rats on both training period and the probe test. We also observed reduced lesion volume in hydrogel-treated groups compared to the untreated TBI group by NISSL staining. Currently, we are performing IHC staining for biomarkers related to the inflammatory response and neuroprotection. Funding: US Department of ACCCRP award number W81XWH-20-C-0114

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.06

Topic: C.10. Brain Injury and Trauma

Support: T32GM007250
NS077888
P30CA43703

Title: Acute inhibition of mitochondrial fission confers long-lasting neuroprotection after traumatic brain injury

Authors: *P. S. SRIDHARAN¹, Y. KOH², E. MILLER¹, M. DHAR³, E. F. VAZQUEZ-ROSA³, M.-K. SHIN³, K. FRANKE³, H. FUJIOKA⁵, T. KEE^{2,8}, D. E. KANG^{2,9}, J. WOO^{2,9}, X. QI⁴, A. A. PIEPER^{6,1,10,11,7},

¹Dept. of Neurosciences, ²Dept. of Pathology, ³Dept. of Psychiatry, ⁴Dept. of Biophysics, Case Western Reserve Univ. Sch. of Med., Cleveland, OH; ⁵Cryo-Electron Microscopy Core, ⁶Dept. of Psychiatry, ⁷Inst. for Transformative Mol. Med., Case Western Reserve Univ., Cleveland, OH; ⁸Dept. of Mol. Med., Univ. of Southern Florida Col. of Med., Tampa, FL; ⁹USF Hlth. Byrd Inst., USF Hlth. Byrd Inst., Tampa, FL; ¹⁰Harrington Discovery InstituteHarrington Discovery Inst., Univ. Hosp., Cleveland, OH; ¹¹Geriatric Res. Educ. and Clin. Ctr. (GRECC), Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH

Abstract: In the past decade, there has been an increasing incidence of traumatic brain injury (TBI), which is associated with a 2-4-fold increase in risk for developing dementia later in life. TBI is a chronic neurodegenerative condition, and there are no available treatments that slow its progression. We are modeling TBI in our laboratory using an injury system that induces components of global concussion, acceleration/deceleration, and early blast wave exposure. TBI patients are at an especially elevated risk of developing Alzheimer's disease (AD), and mitochondrial fission has been identified as an important component of pathogenesis in AD. Here, we have investigated the potential efficacy of a pharmacologic peptide inhibitor of mitochondrial fission, called P110, in mitigating the progression of neurodegeneration and symptoms after TBI. Male and female C57BL/6J mice were subjected to either TBI or sham injury at 2 months of age, and subsequently treated daily with intraperitoneal injection of either P110 or vehicle control. Mice were later evaluated in the novel object recognition (NOR) and open field tests, and brain tissue was processed for biochemistry, transmission electron microscopy (TEM), immunohistochemistry, and mitochondrial bioenergetics. Drp1, the primary mediator of mitochondrial fission, is the target of P110. After TBI, mice show an elevation in Drp1 expression in the brain 24 hours later, with levels returning to normal after 2 weeks. At the two week time point, mitochondrial bioenergetics and silver stain analyses show hippocampal impairment, and mice perform poorly in the NOR test of cognition. Mitochondrial fragmentation is also prominent at this time point. Notably, the acute treatments with P110 blocked all these deleterious effects after TBI. Moreover, when treatment with P110 was ceased at the two week time point after injury, neurocognitive protection, reduced axonal degeneration, and normal mitochondrial morphology was still observed 9 months later. When P110 treatment initiation was delayed until 8 months after injury, it did not restore normal cognition, suggesting a key therapeutic window for inhibition of mitochondrial fission at the acute stage of injury. All experiments were blinded for analysis. Our results indicate that early inhibition of mitochondrial fission after TBI is protective against neurodegeneration and cognitive impairment, and that this

brief treatment produces a lasting protective effect from chronic memory deficits as well. Future directions will focus on investigation of the mechanisms by which acute imbalance of mitochondrial fission and fusion after TBI mediates progressive neurodegeneration.

Disclosures: P.S. Sridharan: None. Y. Koh: None. E. Miller: None. M. Dhar: None. E.F. Vazquez-Rosa: None. M. Shin: None. K. Franke: None. H. Fujioka: None. T. Kee: None. D.E. Kang: None. J. Woo: None. X. Qi: None. A.A. Pieper: None.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.07

Topic: C.10. Brain Injury and Trauma

Support: NRF Grant 2020R1A2C2008480
KHIDI Grant HI19C1347
NRF Grant 2022R1C1C2006049

Title: Focused ultrasound mediated Temozolomide delivery into intact blood-brain barrier tissue improves survival of patient derived xenograft rat model of glioblastoma

Authors: *J. SHIN^{1,2}, J.-K. SIM¹, C. KONG¹, Y. SEO¹, J. CHANG¹, S.-G. KANG¹, W. CHANG¹;

¹Dept. of Neurosurg., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; ²Biol. Sci., Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: Objective: Glioblastoma (GBM) is the most common and aggressive type of primary brain tumor in adult, characterized by highly proliferative and infiltrative activity into the normal brain. Currently, GBM shows >80% of recurrences in the area adjacent to the resection site even after the maximal resection of tumor and temozolomide (TMZ) chemotherapy. The effectiveness of treatment is limited not only due to drug resistance but also due to restriction of passage due to the blood-brain barrier (BBB). In this study, we investigated the effect of FUS to transiently open BBB and optimal delivery time point of the TMZ to the human GBM infiltrated into the normal brain before tumor neo-vascularization in patient derived xenograft models (PDX).

Methods: PDX model was made by injecting human GBM tumor-spheres (TSs; TS 15-88) into the striatum of male athymic nude mice (4-8 weeks). For the early stage of PDX model, TMZ was injected and FUS was applied to the striatum a week after GBM TSs implantation. BBB permeability was identified with Evans blue (EB) extravasation, MRI with gadolinium-enhanced T1-weighted image and tight junction protein of ZO-1 expression level, and infiltrated GBM TSs into brain tissue was confirmed by IHC of ZEB-1 staining and H&E staining in wild type, control PDX, and the FUS PDX group. To evaluate the therapeutic effect of combined treatment of GBM TSs with FUS and TMZ, bioluminescence and survival rate were analyzed. **Results:** We confirmed that BBB permeability was not significantly different between wild type and PDX

models. The FUS-induced BBB opening significantly increased EB extravasation and significantly reduced the expression levels of ZO-1. Bioluminescence imaging showed that the combination of FUS and TMZ considerably reduced the proliferation of GBM TSs in PDX model. Combined treatment of GBM TSs with FUS and TMZ significantly increase in survival rate compared to the control and TMZ single treatment. **Conclusions:** In the present study, we demonstrated that FUS with TMZ can enhance treatment effect more than standard chemotherapy in PDX model for therapy of GBM TSs before tumor neo-vascularization. This combined therapy has the potential to reduce recurrences and tumor growth and serve as a novel therapeutic protocol for the treatment of GBM patients.

Disclosures: **J. Shin:** None. **J. Sim:** None. **C. Kong:** None. **Y. Seo:** None. **J. Chang:** None. **S. Kang:** None. **W. Chang:** None.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

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Program #/Poster #: 460.08

Topic: C.10. Brain Injury and Trauma

Support: NIA Intramural Research Program
NIDA Intramural Research Program
MOST Taiwan

Title: Intravenous administration of pomalidomide protects cerebral cortex and striatum from neurodegeneration, oxidative damage and neuroinflammation after traumatic brain injury

Authors: N. H. GREIG¹, B. HOFFER², *B. HARVEY², Y.-H. CHIANG³, **J.-Y. WANG³**;
¹Natl. Inst. on Aging, Baltimore, MD; ²NIDA - NIH, Baltimore, MD; ³Taipei Med. Univ., Taipei, Taiwan

Abstract: Neuronal damage resulting from traumatic brain injury (TBI) causes disruption of neuronal projections and neurotransmission that contribute to behavioral deficits. Generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is an early event following TBI. ROS cause the oxidation of lipids and proteins while RNS attack proteins. The products of lipid peroxidation 4-hydroxynonenal (4-HNE) and protein nitration 3-nitrotyrosine (3-NT) are often used as an index of oxidative damage. Oxidative injury is a major pathway driving neurodegeneration. Here we further compared the effects of Pom on cortical and striatal tissue focusing on neurodegeneration, oxidative damage, and neuroinflammation following TBI. Sprague-Dawley rats, subjected to a controlled cortical impact, were used as the TBI model. Pom (0.5 mg/kg, i.v.) given at 5 h after TBI significantly reduced neurological deficits, contusion volume, and degenerating neurons stained by Fluoro-Jade C at 24 h post-injury. Pom treatment alleviated TBI-induced oxidative damage is evidenced by fewer cortical and striatal neurons exhibiting 4-HNE and 3-NT. Additionally, Pom attenuated microgliosis, astrogliosis and

elevations of proinflammatory cytokines in cortical and striatal tissue. We conclude that Pom represents a potential therapy to mitigate TBI-induced neurodegeneration, oxidative damage and neuroinflammation in cortical-striatum resulting in improved behavioral outcome.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.09

Topic: C.10. Brain Injury and Trauma

Support: Intramural Research Program NIH

Title: Activity of a novel anti-inflammatory agent F-3,6'-dithiopomalidomide as a treatment for traumatic brain injury

Authors: *B. J. HOFFER¹, W. R. SELMAN¹, S.-C. HSUEH², D. KIM³, N. H. GREIG²;
¹Dept. of Neurosurg., Case Western Reserve Univ. Sch. of Med., Cleveland, OH; ²Drug Design & Develop. Section, Intramural Res. Program Natl. Inst. on Aging, NIH, Baltimore, MD;
³Aevisbio Inc, Gaithersburg, MD

Abstract: Background: Traumatic brain injury (TBI) has been identified as a major risk factor for several neurodegenerative disorders, including Parkinson's Disease (PD) and Alzheimer's Disease (AD). Neuroinflammation is considered the cause of later secondary cell death following TBI, has the potential to chronically aggravate the initial impact, and provides a therapeutic target albeit that has widely failed to translate into clinical trial success. Thalidomide-like compounds have well-documented neuroinflammation reduction properties across cellular and animal models of TBI and neurodegenerative disorders. They lower the generation of proinflammatory cytokines, particularly TNF- α that is pivotal in microglial cell activation. Unfortunately, thalidomide-like drugs possess adverse effects in humans before achieving anti-inflammatory drug levels. Methods and Results: We developed F-3,6'-dithiopomalidomide (F-3,6'-DP) as a novel thalidomide-like compound to ameliorate inflammation that binds to the key protein cereblon, but does not trigger the ubiquitination of transcription factors (SALL4, Ikaros and Aiolos) associated with the teratogenic, anti-proliferative, and anti-angiogenic responses induced by this drug class. We utilized a phenotypic drug discovery approach that employed multiple cellular and animal models. All protocols were fully approved by the IACUC of NIA. Only male animals were used to avoid estrogen neuroprotection. Sample size was based on our previous studies. F-3,6'-DP significantly mitigated LPS-induced inflammation and TNF- α levels in F344 8 week old rats. We subsequently examined immunohistochemical, biochemical and behavioral measures following controlled cortical impact (CCI) in C57Bl6 8 week old mice, a well-characterized model of moderate TBI. F-3,6'-DP decreased CCI-induced

neuroinflammation, neuronal loss and behavioral deficits when administered after TBI, using commercial available reagents and blinded observers. In conclusion: F-3,6'-DP represents a novel class of thalidomide-like drugs with anti-inflammatory actions that possesses promising efficacy in the treatment of TBI and potentially longer-term neurodegenerative disorders. Funding: Intramural Research Program, NIA, NIH

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.10

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 1 R21 EB028055-01

Title: Delivery of Low-Intensity Pulsed Ultrasound in the Cortex to Improve Longevity and Performance of Neural Interfaces

Authors: ***R. BAGWELL**¹, N. N. TIRKO², A. S. ALSUBHI¹, J. K. GREASER¹, K. A. SNOOK¹, M. L. MULVIHILL¹;

¹Actuated Medical, Inc., Bellefonte, PA; ²Eberly Col. of Sci., The Pennsylvania State Univ., University Park, PA

Abstract: Chronic neural implants hold great potential for illuminating features of neural function, treating neurological disorders, and enabling the next generation of neuroprosthetics. Penetrating electrode arrays provide direct access to neural signals across the central and peripheral nervous system with high temporospatial resolution. However, a consistent point of failure for chronically implanted microelectrode arrays is poor longevity and variability in functionality of these devices. The foreign body response (FBR) can cause glial scarring and neural cell loss near the electrode sites. The FBR begins with electrode insertion, when damage to the blood brain barrier activates astrocytes and microglia, and continues throughout the lifetime of the implant due to the persistent presence of the foreign material in the tissue. Efforts to mitigate the FBR have focused on things like: limiting insertion damage during implantation, reducing the mechanical mismatch between brain and implant, and suppressing the FBR by incorporating exogenous chemicals.

Rather than relying on temporary interventions to limit the FBR, we proposed to chronically harness endogenous cortical function to improve the neural interface microenvironment. Here, we investigate the use of sub-threshold low-intensity pulsed ultrasound (LIPUS) to improve tissue health at the neural interface. LIPUS has recently been shown to have protective and healing effects in models of cerebral disease and injury, through promotion of brain-derived neurotrophic factor (BDNF) and other neurotrophic factors. Our studies demonstrate that

periodic application of localized LIPUS to tissue at the neural interface can promote improved electrophysiology signal quality, as measured via signal-to-noise ratios, electrode single-unit yields, and histological evaluation of glial scarring.

Disclosures: **R. Bagwell:** A. Employment/Salary (full or part-time);; Actuated Medical Inc. **N.N. Tirko:** F. Consulting Fees (e.g., advisory boards); Actuated Medical Inc. **A.S. Alsubhi:** A. Employment/Salary (full or part-time);; Actuated Medical Inc. **J.K. Greaser:** A. Employment/Salary (full or part-time);; Actuated Medical Inc. **K.A. Snook:** A. Employment/Salary (full or part-time);; Actuated Medical Inc. **M.L. Mulvihill:** A. Employment/Salary (full or part-time);; Actuated Medical Inc..

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.11

Topic: C.10. Brain Injury and Trauma

Title: Effect of SUN11602, a bFGF mimetic, in an in vivo model of Traumatic Brain Injury.

Authors: ***E. ESPOSITO**, A. FILIPPONE, A. CAPRA, M. CAMPOLO, I. PATERNITI, S. CUZZOCREA;
Univ. of Messina, Messina, Italy

Abstract: Background: Traumatic Brain Injury represents the main cause of death among young in the industrialized societies and compromises tissue integrity by causing the release of mediators of inflammation and apoptosis. Recently, basic fibroblast growth factor (bFGF) influences various biological processes such as cell growth and tissue repair, exerting neurotrophic activity in the Central Nervous System (CNS) by promoting neurites survival. The mimetic of bFGF, SUN11602, has been reported to show neuroprotective activities similar to those of bFGF, but with greater safety. Therefore, the aim of this study was to investigate the neuroprotective effects of SUN11602 in a mouse model of TBI. Methods: Traumatic Brain Injury was induced in animals by a controlled cortical impactor (CCI) by using the controlled impactor device Impact One™ Stereotaxic impactor for CCI. The craniotomy of the right hemisphere including the bregma and lambda between the sagittal suture and the coronal ridge was executed with a micro-motor handpiece and drill. A cortical contusion was performed through the controlled stereotaxic impactor on the uncovered cortex. This produced brain injury of moderate severity. Closely after injury, the skin cut was sutured with surgical staples by nylon thread and was applied 2% lidocaine jelly in the lesion in order to decrease pain. SUN11602 (1, 2.5, and 5 mg/kg) was administered intraperitoneally at 1hr and 4 hr after CCI. All groups were sacrificed after 24 h post-TBI-injury for histopathological and biochemical examinations. Results: SUN11602 treatment significantly reduced behavioral impairments and histological damage. Moreover, SUN11602 modulated neurotrophic factors as evidenced by Immunofluorescence staining. Furthermore, SUN11602 treatment attenuated the

neuroinflammatory and apoptosis states via modulation of glial activation, NF-kB pathway, cytokine overexpression such as Interleukin-1 β (IL-1 β) and IL-6, and Bcl-2, Bax, Caspase-3 expression. Additionally, we demonstrated that SUN11602 treatment rebalanced Ca²⁺ overload in neurons by regulating Ca²⁺-binding proteins. **Conclusions:** In conclusion, SUN11602 exerted great abilities for counteracting inflammation of CNS, apoptotic processes activation and also preserving neuronal survival by modulating neurotrophic factors in a mouse model of TBI. Thus, bFGF mimetic use could represent a potential therapeutic approach in resolving CNS traumatic events.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

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Program #/Poster #: 460.12

Topic: C.10. Brain Injury and Trauma

Support: New Jersey Health Foundation PC 29-21

Title: Simultaneous Motor-Cognitive Virtual Reality Training to Improve Ambulation in Young Adults with TBI

Authors: *K. J. NOLAN, K. K. KARUNAKARAN;
Ctr. for Mobility and Rehabil. Engin., Kessler Fndn., West Orange, NJ

Abstract: Traumatic Brain Injury (TBI) is one of the leading causes of motor and cognitive deficits in young adults. It often results in lower extremity motor control and balance impairments due to cortical changes induced by injury. Evidence suggests that cognitive function is positively correlated to physical function and that cognitive effort contributes to motor recovery, the ability of motor control, and the performance of activities of daily living. There is also a strong relationship between cognitive and motor deficits, where individuals with cognitive deficits may not proficiently perform motor rehabilitation, reducing its impact. Therefore, training programs that increase the cognitive effort during motor training may provide a better approach for achieving maximum ambulation recovery, especially during the chronic stages of traumatic brain injury. The objective of the investigation was to evaluate the efficacy of simultaneous motor & cognitive training (SMCT) using virtual reality to improve ambulation using biomechanical, functional, and cortical measures using functional near-infrared spectroscopy (block averaged hemodynamic response). Preliminary data are presented for two participants with chronic traumatic brain injury who utilized a virtual reality integrated treadmill for 12 sessions (3 sessions/ week for 4 weeks) to progressively increase the cognitive and motor effort during the training and one healthy control (HC). The results from this study demonstrated improved biomechanical (step length, spatial symmetry, gait cycle time, and an overall

progression towards healthy bilateral loading) and functional (speed) changes with associated improved hemodynamic response in the motor control network (bilateral supplementary motor area, secondary somatosensory cortex, premotor areas, and primary motor cortex) after training in the participant with TBI compared to HC. These preliminary results suggest that increasing the cognitive effort during motor training has the potential to induce recovery of motor function in a young adults diagnosed with Traumatic Brain Injury.

Disclosures: **K.J. Nolan:** None. **K.K. Karunakaran:** None.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.13

Topic: C.10. Brain Injury and Trauma

Support: KMDF_PR_20200901_0103

Title: Ambivalent effect of applying focused ultrasound to photodynamic therapy for brain tumors using C6 glioblastoma rat model

Authors: ***J. PARK**¹, C. KONG¹, J. SHIN¹, Y. NA², S. HAN³, J. CHANG¹, W. CHANG¹;
¹Dept. of Neurosurg., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; ²Catholic Kwandong Univ. Col. of Med., Incheon, Korea, Republic of; ³Univ. of Toronto, Toronto, ON, Canada

Abstract: Glioblastoma (GBM) is a typical intractable disease, and various treatments have been attempted to date, but with slight effect. Photodynamic therapy (PDT) is a way to treat tumors under certain conditions. However, problem of light transmission decreases its usage. Similar to PDT, Sonodynamic therapy (SDT) generates reactive oxygen species by ultrasonic excitation, ultimately killing tumor cells. Some studies have found that focused ultrasound can also affect more light penetration into tissues. Based on this background, we conducted studies to demonstrate the possibility of combined therapy, which can increase the energy transmission rate and effectively stimulate the sensitizer by simultaneously applying ultrasound energy and light energy. In this study, C6 cells were transplanted into the brain of SD rats (n=42). 5-aminolevulinic acid hydrochloride (5-ALA, 60 mg/kg) was intravenously injected 6 h before treatments, and the rats were divided into 5-ALA alone PDT, SDT, and SPDT groups. Treatments were administered 9 days after tumor transplantation. The acoustic power used for the SDT was 5.5 W/cm² using a 0.5-MHz single-element spherically focused transducer for 20 min. The 633 nm laser was illuminated at 100 J/cm². Magnetic resonance imaging was performed to confirm the tumor volume after treatment each week. Also, FDG-PET was performed to observe treatment-induced changes in tumor activity. In MRI image, there were differences on day 21, which showed a significant decrease in the PDT group (p<0.05) compared with the 5-ALA and SDT groups. In PET image, activation of metabolism at the tumor site was

observed in the 5-ALA and SDT groups. In addition, high expression rates of reactive oxygen species-related factors in SPDT were observed through Immunohistochemistry ($p < 0.01$, $p < 0.001$). Our results suggest that the PDT regimen of 5-ALA combined with laser therapy can significantly inhibit tumor growth in the rat brain. The tumor increased in size in SDT due to the misapplication of ultrasound. However, in SPDT, a decrease in tumor size and high oxidative stress due to treatment were observed. This indicates that SPDT has great potential as a therapeutic method for GBM. Further studies are needed to investigate the safety parameters to improve this combination method.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.14

Topic: C.10. Brain Injury and Trauma

Support: CIHR Project Grant 364131

Title: Idol inhibition ameliorates neurological dysfunction after experimental traumatic brain injury

Authors: M. SADEK¹, C. LIU², K. STOVER², M. REED², D. WEAVER², *A. Y. REID²;
¹Univ. of Toronto, Toronto, ON, Canada; ²Univ. Hlth. Network, Toronto, ON, Canada

Abstract: Background: Traumatic brain injury (TBI) affects millions of people every year and can lead to devastating neurological consequences. Secondary injuries that occur after TBI, such as neuroinflammation and vascular impairment, are potentially reversible targets for treatment. The kynurenine pathway (KP), a metabolic pathway involved in the breakdown of tryptophan, produces toxic and protective metabolites and is upregulated after TBI. Indoleamine 2,3-dioxygenase (IDO1) is the initial rate-limiting enzyme in the KP. Inhibition of IDO1 and the KP is a novel strategy to attempt to improve functional outcomes after TBI.

Objectives: Determine the effects of IDO1 inhibition on neuroinflammation and KP activity after experimental TBI, and the impact on neurological functions such as motor ability, memory, and anxiety.

Methods: Young adult male Sprague-Dawley rats underwent either fluid percussion injury (FPI) or sham injury. Intracerebral microdialysis and brain tissue samples were collected from a subset of animals at various timepoints in the acute-subacute post-injury period to assess alterations in KP activity using liquid chromatography mass spectrometry (LCMS) techniques (n=5 per group/timepoint). Another subset of rats received twice daily oral dosing of an IDO1 inhibitor (100 mg/kg PF-06840003) or vehicle for 28 days (n=8). During this period rats were tested on the Barnes Maze, rotarod, neuroscore, and open field test. Rats were then sacrificed for brain

tissue collection to assess KP activity and inflammatory markers.

Results: FPI led to increased KP activity and neuroinflammation acutely after injury as compared to sham injury. Treatment with the IDO1 inhibitor PF-06840003 for 28 days post-injury led to improvements in motor ability in the neuroscore and rotarod tests as well as improved results on long-term memory trials of the Barnes Maze versus injured rats receiving vehicle. There was no affect on measure of anxiety. KP activity was no longer elevated at 28 days post-injury and at that timepoint was not different between groups receiving IDO1 inhibitor or vehicle.

Conclusions: The use of IDO1 inhibitors to disrupt KP activity after experimental TBI partially ameliorates neurological recovery. This may prove to be a useful treatment strategy to improve function in patients after injury.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.15

Topic: C.10. Brain Injury and Trauma

Title: Cannabidiol (CBD) leaf extract is a more efficacious neuroprotectant than hemp CBD following traumatic brain injury

Authors: ***L. K. FRIEDMAN**¹, **H. PENG**², **R. J. ZEMAN**³;

¹New York Med. Col., ³Cell Biol. and Anat., ²New York Med. Col., Valhalla, NY

Abstract: Our dual dispensary system of cannabidiol (CBD) leaf (CBD_{leaf}) extract applied directly to the injured region and with oil injection (IP) following traumatic brain injury (TBI) reduced lesion volume and restored vestibulomotor and cognitive clinical functions. To determine whether CBD_{leaf} containing tetrahydrocannabinol (THC) has superior behavioral and neuroprotective effects than hemp derived CBD (CBD_p) lacking THC, two extracts at different ratio concentrations of CBD:THC (300:1 and 10:1) were compared to CBD_p in a pre-clinical TBI model. Brains were evaluated histologically with hematoxylin and eosin and immunohistochemically with NeuN, GFAP, and parvalbumin (PV). Behavioral performance was restored to a greater degree with either of the CBD_{leaf} extracts compared to CBD_p, one being more efficacious than the other depending on the task. On the beam balance, vestibulomotor recovery was reached at 12 days with 300:1, 14 days with 10:1, and 21 days with CBD_p. In the alternating T-maze, only the TBI untreated group exhibited significant reduction in spontaneous alternation; the highest rates were observed with both CBD_{leaf} extracts. In the novel object recognition test (NOR), time spent with novel objects was greatest with CBD_{leaf} 300:1, exhibiting a similar index ratio as sham controls. Although CBD_p treated rats spent more time with objects than the TBI group, the amount of time spent with novel and familiar objects was equal thereby lacking recognition discrimination. In the elevated plus maze (EPM), sham

animals spent most of time in open arms, TBI and CBDp groups spent most of the time in closed arms, whereas the CBD_{leaf} groups spent similar time in closed and open arms. In the forced swim test (FST), reduced floating was observed with CBD_{leaf} 300:1. Both tests suggest reduced anxiety level was THC concentration dependent. Moreover, lesion volume and gliosis were reduced to a greater extent with preserved hippocampal neuronal labeling with CBD_{leaf} compared to CBDp. Concomitant reduction in PV labeling was observed not only within the hippocampus on the side of contusion, but also within the contralateral hippocampus. With CBDp and either CBD_{leaf} extracts, PV cell counts were similar to controls on the contralateral side and partly restored ipsilaterally suggesting that CBD was responsible for their rescue. The sparing of neurons and glia following TBI was correlated with preservation of fast spiking inhibitory PV interneurons which may contribute to their protection. In contrast, the ratio of CBD:THC appears critical for optimal recovery of locomotor and cognitive functions compared to CBDp suggesting multiple mechanisms of protection are involved.

Disclosures: L.K. Friedman: None. H. Peng: None. R.J. Zeman: None.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.16

Topic: C.10. Brain Injury and Trauma

Title: Recovery of optic nerve projection in a mouse model of normal tension glaucoma

Authors: *S. SHIBUE, C. TOHDA;
Univ. of Toyama, Toyama city, Japan

Abstract: Glaucoma is a leading cause of irreversible blindness in the world. It is a progressive disorder of vision caused by optic nerve atrophy and loss of retinal ganglion cells (RGC). The number of patients with normal tension glaucoma is high. The only evidence-established treatment for normal tension glaucoma is intraocular pressure reduction, which probably inhibit further progression, but does not recover lost vision. Therefore, in this study, we focused on Drug A (name is closed due to the patent) as a potential candidate of a therapeutic drug because it has an axonal growth activity. Treatment with Drug A for 4 days significantly elongated axons and dendrites in primary cultured RGC. Optic nerve crushing was conducted in mice to produce a model of normal tension glaucoma. Although intravitreal administration of Drug A tended to increase the optic nerve density, we approached oral administration of Drug A to achieve more potent effect. LC-MS/MS detection revealed that Drug A was distributed in the retina, optic nerve and whole brain at least 6 h after oral administration. Drug A was orally administered for 3 weeks to optic nerve crush mice expecting that Drug A would act on the entire visual pathway. Before sacrificing, anterograde tracer dye-conjugated cholera toxin B was intravitreally injected, and retrograde tracer Fluoro-Gold was injected to the primary visual cortex. Drug A increased the optic nerve density and its projection to the lateral geniculate nucleus cells which terminate

the primary visual cortex. RGC reduction was also significantly inhibited by Drug A. Intraocular pressure was not altered by optic nerve crush or by Drug A treatment. This study showed that Drug A may improve optic nerve damage and RGC reduction. The effects of Drug A on vision and the molecular mechanism for axonal growth are currently under investigation. This study has a potential to develop a new oral therapeutic drug for the vision recovery in glaucoma.

Disclosures: S. Shibue: None. C. Tohda: None.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.17

Topic: C.10. Brain Injury and Trauma

Support: FAPESP 2022/01718-1

Title: Anticonvulsant effects of cannabidiol after lateral fluid percussion injury in rats

Authors: *M. B. BRAGA¹, S. ROMARIZ¹, C. GIMENES¹, A. KATANOSAKA², R. S. POLLÍ², M. FORESTI¹, B. M. LONGO¹;

¹Physiol., Univ. Federal of São Paulo (UNIFESP), São Paulo, Brazil; ²Inst. of Sci. and Technol., Univ. Federal of São Paulo (UNIFESP), São José dos Campos – SP, Brazil

Abstract: Title: Anticonvulsant effects of cannabidiol after lateral fluid percussion injury in rats

Authors: Braga, MB¹; Romariz, SAA¹; Gimenes, C¹; Katanosaka, A²; Polli, RS²; Foresti, M³; Longo, BM¹ Laboratory of Neurophysiology, Department of Physiology, University Federal of São Paulo (UNIFESP) - São Paulo - SP, Brazil² Institute of Science and Technology, University Federal of São Paulo (UNIFESP) - São José dos Campos - SP, Brazil³ Laboratory of Neurobiology, Department of Physiology, University Federal of São Paulo (UNIFESP) - São Paulo - SP, Brazil **Introduction:** Traumatic brain injury (TBI) is considered the leading cause of death and disability in the world. TBI can cause cognitive impairment, sleep disturbance, and epilepsy. Lateral fluid percussion (LFP) is one of the most used animal models of TBI to investigate seizure susceptibility. Cannabidiol (CBD) have been shown protective effects in several animal models and clinical

trials. However, its anticonvulsant effect after TBI remains poorly understood. **Objective:** Investigate the susceptibility to seizures and the anticonvulsant effects of CBD after TBI. **Methods:** Male Wistar rats received the trauma by the LFP model (TBI) or were craniotomized without trauma (Sham). After 24 hours, both TBI and Sham animals were treated with 5mg/kg CBD or vehicle (VEH) for 5 days, constituting the groups TBI-CBD (n=6), TBI-VEH (n=5), Sham-CBD (n=6) and Sham-VEH (n=6). Thirty-days after TBI, all animals were challenged with pentylenetetrazole (PTZ, 35 mg/kg) as a second hit, and the incidence and severity of seizures were analyzed. This research was approved by the CEUA (Committee on Ethics and Use of Animals) N°6764080317.

Results: All animals of the Sham-VEH (100%) and 80% of the TBI-VEH groups showed

seizures, whereas only 50% and 33% of Sham-CBD and TBI-CBD groups, respectively, presented seizures. According to Racine's scale, seizure type 4 and 5 were classified as severe seizures. Only 33% of the animals treated with CBD (TBI-CBD and Sham-CBD) showed severe seizures type 4 and 5 compared to 67% from Sham-VEH and 80% from TBI-VEH groups.

Conclusions: Our data suggest that the treatment with CBD immediately after the TBI was able to reduce the incidence, severity, and susceptibility of seizures, and since it was given 30 days before PTZ induction, CBD showed prolonged protective effects suggesting a therapeutic potential in TBI complications such as the increase of seizure susceptibility.

Disclosures: M.B. Braga: None. S. Romariz: None. C. Gimenes: None. A. Katanosaka: None. R.S. Polli: None. M. Foresti: None. B.M. Longo: None.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

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Program #/Poster #: 460.18

Topic: C.10. Brain Injury and Trauma

Support: Department of Defense W81XWH1920011
N.K. was supported by the National Science Foundation Graduate Research Fellowship under Grant No. 1122374

Title: "Backpack" cell-mediated therapy in a contusion model of traumatic brain injury

Authors: *B. GOLEMB¹, R. H. PETRILLO¹, T. STINSON¹, M. TYNAN¹, R. LIAO², N. KAPATE², S. MITRAGOTRI², B. COSTINE-BARTELL¹;

¹Neurosurg., Massachusetts Gen. Hosp., Charlestown, MA; ²John A. Paulson Sch. of Engin. & Applied Sci., Harvard Univ., Cambridge, MA

Abstract: Rationale:

There has been little success in developing treatments for traumatic brain injury in human patients beyond clinical management. Macrophages have been explored as a treatment modality for cancers. However, cell-based delivery methods that encapsulate therapeutics within the cell have limited control over therapy dosage and release profile. Here, we test if macrophages carrying "backpacks" hone to the contusion site, polarize the macrophage population at the contusion site into an M2-like anti-inflammatory phenotype, reduce inflammation, and reduce lesion size in a piglet model of cortical impact.

Methods:

Backpacks are discoidal particles designed for cell-mediated drug delivery that resist phagocytosis. Fluorescently-labelled backpacks were prepared from biodegradable polymers via spin-coating and microcontact printing, loaded with interleukin-4 and dexamethasone. Porcine bone marrow derived monocytes were differentiated into macrophages and incubated with backpacks to promote binding. Male, 30-day old, Yorkshire piglets were infused at 1 or 4 hours

after cortical impact with either cells with backpacks ($1.10\text{-}1.58 \times 10^8$ cells; $N = 6$) or vehicle ($N = 7$) and organs were collected 6-7 days later. A total of 26 piglets are planned. In this interim analysis, we estimated lesion area as a ratio of the uninjured contralateral hemorrhage via photographs of coronal brain slabs while histopathology is underway. Lesions were categorized into having a large hemorrhage vs. none to little. Statistical comparisons of groups will be conducted when the experiment is completed.

Results:

Approximately, 10-25% of macrophages had bound backpacks. Therapies were released from the backpacks by 72 hours. Piglets receiving cells with backpacks did not display any clinical signs of adverse reactions. Fluorescently labelled backpacks were observed at the site of brain contusion and in the lung, spleen, liver, and kidney. In subjects receiving vehicle, 57% of lesions were large and hemorrhagic and the lesion was $24.5 \pm 8.5\%$ (means \pm SEM) of the area of the contralateral hemisphere. In subjects receiving macrophages with therapeutic cellular backpacks, only 16% of lesions were large and hemorrhagic and lesion area was $10.8 \pm 5.5\%$. The histopathology results of lesion size and organ backpack accumulation will be presented.

Conclusions:

Backpack-carrying macrophages show promise in honing to the contusion site, inhibiting conversion to hemorrhage, and reducing lesion size. Work is ongoing to evaluate macrophage phenotype and markers of inflammation at the contusion site, organ toxicity, optimal time of administration, and the mechanisms of action.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

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Program #/Poster #: 460.19

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 1R01NS100710-01A1 (YX)

Title: Ablation of microRNAs diminishes therapeutic effects of exosomes derived from human mesenchymal stromal cells on improving functional recovery in rats after traumatic brain injury

Authors: ***Y. ZHANG**¹, **Y. ZHANG**², **M. CHOPP**^{2,3}, **H. PANG**¹, **L. CHEN**¹, **Z. ZHANG**², **A. MAHMOOD**¹, **Y. XIONG**¹;

¹Neurosurg., ²Neurol., Henry Ford Hosp., Detroit, MI; ³Physics, Oakland Univ., Rochester, MI

Abstract: Mesenchymal stromal cell (MSC)-derived exosomes promote functional recovery after experimental traumatic brain injury (TBI). Argonaut 2 (Ago2) is one of the primary miRNA machinery proteins that is required for packaging miRNAs into exosomes and activities in the recipient cells. This study was performed to determine the effects of exosomes with reduced

microRNAs harvested from human bone marrow MSCs with Ago2 knockdown on brain remodeling, neuroinflammation, and neurological recovery after TBI. Therapeutic effects of exosomes derived from naïve MSCs (naïve-Exo), MSCs transfected with a vector carrying scramble control shRNA (Vector-Exo), MSCs transfected with a lentiviral vector based shRNA against Ago2 to knock down Ago2 (Ago2-KD-Exo) were determined in adult male rats subjected to a moderate controlled cortical injury (CCI). A single intravenous injection of exosomes (naïve-Exo, Vector-Exo, and Ago2-KD-Exo) and Vehicle (phosphate-buffered solution) was given via tail vein 1 day after injury. Multiple neurological functional tests were performed weekly after TBI for 5 weeks. The Morris water maze test was performed for spatial learning and memory on days 31-35 after TBI. All animals were euthanized 5 weeks after injury and the brains were collected for histopathological and immunohistochemical analyses of lesion volume, cell loss, angiogenesis, neurogenesis and neuroinflammation. Down-regulation of Ago2 reduced miRNA levels in exosomes, with a selective reduction of miR-17-92 cluster. Compared to the vehicle treatment, both naïve-Exo and Vector-Exo treatments significantly improved sensorimotor and cognitive function, reduced hippocampal neuronal cell loss and neuroinflammation, and promoted neurovascular remodeling (angiogenesis and neurogenesis) without effects on the lesion volume. Moreover, Ago2-KD-Exo treatment exhibited a significantly less therapeutic effect on all the parameters measured above than did naïve-Exo and Vector-Exo treatments. The therapeutic effects of Ago2-KD-Exo are comparable to that of Vehicle treatment. One-way ANOVA followed by post hoc Tukey's tests was used to compare the differences in functional and histological outcomes. P value <0.05 was considered significant. Our findings demonstrate that attenuation of Ago2 protein in MSCs reduces miRNAs in MSC-derived exosomes and abolishes exosome treatment-induced beneficial effects in TBI recovery, suggesting that miRNAs in MSC-derived exosomes play a critical role in reducing cell loss and neuroinflammation, enhancing angiogenesis and neurogenesis as well as improving functional recovery after TBI.

Disclosures: Y. Zhang: None. Y. Zhang: None. M. Chopp: None. H. Pang: None. L. Chen: None. Z. Zhang: None. A. Mahmood: None. Y. Xiong: None.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

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Program #/Poster #: 460.20

Topic: C.10. Brain Injury and Trauma

Support: Burn Shock Trauma Research Institute
Otolaryngology-Head and Neck Surgery Department

Title: Delayed testosterone treatment reduces neuronal loss in the vestibular nucleus by ameliorating oxidative stress and pyroptosis following repetitive mild traumatic brain injury

Authors: *M. VOLYANYUK^{1,4}, A. B. SEGISMUNDO^{1,4}, T. K. PETER⁵, S. C. BYRAM^{4,6}, J. E. EXLINE^{1,4}, K. M. LOTESTO^{2,4}, E. M. FOECKING^{2,3,4,5};

¹Neurosci. Grad. Program, ²Burn and Shock Trauma Res. Inst., ³Dept. of Mol. Pharmacol. and Neurosci., Loyola Univ. Chicago, Maywood, IL; ⁴Res. and Develop. Services, Edward Hines Jr. VA Hosp., Hines, IL; ⁵Dept. of Otolaryngology-Head and Neck Surgery, ⁶Dept. of Anesthesiol. and Perioperative Med., Loyola Univ. Med. Ctr., Maywood, IL

Abstract: Repetitive mild traumatic brain injury (rmTBI) results in a wide array of physical, emotional, and cognitive deficits. A common complaint after rmTBI is vestibular dysfunction, which can lead to debilitating balance issues that reduce overall quality of life. Our lab has shown that delayed testosterone treatment improves prolonged vestibular functional deficits in a rodent model of rmTBI. The current research aims to uncover the molecular mechanisms associated with this vestibular functional improvement following delayed testosterone treatment in the vestibular nucleus. Eight-week-old male Long-Evans rats received 5 closed-head rmTBIs spaced 48 hours apart. Sham rats received similar anesthesia but with no impacts. At 35 days post the final rmTBI (DPI), a subset of rmTBI rats received a capsule producing physiological levels of testosterone, and brainstems were collected at 0, 2, 7, 28, and 140 days after treatment, equating to 35, 37, 42, 63, and 175 DPI. In one cohort of animals, brainstems were sectioned and stained with thionin to quantify neuronal cell counts through the entire vestibular nuclei. In a second cohort, vestibular nuclei were extracted, and mRNA and protein were analyzed via RT-qPCR and western blotting. At 175 DPI, a significant 28% and 25% reduction in total neurons was quantified in the ipsilateral and contralateral vestibular nuclei, respectively. Testosterone treatment significantly improved neuronal survival by 10-11%. Gene expression for NADPH Oxidase 4 (*Nox4*) and Gasdermin D (*Gsdmd*) was shown to be statistically elevated at 42 DPI, suggestive of persistently increased oxidative stress and pyroptosis at extended timepoints after rmTBI. Testosterone treatment ameliorated the elevations in expression for *Nox4* and *Gsdmd* when compared to injured controls. Proteomic results corroborated these findings by demonstrating nearly a 12- fold increase in NOX4 levels at 35 DPI, and testosterone produced a 64% reduction in these levels 28 days after treatment. This study provides novel insight into the temporal profile of molecule sequelae associated with neuronal loss in the vestibular nuclei following rmTBI and demonstrates the therapeutic potential of delayed testosterone treatment.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

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Program #/Poster #: 460.21

Topic: C.10. Brain Injury and Trauma

Support: US Department of ACCCRP award number W81XWH-20-C-0114

Title: Peg hydrogel containing hyaluronic acid (ha)-dexamethasone (DX) reduces inflammatory response and improves motor function in a rat moderate controlled cortical impact (cci) tbi model

Authors: *C. JONES¹, B. ELLIOTT¹, F. MA¹, Z. JOHNSON¹, Z. LIAO¹, K. WEBB¹, Z. S. BAILEY², A. SCULTETUS², J. GILSDORF², D. A. SHEAR², J. LEE¹;

¹Bioengineering, Clemson Univ., Clemson, SC; ²Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Traumatic brain injury (TBI) causes worsening conditions in the nervous system due to the natural inflammatory response that causes progressively damaging conditions in the surrounding tissue (secondary injury). Dexamethasone (DX), a synthetic glucocorticoid, has been shown to minimize neuroinflammation after injury and improve recovery. In our published work, we demonstrated that local application of PEG-bis-AA/HA-DXM hydrogels composed of polyethyleneglycol-bis-(acryloyloxy acetate) (PEG-bis-AA) and dexamethasone-conjugated hyaluronic acid (HA-DXM) reduced neuroinflammation, apoptosis, and lesion volume and improved neuronal cell survival and motor functional recovery at 7 days post-injury (DPI) in a rat mild controlled cortical impact (CCI) TBI model *in vivo*. In this study, we evaluated the effect of PEG-bis-AA/HA-DXM hydrogel on motor function and secondary injury after moderate injury at 7 DPI (acute phase). PEG-bis-AA/HA-DXM was photopolymerized to form hydrogel discs approximately 5.5 mm in diameter and 2 mm in thickness. The moderate CCI TBI model was generated using a CCI device armed with a 5 mm blunt tip to deliver an injury at a velocity of 4 m/sec and a depth of 2.5 mm after the craniectomy (performed using a 6 mm diameter Trephine bur tip, over the right cortex at 1mm posterior and 3 mm lateral to bregma). Male *SD* rats were divided into 3 groups: 1) Normal group: no surgery, 2) TBI untreated group:, and 3) PEG-bis-AA/HA-DXM gel treated group (3µg of DX/hydrogel). Motor function recovery after TBI was evaluated at 1, 2, 3, 5 and 7 DPI. After functional studies, rats were sacrificed under deep anesthesia via cardiac perfusion with 4% PFA. Brains were fixed and cryo-sectioned and stained for histological and immunohistochemical analysis of lesion volume, neuro-inflammation, neuronal survivability and apoptosis. We observed that PEG-bis-AA/HA-DXM hydrogel treatment significantly improved motor function by rotarod and beam walk. We observed significantly reduced lesion volume in hydrogel-treated groups compared to that in untreated TBI group using NISSL staining. For inflammatory response, we observed a reduced number of ED1⁺ (M1 marker,) cells and an increased number of Arg 1⁺ (M2 marker) cells. For neuronal cell survival, a significant increase in NeuN⁺ cells was observed in treated rats compared to untreated TBI group. We also observed fewer apoptotic TUNEL⁺ cells and GFAP⁺ cells in the hydrogel treated group compared to untreated TBI group. These results suggest that localized dexamethasone delivery from PEG-bis-AA/HA-DXM hydrogels can mitigate acute secondary injury responses and improve motor recovery following moderate TBI.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

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Program #/Poster #: 460.22

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 5R01NS109477-02 (YZ)

Title: Vepoloxamer Improves Functional Recovery in Rat After Traumatic Brain Injury: A Dose-Response and Therapeutic Window Study

Authors: *L. CHEN¹, Y. XIONG¹, M. CHOPP^{1,2}, H. PANG¹, Z. ZHANG¹, A. MAHMOOD¹, Y. ZHANG¹;

¹Henry Ford Hosp., Detroit, MI; ²Oakland Univ., Rochester, MI

Abstract: Background: Traumatic brain injury (TBI) is a major cause of death and disability worldwide. There are no effective therapies available for TBI patients. Vepoloxamer is an amphiphilic polyethylene-polypropylene-polyethylene tri-block copolymer that is reported to seal membranes and restore plasma membrane integrity in damaged cells. In this study, we conducted a dose-finding study to identify a dose-response and therapeutic window effect of Vepoloxamer on functional recovery in young rats with TBI. **Methods:** Male Wistar young rats, subjected to moderate TBI induced by controlled cortical impact injury, were treated randomly with 0 (saline as vehicle), 100, 300, or 600 mg/kg of Vepoloxamer intravenously (IV) 2, 4 h, 1 day or 3 days after TBI. A battery of cognitive and neurological functional tests was performed weekly after injury for 5 weeks. Spatial learning and memory were measured on days 31-35 after TBI using the Morris water maze test. Animals were killed 35 days after TBI and brain sections were stained for the analyses of lesion volume. **Results:** IV administration of Vepoloxamer (100, 300, 600 mg/kg) 2 h post injury significantly improved cognitive functional recovery while Vepoloxamer at doses of 300 and 600 mg/kg significantly reduced lesion volume compared to saline treatment. Our data demonstrate that Vepoloxamer 300 mg/kg is the most effective dose to improve neurological and cognitive functional recovery after TBI. Our data also demonstrated that IV Vepoloxamer (300 mg/kg) treatment initiated at 2, 4 h, 1 day and 3 days post injury significantly improved neurological functional recovery while Vepoloxamer treatment initiated at 2 or 4 h post injury significantly improved cognitive functional recovery after TBI. Vepoloxamer administration at 2 h post injury is the optimal time point to improve neurological and cognitive function recovery after TBI. One-way ANOVA followed by post hoc Tukey's tests was used to compare the differences in functional and histological outcomes. P value <0.05 was considered significant. **Conclusion:** We have demonstrated that IV Vepoloxamer at a dose range of 100 mg/kg to 600 mg/kg is safe and has a therapeutic effect on TBI. Vepoloxamer 300 mg/kg is likely an optimal dose and 2 h post injury is an optimal time to reduce the lesion volume and improve neurological and cognitive functional recovery after TBI. Our study suggests that Vepoloxamer treatment improves functional recovery in a dose-and time-dependent manner in rats after TBI. The therapy is more efficient when initiated 2 h post injury at 300 mg/kg.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

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Program #/Poster #: 460.23

Topic: C.10. Brain Injury and Trauma

Support: NSERC Discovery Grant (Staines)
NSERC Discovery Grant (Meehan)

Title: Effect of Acute Aerobic Exercise on Motor Cortex Plasticity in Individuals with a History of Concussion

Authors: *M. E. R. KHAN, K. D. HAYES, K. R. GRAHAM, R. STAINES, S. K. MEEHAN; Kinesiology and Hlth. Sci., Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Neuropsychological and physiological tools have revealed that long-term cognitive and motor abnormalities persist past the acute phase of a concussive injury. For example, transcranial magnetic stimulation (TMS) studies demonstrate long-term increases in gamma-aminobutyric acid (GABA) mediated intracortical inhibition more than 6-month after injury. Elevated GABAergic activity suppresses plasticity of motor cortex. In healthy populations, acute aerobic exercise reduces GABAergic activity and enhances synaptic plasticity. Therefore, aerobic exercise may enhance the potential for motor cortex plasticity in those with a history of chronic concussion. This study used TMS to investigate the benefits of acute aerobic exercise on M1 plasticity in individuals with a history of chronic concussion (>six months post-concussion). In a crossover design, the potential for plasticity was assessed using a plasticity-inducing method known as paired associative stimulation (PAS). In one arm of the crossover, participants received PAS after a period of minimal physical activity (Rest+PAS). In the other arm of the crossover, participants received PAS after a single bout of 20-minutes of moderate-intensity biking (Exercise+PAS). Cortical and intracortical excitability were measured before and at five and thirty minutes after PAS. Cortical excitability was assessed using motor evoked potential (MEP) amplitude. Intracortical network excitability was measured using short-interval intracortical inhibition (SICI, 2ms), intracortical facilitation (ICF, 12ms), long-interval intracortical inhibition (LICI, 100ms) and the cortical silent period (CSP). Preliminary results (n=9) suggest that Exercise+PAS decreased SICI compared to Rest+PAS immediately following PAS, but that SICI returned to baseline by 30-minutes. In contrast, CSP duration and LICI were similarly increased following Exercise+PAS and Rest+PAS. No changes in MEP amplitude or ICF were detected in either session. Therefore, exercise enhanced the plastic response of GABA_A-mediated intracortical inhibition following chronic concussion. The alteration in GABA_A-mediated intracortical inhibition could be an important substrate to enhance motor control and normalize persistent subclinical motor declines following chronic concussion.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.24

Topic: C.10. Brain Injury and Trauma

Support: IRSC Grant

Title: Cannabidiol as potential therapeutic agent in mouse model of multi-traumatic injuries

Authors: *M. REGNIEZ¹, I. MASSE¹, L. DE BEAUMONT^{2,1};

¹CIUSSS-NIM - Res. center, Montreal, QC, Canada; ²Univ. of Montreal, Montreal, QC, Canada

Abstract: Recent studies documented the anti-inflammatory properties of cannabidiol (CBD), the main non-psychoactive cannabinoid extract, making it a promising neuroprotective agent in a variety of neurological conditions such as mild traumatic brain injuries (mTBI). The main objective of this study was to evaluate the effects of a 7-day CBD treatment on neuroinflammation, pain sensitivity and behavioral recovery after multitrauma. Validated mouse models of trauma were used to combine closed tibial fracture with concomitant closed head mTBI. Male C57Bl6 mice (n = 88) were divided into 8 groups according to 3 independent variables: Injury (mTBI+fracture vs. sham) X Treatment (CBD vs. vehicle) X Time (acute vs. chronic). Pain sensitivity was assessed with the Mouse Grimace Scale and mechanical (von Frey) nociceptive withdrawal threshold tests before and following treatment (D7). Orthopedic and cognitive functions were assessed with open field, Y-maze and rotarod tests for 3 consecutive days from D30. Neuroinflammation was evaluated by 24h following treatment and longitudinally at D35 following multi-trauma by immunohistochemistry against GFAP and IBA-1 protein. We hypothesized that relative to placebo, CBD treatment will significantly reduce baseline-adjusted pain on D7, neuroinflammation on D8 and 35; and functional impairment on D30. This project could provide objective animal evidence on the clinical utility of CBD interventions in treating neuroinflammatory conditions

Disclosures: M. Regniez: None. I. Masse: None. L. De Beaumont: None.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

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Program #/Poster #: 460.25

Topic: C.10. Brain Injury and Trauma

Support: CTSI Grant: TL1TR002531
W81XWH-18-1-0433 Department of Army
Indiana State Department of Health

Title: Mild traumatic brain injury induced systemic changes in peripheral blood mononuclear cell are influenced by a single propranolol injection

Authors: ***J. SMITH**¹, N. NGUYEN², T. NGUYEN², F. A. WHITE²;
¹Med. Neurosci. Program, ²Anesthesia, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: There are 1.5 million new mild traumatic brain injuries (mTBI) annually in the US with many of those impacted experiencing long-term consequences months after the injury. Although the post injury mechanisms are not well understood, current knowledge indicates peripheral immune system activation as a causal link between mTBI and long-term side effects. Through a variety of mechanisms, peripheral innate immune cells are recruited to the CNS after TBI to repair and heal the injured tissue; however, the recruitment and activation of these cells leads to further inflammation. Emerging evidence suggest sympathetic nervous system (SNS) activity plays a substantial role in the recruitment of immune cells post injury. We sought to identify the peripheral innate immune response after repeated TBIs in addition to repurposing the nonselective beta blocker propranolol as a novel mTBI therapy to limit SNS activity and mTBI pathophysiology. Accordingly, innate immune cells were isolated from blood, spleen, brain, and bone marrow for flow cytometry and RNA sequencing assays at 1-, 7-, and 28-days. Our data depicts bone marrow RNA modifications 1 day post injury; however, spleen and brain RNA changes did not occur until the 7- and 28-days. Additionally, our spleen and blood flow cytometry data display monocyte population alterations most significantly at 7- and 28-days. In summary, our data displays changes at both the RNA and cellular levels at various timepoints, most pronounced in the mTBI propranolol group, suggesting a single dose propranolol injection as a viable future mTBI therapy in the acute setting.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.26

Topic: C.10. Brain Injury and Trauma

Support: Grant #BA170606

Title: Identification and properties of NA-184, a selective calpain-2 inhibitor, and its use for TBI treatment

Authors: ***M. BAUDRY**¹, Y. WANG¹, X. BI², Y. LUO¹, Z. KAMAL³, A. SHIROKOV³, E. SULLIVAN³, D. LAGASCA³, H. KHALIL³, G. LEE³, K. FOSNAUGH⁴, P. BEY⁴, S. MEHDI⁴,

G. COULTER⁴;

¹Western Univ. of Hlth. Sci., Pomona, CA; ²Western Univ. Hlth. Sci., Pomona, CA; ³Nanosyn, Santa Clara, CA; ⁴NeurAegis, Inc, Irvine, CA

Abstract: The roles of calpain in neurodegeneration in general, and in stroke and traumatic brain injury (TBI), have long been reported and confirmed in different mammals, including humans. Consequently, numerous studies have attempted to use calpain inhibitors to reduce neurodegeneration in both stroke and TBI. More recently, our laboratory has shown that calpain-2 activation in the brain following acute injury is directly related to neuronal damage and the long-term functional consequences on the injury, while calpain-1 activation is generally neuroprotective and calpain-1 deletion exacerbates neuronal injury. We have also shown that a relatively selective calpain-2 inhibitor, referred to as C2I enhanced long-term potentiation and learning and memory and provided neuroprotection in the controlled cortical impact (CCI) model of TBI in mice. Through an extensive medicinal chemistry optimization program, we have now selected the selective calpain-2 inhibitor, NA-184, (S)-2-(3-benzylureido)-N-((R,S)-1-((3-chloro-2-methoxybenzyl)amino)-1,2-dioxopentan-3-yl)-4-methylpentanamide diastereomer mixture), as our lead clinical candidate. Its K_i against human calpain-2 is 50 nM as compared to 243 nM for calpain-1. The in vivo IC_{50} of NA-184 for mouse calpain-2 is about 130 nM, as compared to 2826 nM for calpain-1. However, when measured in human cell lines against the degradation of spectrin, the IC_{50} for calpain-2 is about 10 nM and no inhibition of calpain-1 is observed up to 30 μ M. NA-184 IC_{50} to inhibit neuronal death in the CCI model of TBI is about 0.15 mg/kg when injected ip, with a maximal effect observed at 1 mg/kg, with no inhibition of calpain-1 at doses up to 10 mg/kg. Its half-life in the plasma is about 5 h, and its half-life in the brain is about 2.5 h. However, the half-life of NA184 measured by calpain-2 inhibition in the brain is somewhat longer, about 8 h, which is probably due to the fact that NA184 forms a reversible covalent bond at the active site of calpain, which is slow to dissociate. NA184 is a mixture of two diastereoisomers (R-R and R-S), which exhibit rapid interconversion through epimerization in PBS and plasma. We are therefore planning to use the mixture of the diastereoisomers for our clinical studies with NA-184 for TBI, which we are planning to initiate late in 2023. Work supported by the Office of the Assistant Secretary of Defense for Health Affairs through The DMRD Program (Award No. W81XWH-19-1-0329). Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the DoD. Grant #BA170606. "Optimization of a selective calpain-2 inhibitor for prolonged field care in Traumatic Brain Injury".

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NeurAegis, Inc. **S. Mehdi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurAegis, Inc. **G. Coulter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurAegis, Inc.

Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.27

Topic: C.10. Brain Injury and Trauma

Support: Chaire Fondation Caroline Durand en traumatologie aigüe de l'UdeM

Title: Efficacy of intranasal administration of the NMDA receptor antagonist MK-801 on the acute neurochemical response to a concussion in a rat model combining force and rotation

Authors: *I. MASSE¹, L. MOQUIN², C. BOUCHARD¹, A. P. GRATTON², L. DE BEAUMONT³;

¹Hop. du Sacre-Coeur Montreal, Montréal, QC, Canada; ²Douglas Hosp. Res. Ctr., Verdun, QC, Canada; ³Univ. of Montreal, Montreal, QC, Canada

Abstract: Following concussion, there are marked alterations in the extracellular amino acid concentrations, which in turn contribute to the delayed neuronal damage mostly *via* overactivation of the N-methyl-D-aspartate (NMDA) glutamatergic receptors. While acute therapies with NMDA receptor antagonists could be beneficial to concussed patients, previous failures with rodent models and human patients have highlighted the need for alternative, faster routes of administration. The aim of the present study was to investigate the efficacy of intranasal administration of MK-801, a promising NMDA antagonist, in a novel formula on the acute changes in amino acid extracellular concentrations involved in excitotoxicity resulting from a concussive trauma. Our previously validated combination of a weight-drop concussion rat model and *in vivo* cerebral microdialysis was used. The microdialysis probe was inserted inside the hippocampus and left inserted at impact to allow uninterrupted sampling of amino acids of interest immediately after concussion. The primary outcome included amino acid concentrations and the secondary outcome included righting time. Samples were taken in 10-minute increments for 60 minutes before, during, and 60 minutes after impact, and analyzed for glutamate, gamma-aminobutyric acid, taurine, glycine, glutamine, and serine using high-performance liquid chromatography. Righting time was acquired as a neurological restoration indicator. Vehicle or 10mg/kg MK-801 was administered intranasally immediately following induction of sham injury or concussion. Compared to sham-injured animals, glutamate, taurine, and glycine levels as well as righting times were significantly increased in cases from the vehicle-treated concussion group. In contrast, righting times and amino acid concentrations observed within the first 10 minutes after induction of concussion in cases assigned to the MK-801-treated concussion group were comparable to sham-injured animals. These results suggest that

presynaptic actions and availability of MK-801 following intranasal administration significantly inhibit the immediate and indiscriminate release of glutamate, taurine, and glycine in extracellular fluid after a concussion.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.28

Topic: C.10. Brain Injury and Trauma

Support: KHIDI: HI18C2196, NRF 2021R1I1A1A0106129911

Title: Finding the beneficial targeted hyperbaric oxygen treatment pressure to preserve brain injury in acute carbon monoxide poisoning in rat model

Authors: *Y. LEE^{1,4}, J. LEE², H. KIM⁴, J. KIM⁴, H. KIM^{1,4}, Y. CHA^{1,4}, K.-S. PARK³, S.-K. CHA³, M. KIM²;

¹Dept. of Emergency Medicine, Res. Inst. of Hyperbaric Med. and Sci., ²Res. Inst. of Hyperbaric Med. and Sci., ³Dept. of Physiol., Yonsei University, Wonju Col. of Med., Wonju, Korea, Republic of; ⁴Dept. of Emergency Med., Yonsei University, Wonju Severance Christian Hosp., Wonju, Korea, Republic of

Abstract: Introduction Poisoning with carbon monoxide (CO) remains a significant cause of accidental and intentional injury worldwide. The progressive delayed neuropsychological damage in carbon monoxide (CO) poisoning may be due to neuron apoptosis. Applying high-concentrated oxygen is a standard and widely used treatment for CO poisoning. Several studies have suggested that hyperbaric oxygen therapy (HBOT) prevents the development of delayed neuropsychological sequelae. However, different treated target pressures used after CO exposure for evaluating the effects of HBOT on injured neuron recovery and reducing neuronal cell apoptosis have not been well studied thus far. In addition, the function of mitochondria according to the oxygen pressure difference also has not been evaluated. This study aims to evaluate the efficacy of HBOT based on the different treated target pressures in neurological functions related to mitochondria function after CO poisoning by using the rat model. **Materials and Methods** For this aim, thirty male Sprague-Dawley (SD) rats were divided into five groups of post-CO treated with room air (RA), 100% O₂, HBOT at 2.5 ATA, HBOT at 2.7 ATA, and HBOT at 3.0 ATA. The rats were exposed to continuously 2700 ppm CO for 25 min in the HBOT chamber. Following CO poisoning, we treated with RA, 100% O₂, 2.5 ATA HBOT, 2.7 ATA HBOT, and 3.0 ATA HBOT for 90min. We performed the open field test and the plus-maze test to evaluate the neurobehavioral function of rats before and after CO poisoning. Within 1 hour after HBOT, rats were sacrificed, and histological analysis and western blot analysis were performed to

confirm the neuronal cell death and mitochondrial function. **Results** HBOT overall immediately improved CO-induced pathologic condition, including motor and mood performance, compared to the RA group. In particular, 3.0 ATA HBOT showed the best improvements among the other interventions. The mitochondrial function increased in 2.5, 2.7, and 3.0 ATA HBOT groups than in the RA group. However, there was no significance in histologic findings to confirm neuronal cell death among the interventions. **Conclusions.** HBOT has a pressure-dependent protective effect on CO-induced neuro-behavior and mitochondrial function, with the highest effect in 3.0 ATA after CO poisoning. Because CO-poisoned rats immediately were sacrificed after HBOT, we could not confirm the histological differences among groups, but considering the mitochondrial response, it could be presumed that there will be an effect related to neuronal protection during long-term follow-up.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.29

Topic: C.10. Brain Injury and Trauma

Title: Combined nutrition and lifestyle treatment to reduce the risk of dementia in persons with traumatic brain injury

Authors: *P. F. ARAVICH¹, J. MANCUSO³, P. GOODALL⁴, A. NIAZ², A. MCDONNELL⁵; ¹Pathology and Anat., ²Med. Student, Eastern Virginia Med. Sch., Norfolk, VA; ³Former Director, Beacon House and Brain Injury Services, Virginia Beach, VA; ⁴Former Program Director, Brain Injury Services, Virginia Dept. of Aging and Rehabilitative Services, Richmond, VA; ⁵Executive Director, Brain Injury Assn. of Virginia, Richmond, VA

Abstract: The 2020 Lancet Commission on Dementia lists 12 preventable causes of dementia, which if addressed could reduce global dementia by 40% (Livingston et al. Lancet. 2020 Aug 8;396(10248):413-446). Traumatic brain injury (TBI) is one of these factors since it increases the risk of, e.g., Alzheimer's disease, vascular dementia, Lewy Body dementia, Parkinson's dementia and frontal-temporal dementia. Indeed, some experts describe TBI as the number one environmental risk factor for the dementias. A recent paradigm shift in dementia research and treatment focuses on prevention using a combination of lifestyle factors and cardiovascular disease management. This "multidomain" lifestyle approach is based on a randomized controlled trial known as the FINGER study (Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability). It found cognitive benefits in older persons at risk for the dementias compared to controls (Ngandu et al. Lancet. 2015 Jun 6;385(9984):2255-63). The FINGER study combined four behavioral lifestyle domains with cardiovascular disease management: Good nutrition, physical exercise, cognitive exercise and social engagement. The World Wide

FINGERS network is attempting to replicate these data across various cultures. The current POINTER study (Protect Brain Health Through Lifestyle Intervention to Reduce Risk) is the US portion of this network. Its four multidomain behavioral interventions are the so-called MIND diet (Mediterranean-DASH Intervention for Neurodegenerative Delay) together with moderate physical exercise, cognitive exercise and social engagement. The MIND diet is the combination of a Mediterranean-type diet with a DASH diet (Dietary Approaches to Stop Hypertension) and is high in vegetables, grains, legumes, nuts, berries and olive oil; low in sugar and salt; and substitutes poultry and fish for red/processed meats (Morris et al. *Alzheimers Dement.* 2015 Sep;11(9):1015-22). Unfortunately, none of the multidomain dementia prevention trials included persons with TBI. Nonetheless, the existing data are a call-to-action to reduce dementia risk in persons with TBI by a multidomain lifestyle care and treatment approach at home, in clubhouses and in long-term care facilities.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.30

Topic: C.10. Brain Injury and Trauma

Support: 2022 CIHR-BC Support Unit Patient Oriented Research Fellowship to TS

Title: Train your brain: a patient-partnered study to determine if at-home three-dimensional multiple object tracking improves cognitive function and adaptability in individuals with moderate to severe brain injury.

Authors: *T. SNOWDEN¹, L. OHLHAUSER¹, J. MORRISON¹, M. YANISH¹, K. MUIRHEAD¹, E. BOSDACHIN¹, B. MAYOH¹, J. GAWRYLUK¹, B. R. CHRISTIE²;
²Div. of Med. Sci., ¹Univ. of Victoria, Victoria, BC, Canada

Abstract: Traumatic brain injury (TBI) is a leading cause of disability. Three-Dimensional Multiple Object Tracking (3DMOT) is an adaptive cognitive training tool that shows promise for individuals with TBI. Our work suggests that bi-weekly 3DMOT improves cognitive functions like memory, attention, and executive functions in TBI survivors. Feedback from our patient-partnered panel revealed that an *at-home* intervention would be preferable since travel and locomotion can be difficult for TBI survivors. The objective of this study is to explore 3DMOT as an accessible therapeutic tool for TBI survivors. In phase 1 of this confirmatory patient-partnered study, 13 TBI survivors were randomly assigned to a 5-week waitlist-control or intervention group. The intervention consisted of two in-lab 3DMOT sessions per week. TBI survivors demonstrated an average 124% improvement on 3DMOT, similar to cognitively healthy individuals. Estimation statistics were used for all analyses, 5000 bootstrap samples were

taken, and the confidence intervals were bias-corrected and accelerated. Results are presented as (mean-difference from pre-intervention to post-intervention [95%CI]), (mean-difference from pre-waiting period to post-waiting period [95%CI]). The intervention group had decreased proactive interference compared to the waitlisted group (-0.332 [95CI -0.873; 0.0523]) (0.161 [95CI -0.0846; 0.435]), greater inhibition on the STROOP task, demonstrated by fewer trial repetitions at the follow-up time point (-1.78 [95CI -4; -0.556]) (2.0 [95CI -0.75; 4.75]), improved digit span sequencing scores following the intervention (1.67 [95CI 0.111; 3.22]) (-0.5 [95CI -1.75; 0.277]), and demonstrated greater verbal fluency (3.56 [95CI -7.56; 15.1]) (-3.25 [95CI -9.0-6.64]). The intervention group's TBI-related symptoms decreased near 50% (-15.9 [95CI -36.6; 4.89]) (3.25 [95CI -8.25; 12]), suggesting that 3DMOT may be a therapeutic tool. In phase 2 (ongoing; data collection ends June 20, 2022), 19 TBI survivors were randomly assigned to a waitlist control or *at-home* 3DMOT intervention group. Participants improved on 3DMOT an average of 165%, outperforming the in-lab group at baseline (mean difference between in lab versus at home; -0.391 [95CI -0.698; -0.185]) and the final session (mean difference; -0.606 [95CI -1.02; -0.272]). Using estimation statistics, TBI survivors' cognitive functions, symptoms and adaptability will be assessed pre- and post-intervention and compared to in-lab participants. 3DMOT may improve various TBI-related cognitive challenges, and providing an at-home, accessible training tool may better support TBI survivors.

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Poster

460. Traumatic Brain Injury: Developing Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 460.31

Topic: C.10. Brain Injury and Trauma

Support: Department of Veterans Affairs RR&D IK2-RX003376 (O'Donnell)
Department of Veterans Affairs [BLR&D I01-BX005017 (Cullen)]

Title: Neurocritical care and multimodal neuromonitoring in comatose swine following TBI

Authors: *J. C. O'DONNELL^{1,2}, K. D. BROWNE^{1,2}, S. KVINT², S. KARANDIKAR^{1,3}, D. HAN^{1,2}, M. R. GROVOLA^{1,2}, T. J. KILBAUGH^{2,4}, D. CULLEN^{1,2,3}, D. PETROV²;

¹Ctr. for Neurotrauma, Neurodegeneration, & Restoration, CMC-VA Med. Ctr., Philadelphia, PA; ²Dept. of Neurosurg., Ctr. for Brain Injury & Repair, Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA; ³Dept. of Bioengineering, Sch. of Engin. and Applied Science, Univ. of Pennsylvania, Philadelphia, PA; ⁴Dept. of Anesthesiol. and Critical Care Med., Perelman Sch. of Med., Univ. of Pennsylvania, The Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Achieving a positive outcome following moderate-to-severe traumatic brain injury (TBI) often requires monitoring and responding to secondary injury processes, such as altered intracranial pressure (ICP) and brain tissue oxygen ($P_{bt}O_2$). Recapitulating the injury mechanisms (e.g., rotational loading) and responses (e.g., coma) observed in humans is necessary for preclinical study of the resultant neurocritical care phase and beyond. Herein we report on our methods development study applying clinical multimodal neuromonitoring and neurocritical care in the swine rotational head acceleration model of TBI. Female swine (25-30kg) were induced via ketamine/midazolam, intubated, and maintained with isoflurane anesthesia. Femoral artery and internal jugular vein catheterizations were performed to allow for continuous blood pressure monitoring and drug administration, respectively. A lumbar drain was placed to facilitate cerebrospinal fluid (CSF) sampling. Anesthetized subjects were then secured to a pneumatic actuator that provided rapid rotational acceleration of the head in the sagittal plane (sham subjects were secured to the device without activating it). Immediately following TBI or sham injury subjects were transferred to our swine neuroICU and a quad-lumen bolt was secured 1cm rostral to bregma for placement of a parenchymal ICP probe, $P_{bt}O_2$ /temperature sensors, depth electrode, and microdialysis probe. After probes were placed and subjects were stable, total intravenous anesthesia with propofol/fentanyl was initiated and isoflurane was shut off. Animals were monitored continuously up to 36h. EKG, SpO_2 , capnography, blood pressure, ICP, $P_{bt}O_2$ /temperature, and EEG (depth+scalp) were time-synchronized and continuously recorded with waveform resolution on a Moberg CNS-200, and we collected arterial blood, CSF, microdialysate, and urine. Patterns and events mirrored those seen clinically (e.g., apnea, coma, increased ICP, etc.) and were managed according to treatment algorithms adapted from the clinic (e.g. mechanical ventilation, sedation, hypertonic saline, vasopressors, etc.). A large animal model replicating the mechanisms and manifestations of human TBI is essential to bridge the translational gap between rodent studies and clinical trials. The integration of neuromonitoring and critical care into such a model further increases translational relevance and allows for preclinical study of neurocritical care, while extending the study period for moderate-to-severe TBI with coma.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 461.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH-NINDS Grant R01NS102920-02

Title: Restoring functional activation patterns during locomotion after motor complete paraplegia through non-invasive spinal stimulation, activity-based training, and pharmacological intervention

Authors: *A. WILLHITE¹, R. SIU¹, A. V. OVECHKIN², V. EDGERTON⁴, Y. GERASIMENKO³;

¹Kentucky Spinal Cord Injury Res. Ctr., Univ. of Louisville, Louisville, KY; ²Neurolog. Surgery, Univ. of Louisville, Louisville, KY; ³Physiol., Univ. of Louisville, Louisville, KY; ⁴Rancho Res. Inst., Downy, CA

Abstract: Intense step training of increasing speed, body-weight load, and independence after spinal cord injury (SCI) can lead to improvements in motor pool activation which can contribute to functional changes. In combination with neuroplasticity-promoting neuromodulation, activity-based recovery training for locomotion can reactivate dormant neuronal circuitry after SCI. These neuromodulatory approaches include spinal cord transcutaneous electrical stimulation and pharmaceutical agents that enhance spinal plasticity. We hypothesized that a multi-modal approach that synergistically merges these specific neuromodulatory approaches can bring about sufficient spinal plasticity to restore components of locomotion despite clinically “complete” motor paraplegia. Here, we assessed this multi-modal approach on a 27 y/o male categorized with AIS-A motor complete paraplegia at the T8 level. The participant was unable to elicit voluntary movement of any joint below the level of injury at the time of study enrollment. The intervention period consisted of a progressive locomotor training program in which assistance was reduced over the course of 17 months using a variety of assistive devices and systems. Throughout this period, non-invasive transcutaneous spinal cord stimulation (scTS) was delivered to various sites along the spinal cord to enhance spinal excitability of multiple spinal networks during the locomotor training sessions. A serotonin receptor agonist, buspirone, was also ingested twice daily for periods of 2 months across various intervention periods in the study. Functional movement assessments were performed across multiple time points to assess changes in motor pool recruitment, activation patterns, and coordination. Inter- and intralimb coordination were assessed in a gravity-neutral environment, in a body-weight support system, and while walking overground with various assistive devices. The results indicate that these rehabilitative interventions facilitated more effective voluntarily driven activation of EMG patterns of lower limb motor pools, temporally and in amplitude. This facilitator effect on rhythmic stepping, even without loadbearing proprioception, suggests that sufficient levels of neuroplasticity can evolve to regain voluntary control and thus reestablish functional brain-spinal network connectivity after chronic, complete paraplegia.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 461.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH-NINDS R01NS102920-02

Title: Restoring locomotion after motor complete paraplegia through non-invasive spinal stimulation, activity-based training, and pharmaceutical intervention

Authors: *R. SIU¹, A. M. WILLHITE¹, A. V. OVECHKIN¹, V. E. EDGERTON², Y. P. GERASIMENKO¹;

¹Kentucky Spinal Cord Injury Res. Ctr., Univ. of Louisville, Louisville, KY; ²Rancho Res. Inst., Downy, CA

Abstract: Trauma to the spinal cord usually leads to damage of ascending and descending spinal tracts, interrupting the flow of information between the brain and spinal cord. This can lead to partial paralysis if some tracts are functionally preserved and complete paralysis if none or a small percentage are preserved. It has been suggested that neuroplasticity-promoting neuromodulation can restore sensory-motor pathways bidirectionally after spinal cord injury (SCI), reactivating the dormant locomotor neuronal circuitry. These neuromodulatory approaches include electrical stimulation of the spinal cord, locomotor training, and pharmaceutical agents that enhance spinal plasticity. We hypothesized that a multi-modal approach that synergistically merges these specific neuromodulatory approaches can bring about sufficient spinal plasticity to restore locomotion despite clinical “complete” motor paraplegia. Here, we assessed this multi-modal approach on a 27 y/o male categorized with AIS-A motor complete paraplegia at the T8 level. The participant was unable to evoke movements in joints below the level of injury at the time of admission into the study. The intervention period consisted of a progressive locomotor training program in which assistance was reduced over the course of 17 months using a variety of assistive devices and systems. Throughout this period, non-invasive transcutaneous spinal cord electrical stimulation was delivered to various sites along the spinal cord to enhance spinal excitability of multiple spinal networks during the training sessions. A serotonin agonist, buspirone, was also ingested twice daily for periods of 2 months across various points in the study. Neurophysiological assessments following a supraspinal conditioning paradigm were performed across multiple time points to assess any changes in supraspinal modulation that may suggest neuroplastic changes across the spinal lesion. The connections assessed were cervico-lumbar connections, propriospinal pathways, reticulospinal pathways, and pathways that play a role in cortical control of the lower limbs. The results indicate, that across the study, general facilitation of the motor evoked responses occurred across all connections as a result of this rehabilitative intervention, with a stronger effect observed in the voluntary task-based connections. This facilitatory effect suggests that neuroplasticity could have contributed to a resurgence of voluntary control after clinically diagnosed complete paraplegia.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 461.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Kosair Charities OGMB141540
National Center of Neuromodulation for Rehabilitation CCDN190765
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Title: Transcutaneous spinal stimulation: Optimal intensity for acute upright sitting posture and trunk control and training effects in children with spinal cord injury

Authors: ***G. SINGH**¹, K. LUCAS², N. STEPP², P. PARIKH², B. UGILIWENEZA³, Y. GERASIMENKO², A. L. BEHRMAN²;

¹Spalding Univ., Louisville, KY; ³Neurosurg., ²Univ. of Louisville, Louisville, KY

Abstract: Objective: Insufficient stimulation of spinal neuronal networks may result in no observable change in sitting posture; inversely, robust stimulation may restrict effort to sit upright. We investigated the acute effects of graded transcutaneous spinal stimulation (scTS) intensities and 60 sessions of activity-based training (ABT) +scTS to enable 1) voluntary upright sitting and 2) trunk control. We hypothesized that optimal scTS intensities applied acutely and following 60 sessions of ABT+ scTS training will enable 1) voluntary upright sitting with improved posture and 2) reduced center of pressure (COP)/kinematic displacement during trunk perturbation in children with SCI and trunk posture/control deficits. **Methods:** Eight participants ages 3-15 years with acquired SCI, and trunk control deficits will be recruited. Currently, 6/8 participants (mean age=10±5 yrs, 3F,3M) and 1/12 participants (4 yrs, F) are enrolled to test acute and training effects, respectively. A 5-channel scTS, BioStim-5, was used to deliver mono/biphasic rectangular waveform current with 1-ms pulse width, 15-30 Hz frequency with 10 kHz modulated carrier frequency. For acute effects, a range of 0-150mA stimulation was delivered at T11 (30 Hz) and L1 (15Hz), singly and paired. For training effects, ABT +scTS was provided 5d/week for 1.5h session. Three trials of upright sitting posture and trunk control during perturbations (anterior and posterior) were assessed at baseline, at varied scTS intensities increased incrementally from 0-150mA, and pre-post 60 training sessions in 1 participant. Trunk kinematics and COP displacement were collected. **Results:** For acute effects, in 2 participants, a combination of T11+L1 scTS sites produced greatest trunk extension whereas a single scTS site (T11 or L1) produced greatest trunk extension in 4 participants. For trunk control, 5 participants produced least COP displacement during anterior perturbation with scTS at L1 or T11 site, whereas only 1 participant produced least COP displacement with two sites scTS (T11+L1). For

training effects, a combination of ABT+scTS improved upright sitting posture (trunk extension) by 9 degrees without using arms to compensate for balance. **Conclusions:** Optimal scTS parameters to achieve upright trunk posture and control are different across individuals and tasks. Thus, testing for optimal scTS parameters at baseline and adjusted throughout training may be necessary for effectiveness.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 461.04

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Acute and chronic effects of spinal cord transcutaneous stimulation on spinal cord excitability and motor activity post cervical spinal cord injury

Authors: ***P. SHARMA**¹, **G. FORREST**², **S. HARKEMA**¹;
¹Univ. of Louisville, Louisville, KY; ²Kessler Fndn., New Jersey, NJ

Abstract: Most spinal cord injuries (SCI) occur at the cervical cord level and regaining upper extremity (UE) function remains the top rehabilitation priority of this population. Non-invasive neuromodulation of the cervical cord using spinal cord transcutaneous stimulation (scTS) and motor rehabilitative training are shown to be effective in restoring UE functions following cervical SCI. However, the recovery mechanisms secondary to cervical scTS remain largely unknown and demand scientific exploration. In the present work, we investigated the effects of the acute and chronic application of cervical scTS on spinal cord excitability and motor activity. Six participants with chronic cervical SCI (C2-C7, AIS A-C) underwent sixty intervention sessions of scTS along with UE motor rehabilitative training. Multi-segmental motor responses (MMR) and electromyographic (EMG) activity during various functional tasks were collected at **1**) pre and post-single intervention sessions **2**) pre and post-sixty intervention sessions. Preliminary findings from the four participants demonstrated a significant increase in the MMR activity following a single intervention session. However, the facilitatory changes were not seen in both UEs, and we did not observe the presence of long-latency responses as reported previously. Additionally, we did not observe differences in the EMG activity. In contrast, sixty

interventions increased the EMG activity of most of the muscles involved in the motor training. Moreover, a greater increase in the activity was observed for muscles with greater residual motor activity. We did not observe the activation of muscles that failed to generate EMG activity during the pre-intervention assessments. The effect of chronic application of scTS on MMR activity was inconclusive and did not show similar trends between participants. The findings of the present work demonstrate an increase in spinal cord excitability secondary to the scTS acute application. However, the motor activity remains largely unaffected. In contrast, chronic application of scTS resulted in increased EMG activity in target UE muscles indicating improved motor unit recruitment. Additionally, effects of chronic scTS on spinal cord excitability cannot be commented based on MMR profiles, and may require involving other neurophysiological assessments. Overall, acute and chronic application of scTS has different effects on spinal cord excitability and motor activity, and results can vary between UEs within an individual. The data can be further exploited in designing optimal scTS strategies for UE recovery post cervical SCI.

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Disclosures: P. Sharma: None. G. Forrest: None. S. Harkema: None.

Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

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Program #/Poster #: 461.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Swiss National Science Foundation

Title: A single cell atlas of mouse spinal cord injury

Authors: *M. GAUTIER¹, J. W. SQUAIR^{1,2}, M. A. SKINNIDER², C. KATHE¹, T. H. HUTSON¹, N. REGAZZI¹, Y. TEO¹, N. D. JAMES¹, Q. BARRAUD¹, M. A. ANDERSON¹, G. COURTINE^{1,3,4};

¹Fac. of Life sciences, Swiss Federal Inst. of Technol. (EPFL), Lausanne, Switzerland; ²MD/PhD Training Program, Fac. of Medicine, Univ. of British Columbia, Vancouver, BC, Canada; ³Fac. of Engineering, Swiss Federal Inst. of Technol. (EPFL), Lausanne, Switzerland; ⁴Dept. of neurosurgery, Univ. Hosp. Lausanne, Lausanne, Switzerland

Abstract: Recent improvements in biological and engineering strategies have opened promising new avenues to treat spinal cord injury. By targeting the spared spinal circuits and residual neural pathways after spinal cord injury, we can improve motor and autonomic functions. Despite these exciting advances, our work has repeatedly shown that the amount of recovery is strongly correlated to the amount of spared spinal cord connections at the lesion site. In spinal cord injury, the initial traumatic insult is rarely itself the primary determinant of neurological outcome, but instead initiate a complex and progressive cascade of secondary injuries involving inflammatory cell infiltration and cytokine release, apoptosis, demyelination, excitotoxicity,

ischemia, and the formation of a glial scar. How the individual cell types and subtypes of the central nervous system collectively orchestrate this response remain poorly understood, leaving researchers and clinicians with few options to influence it. Here, we constructed a comprehensive single cell atlas of the molecular response to spinal cord injury in the mouse model. We sequenced over half a million nuclei, spanning 18 experimental conditions, to carefully dissect the various transcriptional programs triggered in each cell type and subtype of the central nervous system. We charted the severity dependent response, delineated the molecular transitions between acute, subacute and chronic injury processes, investigated sex and age-related differences, and included classical treatments targeting the lesion site that have not translated into successful clinical trials. We used spatial transcriptomic to resolve the topography of these injury processes, building a comprehensive atlas that will provide an unprecedented resource to the field to identify potential therapeutic targets.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 461.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: SNF grant PZ00P3_185728
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Dr. Miriam and Sheldon G. Adelson Medical Foundation

Title: Single-cell topography enables regeneration across a complete spinal cord injury and restores walking

Authors: ***M. MILANO**¹, J. W. SQUAIR¹, M. GAUTIER¹, A. DE COUCY¹, M. A. SKINNIDER¹, N. D. JAMES¹, N. CHO¹, A. LASNE¹, C. KATHE¹, T. HUTSON¹, S. CETO¹, L. BAUD¹, K. GALAN¹, Q. BARRAUD¹, T. J. DEMING², B. SCHNEIDER¹, Z. HE³, M. V. SOFRONIEW⁴, G. COURTINE¹, M. A. ANDERSON¹;
¹EPFL, Genève, Switzerland; ²Univ. of California Los Angeles, Los Angeles, CA; ³Children's Hosp Boston, Boston, MA; ⁴UCLA Schl Med., Los Angeles, CA

Abstract: Although axon regeneration can now be induced experimentally across anatomically complete spinal cord injury (SCI), restoring meaningful function after such injuries has been elusive. This failure contrasts with the spontaneous, naturally occurring repair that restores walking after severe but incomplete SCI. Here, we performed projection specific and comparative single-nucleus RNA sequencing to identify the transcriptional phenotype and connectome of neuronal subpopulations involved in natural spinal cord repair. We identified a

molecularly defined population of excitatory projection neurons in the thoracic spinal cord that extend axons to the lumbar spinal cord where walking execution centers reside. We show that regrowing axons from these neurons across anatomically complete SCI and guiding them to their appropriate region in the lumbar spinal cord restores walking in mice. These results demonstrate that mechanism-based repair strategies that recapitulate the natural topology of molecularly defined neuronal subpopulations can restore neurological functions following anatomically complete SCI.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 461.07

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Neurotechnologies to restore urodynamic function after spinal cord injury

Authors: ***L. MAHE**¹, **R. HUDELLE**², **E. LAVEIX**², **R. TODESCO**², **M. GAUTIER**², **E. SORIANO**², **F. FALLENGER**², **S. KOMI**², **N. JAMES**², **T. HUTSON**², **C. KATHE**², **S. AMIR**², **O. RIZZO**², **L. BAUD**², **Q. BARRAUD**², **G. ROBAIN**², **L. STEPHANIE**², **J. SQUAIR**², **J. BLOCH**², **A. PHILLIPS**², **G. COURTINE**²;

¹Ecole Polytechnique Federale de Lausanne, Geneva, Switzerland; ²Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland

Abstract: A spinal cord injury (SCI) scatters the communication between supraspinal centers and the spinal cord circuitry responsible for maintaining urodynamic functions. This loss of communication disrupts the coordination of detrusor muscle contractions and the necessary relaxation of the external urethral sphincter. Consequently, people with SCI experience incontinence, chronic urine retention, recurrent urinary tract infections, and other uro-renal complications. Here, we developed a conceptual and engineering framework to design an electrical spinal cord neuroprosthesis that targets circuitry in the spinal cord responsible for modulating urodynamic functions. We first established a rodent model of chronic urodynamic dysfunction after SCI. Clinically-inspired assessments of urodynamic functions revealed a complete impairment of normal micturition and significant bladder overactivity that developed over time after injury. To identify the spinal segments most capable of modulating detrusor and external urethral sphincter contractions, we applied epidural electrical stimulation (EES) to each spinal segment, while measuring the activity of both muscles. Chemogenetic experiments

revealed that EES induces detrusor contractions through a circuitry involving large-diameter parvalbumin neurons located in the dorsal root ganglia and excitatory interneurons within the urodynamic hotspots. We then combined CT scans, MRI sequences, and computational modeling to design a neuroprosthesis that targets urodynamic hotspots in the spinal cord. This neuroprosthesis was fabricated using e-dura technology. Closed-loop algorithms adapted the onset and amplitude of EES bursts to restore the coordination between bladder contraction and sphincter relaxation. The restoration of this coordination reduced bladder hyperreflexia and triggered micturition. Future work will aim to provide a foundation to develop a neuroprosthesis to manage bladder dysfunction in people with SCI.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

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Topic: C.11. Spinal Cord Injury and Plasticity

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Wings for Life Foundation
Compute Canada
Natural Sciences and Engineering Research Council
Canadian Institutes of Health Research

Title: Identifying the neurons that cause autonomic dysreflexia after spinal cord injury

Authors: *J. E. SORIANO¹, R. HUDELLE², J. W. SQUAIR², M. A. ANDERSON², C. KATHE², T. H. HUTSON², M. GAUTIER², L. MAHE², M. TSO¹, S. AMIR², K. GALAN², Q. BARRAUD², J. BLOCH³, G. COURTINE², A. A. PHILLIPS¹;

¹Univ. of Calgary, Calgary, AB, Canada; ²École Polytechnique Fédérale de Lausanne, Geneva, Switzerland; ³CHUV, Lausanne, Switzerland

Abstract: Spinal cord injury (SCI) induces a condition called autonomic dysreflexia (AD), in which there is a rapid onset of excessively high, uncontrolled blood pressure. AD can induce severe hypertensive symptomatology leading to stroke and heart attack. We discovered that long-term use of the neuroprosthetic baroreflex has the capacity to reverse the development of AD. However, the neuronal subpopulations that link these two seemingly discrete circuits remain

unknown. To address this, we are conducting experiments to understand the interconnecting circuitry between AD and EES, which may enable the neuroprosthetic baroreflex to reverse or prevent the aberrant circuit development that drives the development of AD after SCI. To enable these experiments, we established a mouse model of upper-thoracic SCI that develops AD over the course of four weeks. First, we asked whether the induction of AD triggers transcriptional activation of neurons in the lower thoracic spinal cord segments since we previously identified these segments as the key hotspots involved in hemodynamic stability. We found that AD elicited neuronal activity primarily in two regions: the hemodynamic hotspots in the lower thoracic spinal cord, and lower lumbar segments, where colorectal afferents are located. This activity coincided with an increased density of synaptic projections from lower lumbar segments to the hemodynamic hotspots in animals with SCI. Subsequent chemogenetic experiments exposed that AD is dependent on the activation of excitatory interneurons embedded within the hemodynamic hotspots. Likewise, chemogenetic inactivation of excitatory interneurons in the hemodynamic hotspots blunted pressor responses induced by EES. Ongoing experiments are aimed at unravelling the precise neuronal subpopulations that account for these findings. These experiments establish a framework that will enable causal experiments leveraging optogenetic and chemogenetic technologies to unravel intersecting circuits in neurological diseases.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

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Support: Swiss National Science Fund (SNF) grant 310030_192558/1
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Title: Uncovering neonatal regenerative growth programs to build next-generation spinal cord repair strategies

Authors: *S. CETO^{1,2,3}, A. CLERFAYT^{1,2,3,4}, Q. BARRAUD^{1,2,3}, L. BAUD^{1,2,3}, M. A. ANDERSON^{1,2,3}, J. W. SQUAIR^{1,2,3,5}, G. COURTINE^{1,2,3,6};

¹Ctr. for Neuroprosthetics and Brain Mind Institute, Sch. of Life Sci., Swiss Federal Inst. of Technol. (EPFL), Lausanne, Switzerland; ²Dept. of Clin. Neurosci., Lausanne Univ. Hosp. (CHUV) and Univ. of Lausanne (UNIL), Lausanne, Switzerland; ³Defitech Ctr. for Interventional Neurotherapies (.NeuroRestore), Lausanne, Switzerland; ⁴Bordeaux Intl. Master of Neurosci. (NeuroBIM), Univ. of Bordeaux, Bordeaux, France; ⁵Intl. Collaboration on Repair

Discoveries (ICORD), Vancouver, BC, Canada; ⁶Dept. of Neurosurg., Lausanne Univ. Hosp. (CHUV), Lausanne, Switzerland

Abstract: Neonatal mammals possess a remarkable capacity for natural spinal cord regeneration. However, the mechanisms supporting the regrowth of axons across the repaired lesion environment remain unknown. Corticospinal and serotonergic axons have previously been reported to grow across and beyond sites of complete spinal cord crush in neonates, but it is unclear which subcortical and propriospinal neuron populations are capable of similar growth. In male and female C57BL/6JRj mice, we successfully reproduced previously reported scar-free neonatal spinal cord repair. Fibronectin(+) bridges formed in the first days after injury and lost most fibronectin immunoreactivity by one week post-injury. To determine which spinal and brain centers were capable of growing axons across sites of neonatal spinal cord injury in an unbiased manner, we injected a retrograde viral tracer, retroAAV-hSyn-GFP, below the injury site of mice that had received postnatal day 2 or 7 (P2 or P7) complete crush at T10. Remarkably, large numbers of retrogradely labeled neurons were observed in the brain and above-lesion spinal cord of both P2 and P7-lesioned animals. Tissue clearing revealed that, while the injury site was typically narrower than the surrounding cord, individual axons and the main corticospinal tract were able to navigate even tortuous paths of growth-permissive tissue to bridge the lesion site. Brain and spinal regions that normally project to the lumbar spinal cord contained retrogradely labeled neurons. To assess the functionality of newly formed anatomical connectivity, we infected propriospinal neurons above the lesion site with AAV5-Syn-ChrimsonR-tdTomato and recorded spinal cord electrophysiological responses to optogenetic stimulation. Propriospinal cell body stimulation above the injury resulted in robust below-lesion responses, and direct stimulation of axon terminals near the recording site elicited reproducible responses of smaller amplitude. Finally, we performed weekly kinematic recordings of voluntary locomotion over four weeks following P2 crush. Despite being injured before learning to walk, pups gained significant hindlimb locomotor function that began to plateau by 3 weeks post-injury. These results independently validate an important new model of mammalian spinal cord repair and reveal the extent to which diverse axon populations grow and/or regenerate through a complete lesion at early postnatal stages. Understanding the molecular programs that enable such growth may lead to new therapies for spinal cord repair.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

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Wings for Life Foundation
Compute Canada
Natural Sciences and Engineering Research Council
Canadian Institutes of Health Research

Title: Targeted neurotechnologies reverse autonomic dysreflexia after spinal cord injury

Authors: ***R. HUDELLE**¹, J. E. SORIANO², J. W. SQUAIR¹, M. A. ANDERSON¹, C. KATHE¹, T. H. HUTSON¹, M. GAUTIER¹, L. MAHE¹, M. TSO², S. AMIR¹, A. BICHAT¹, K. GALAN¹, N. HANKOV¹, R. DEMESMAEKER¹, K. LARKIN-KAISER², L. ASBOTH¹, Q. BARRAUD¹, S. P. LACOUR¹, A. A. PHILLIPS², J. BLOCH³, G. COURTINE¹;
¹École Polytechnique Fédérale De Lausanne, Geneva, Switzerland; ²Univ. of Calgary, Calgary, AB, Canada; ³Ctr. Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Abstract: People with chronic spinal cord injury (SCI) experience daily severe spikes in blood pressure that reduce quality of life, and exacerbate chronic disease. These hypertensive episodes at the chronic stage of SCI are known as “autonomic dysreflexia” (AD). We previously developed an implantable neuroprosthesis based on epidural electrical stimulation that targets the sympathetic circuitry in the thoracic spinal cord to regulate blood pressure in closed-loop. We showed that this neuroprosthetic baroreflex prevents orthostatic hypotension in real-time with high accuracy in rodents, non-human primates, and in one human with SCI. Here, we hypothesized that chronic application of the neuroprosthetic baroreflex, called autonomic neurorehabilitation, could enable a circuitry remodeling that would prevent the development of AD. To test this hypothesis, we developed a protocol enabling 24/7 recording of blood pressure using wireless telemetry technology in rats. We found that as soon as two weeks after the onset of daily autonomic neurorehabilitation, the severity of AD significantly decreased. We found a similar reduction in the severity of AD even when autonomic neurorehabilitation was started in the chronic stage post injury. This reduction in the severity of AD was accompanied by decreased sprouting of ascending propriospinal neurons from L6 to hemodynamic hotspots in the lower thoracic spinal cord. Moreover, we found that the number of Vglut2+ synapses onto sympathetic pre-ganglionic neurons in the lower thoracic spinal cord increased after SCI, and was normalized following autonomic neurorehabilitation. We then translated these findings to two individuals with cervical SCI that led to debilitating, medically refractory autonomic dysreflexia. Two paddle electrode arrays were surgically positioned, with one array below the T10 and T11 vertebral bodies that contain the posterior roots entering lower thoracic segments, and one array placed over the lumbosacral spinal cord. After two months of autonomic neurorehabilitation, we found that the severity of AD triggered during urodynamics evaluations was significantly reduced. These results demonstrate the feasibility of autonomic neurorehabilitation coupled to epidural electrical stimulation of the lower thoracic spinal cord segments to reduce the severity of AD.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

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Title: The recovery of hindlimb locomotion after staggered lateral thoracic hemisections performed on opposite sides of the spinal cord in cats is mediated by a spinal mechanism

Authors: *S. YASSINE¹, J. AUDET¹, C. G. LECOMTE¹, S. MARI¹, A. MERLET¹, J. HARNIE¹, I. A. RYBAK², B. I. PRILUTSKY³, A. FRIGON¹;
¹Dept. of pharmacology-physiology, Univ. de Sherbrooke, Sherbrooke, QC, Canada; ²Drexel Univ., Drexel Univ., Philadelphia, PA; ³Georgia Inst. Technol., Georgia Inst. Technol., Atlanta, GA

Abstract: Mammals, such as mice, rats and cats, recover hindlimb locomotion following spinal transection at thoracic levels. This is due to the presence of the spinal locomotor central pattern generator (CPG) located at lumbar levels that produces the basic locomotor rhythm and pattern. Using a dual spinal lesion paradigm (spinal lateral hemisection followed by spinal transection), Barriere et al. (2008) revealed a prominent role of the lumbar CPG for hindlimb locomotor recovery following an incomplete spinal cord injury (SCI). However, the involvement of the lumbar CPG following other types of incomplete SCIs has not been explored. In this study, we collected kinematic and electromyography data in 3 adult cats before and after two lateral spinal thoracic hemisections (right T5-T6 followed eight weeks later by left T10-T11) during quadrupedal and hindlimb-only (with forelimbs placed on stationary platform) locomotion on a treadmill at 0.4 m/s. Eight weeks after the second hemisection, we performed a complete spinal transection at T12-T13. One day after spinal transection, all 3 cats performed hindlimb locomotion at 0.4 m/s. We observed several changes in temporal characteristics compared to pre-spinal values, such as decreased hindlimb cycle, stance, and swing durations. On the other hand, we did not observe changes in left-right hindlimb coordination, such as phase intervals and double support periods, as well as in overall kinematics. Moreover, two weeks after spinal transection, all 3 cats expressed quadrupedal locomotion at 0.4 m/s with hindquarter support provided by an experimenter. Spatiotemporal analyses showed a significant increase in the hindlimb's proportion of double support periods and a decrease in hindlimb cycle, stance and swing durations, as well as in hindlimb step and stride lengths during quadrupedal locomotion after spinal transection. In contrast, we found no such changes in the forelimbs. Our results indicate that functional changes in the lumbar CPG that lead to greater autonomy following incomplete SCI do not require direct descending inputs from the brain, which were abolished

with staggered hemisections. This suggests the existence of an intrinsic spinal-mediated mechanism involved in the recovery of hindlimb locomotion after incomplete SCI.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: NSERC RGPIN-2016-03790
CIHR PJT-156296
NIH Grant R01NS110550

Title: Obstacle negotiation during overground locomotion before and after incomplete spinal cord injury in adult cats

Authors: *C. G. LECOMTE, S. MARI, J. AUDET, A. N. MERLET, J. HARNIE, C. BEAULIEU, L. GENDRON, A. FRIGON;
Univ. De Sherbrooke, Sherbrooke, QC, Canada

Abstract: During walking, animals frequently negotiate obstacles, which requires voluntary control from the brain to correct limb trajectory. Despite its importance, few studies have investigated obstacle negotiation after incomplete spinal cord injury (SCI). To address this issue, we trained 10 cats to negotiate obstacles of different heights (1-9 cm) on a walkway. We then implanted electrodes to chronically record fore- and hindlimb muscle activity (EMG, electromyography). After obtaining data in the intact state, we performed a right lateral spinal hemisection at mid-thoracic segments (T6-T7). Data collection resumed 1 week later for 8 weeks. Intact cats stepped over obstacles without contact (CC, complete clearance). After hemisection, the right hindpaw (ipsilesional) made frequent contacts (58% and 37% of trials at 2 and 8 weeks post-hemisection, respectively). In these trials, a reflex response allowed the limb to step over the obstacle (SCR, stumbling corrective reaction) while some contacts did not produce an SCR (Other). From 2 to 8 weeks post-hemisection, Other trials decreased (20% to 2%) while CC increased (42% to 63%). In trials with contact (SCR/Other), the right hindpaw at contact was positioned at a greater distance from the obstacle compared to CC with left limbs leading (left limbs first to cross the obstacle) at 2 and 8 weeks post-hemisection. Left limbs leading accounted for 60% and 75% of CC at 2 and 8 weeks after hemisection, respectively. We observed EMG changes after hemisection, such as delayed onset of right hindlimb extensors in the step preceding crossing the obstacle in trials with contact (SCR, Other) compared to CC at 2 and 8 weeks post-hemisection. Extensors of the left forelimb muscles during Other trials had shorter burst durations (19%) and higher amplitudes (34%) 2 weeks after hemisection with left limbs

leading. Burst durations of right forelimb extensors increased (28%) 8 weeks after hemisection with right limbs leading in SCR trials. A lateral spinal hemisection impairs the ability to negotiate obstacles without contact. The increase of CC from 2 to 8 weeks after hemisection reflects recovery of descending control.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

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Title: Control of interlimb coordination after staggered lateral thoracic hemisections performed on opposite sides of the spinal cord in adult cats

Authors: *J. AUDET¹, S. YASSINE¹, C. G. LECOMTE¹, S. MARI¹, A. N. MERLET¹, J. HARNIE¹, I. RYBACK², B. I. PRILUTSKY³, A. FRIGON¹;

¹Univ. de Sherbrooke, Univ. de Sherbrooke, Sherbrooke, QC, Canada; ²Neurobio. and Anatomy, Col. of Med., Drexel Univ., Philadelphia, PA; ³Georgia Inst. Technol., Georgia Inst. Technol., Atlanta, GA

Abstract: The neural control of interlimb coordination is achieved through dynamic interactions between spinal circuits, supraspinal signals, and peripheral sensory inputs. We investigated changes in interlimb coordination during locomotion by performing two lateral thoracic hemisections placed on opposite sides of the spinal cord. These staggered hemisections block direct descending pathways to lumbar levels as well as ascending lumbar pathways. We collected kinematic and EMG data in nine cats during quadrupedal locomotion at 0.4 m/s before (intact), 8 weeks after a first hemisection on the right side of the cord (single-hemisectioned) at T5-T6, and then 8 weeks after a second hemisection performed on the left side (double-hemisectioned) at T10-T11. All cats recovered standing and quadrupedal locomotion in the single-hemisectioned state, while in the double-hemisectioned state, balance and/or weight support provided by an experimenter was required. Several changes in the gait pattern occurred, such as new coordination patterns between the fore- and hindlimbs, where the forelimbs took more steps than the hindlimbs, or a 2:1 fore-hind ratio. Coordination between the fore- and hindlimbs was weakened and became variable after the first hemisection and even more variable after the second one. For left and right homolateral couplings, we noticed a delayed contact of the forelimb after the first hemisection, which was further delayed after the second one. For homolateral couplings, the coefficient of

variation of phase intervals increased after the first hemisection and showed a slight decrease after the second one. We observed an earlier contact of the right forelimb relative to the left hindlimb after the first hemisection, which was further advanced after the second one. We also observed an earlier contact of the left forelimb relative to the right hindlimb compared to intact cats, but no difference between single- and double-hemisected states. The coefficient of variation of phase intervals for these diagonal couplings was higher after the first hemisection and further increased after the second one. Therefore, although cats recovered quadrupedal locomotion after staggered thoracic hemisections, the control of forelimb-hindlimb coordination was impaired.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 461.14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH-R01-NS08111 (Lane)
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Craig H. Neilson Foundation (M.A.L.)
Lisa Dean Moseley Foundation

Title: Engineered Spinal Interneurons to Repair the Injured Cervical Spinal Cord

Authors: ***T. FORTINO**¹, L. ZHOLUDEVA², M. RANDELMAN¹, A. NICEFORO³, A. K. PONNA⁴, S. SAKIYAMA-ELBERT⁶, M. A. LANE⁵;

¹Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; ²Gladstone Inst., San Francisco, CA; ³Dept. of Neurobio. and Anat., Drexel University-College of Med., Philadelphia, PA; ⁵Neurobio. & Anat., ⁴Drexel Univ., Philadelphia, PA; ⁶Bioengineering, Univ. of Washington, Seattle, WA

Abstract: The phrenic motor network is directly compromised by cervical spinal cord injury (SCI), resulting in diaphragm paralysis and impaired breathing. Despite these consequences, some limited plasticity typically occurs weeks post-injury resulting in increased interneuronal integration with the damaged phrenic network and modest improvement of diaphragm recovery. In an effort to harness and enhance this plasticity, we have engineered stem-cell derived spinal interneurons that can be transplanted directly into the injured spinal cord. We recently found that transplanting neural precursor cells (NPCs) comprised of spinal neural precursors promotes phrenic recovery post-SCI. Building on these initial results, the present work tests the hypothesis that enriching these neural precursors with stem cell-derived V2a and V0 spinal interneurons (SpIN), will further enhance diaphragm recovery. Adult, female, Sprague Dawley rats received SpIN-enriched NPC transplants sub-acutely (1 week) following a lateralized, mid-cervical

contusion injury. Donor SpINs were engineered to express the hM4Di receptor, allowing for inhibition of these cells with application of clozapine. Multi-electrode array recordings were used to confirm inhibition of donor SpINs *in vitro* prior to transplantation. Intraspinal clozapine application during diaphragm electromyography was used to silence transplanted SpINs, resulting in decreased diaphragm output following drug delivery. Pseudorabies virus tracing of the injured phrenic network was used to show synaptic integration of donor NPCs and V2a and V0 SpINs with host phrenic circuit. Ongoing analysis of functional data will provide insight into the relative contribution of these integrated cells with phrenic motor recovery while anatomical analysis will provide anatomical insight for a correlation between synaptically integrated cells and functional recovery. This research provides the first in depth assessment of how these donor SpIN populations contribute to phrenic recovery.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 461.15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01-NS104291
Pennsylvania State Funds for Spinal Cord Injury Research

Title: Identifying Spinal Interneurons that Contribute to Respiratory Plasticity after Cervical Spinal Cord Injury

Authors: *K. A. SCHARDIEN¹, T. A. FORTINO¹, A. A. HALL^{1,3}, J. SÁNCHEZ VENTURA³, M. L. RANDELMAN², L. V. ZHOLUDEVA⁴, M. A. LANE⁵;

²Neurobio. and Anat., ¹Drexel Univ. Col. of Med., Philadelphia, PA; ³Neuroplasticity and Regeneration, Autònoma de Barcelona, Barcelona, Spain; ⁴Gladstone Inst., San Francisco, CA;

⁵Neurobio. & Anat., Drexel Univ., Philadelphia, PA

Abstract: Spinal cord injury (SCI) is a devastating and irreversible event that affects thousands of people each year. Most injuries occur at the cervical level, compromising the phrenic motor network, resulting in life-threatening respiratory deficits. Despite the devastating outcomes, there is significant evidence for spontaneous neuroplasticity after cervical SCI. Spinal interneurons are widely recognized as an essential factor in this plasticity, altering their function and connectivity to provide new neuronal pathways to facilitate some functional recovery. While many spinal interneuron subtypes have been identified, their characterization and involvement in respiratory plasticity after injury has remained limited. Building upon our previous studies focused on the role of excitatory V2a interneurons in neuroplasticity after SCI, the present study uses a battery of outcome measures to focus on exploring the role of inhibitory V1 and modulatory V0c

interneurons and their roles in respiratory plasticity following high cervical SCI in mice. Transgenic mice received a lateral left hemisection at the second cervical segment (C2) which denervates the ipsilateral spinal phrenic motor circuit (C3-C5/6) that controls the diaphragm – a primary muscle of breathing. Weeks to months post-injury, following modest neuroplastic changes and partial recovery, a transsynaptic retrograde tracer - pseudorabies virus – was applied to the left hemidiaphragm to label motor and interneurons within the phrenic motor network ipsilateral to injury. Respiratory function was examined either 2- or 12-weeks following injury through terminal diaphragm electromyography under anesthesia. Tissues were perfuse-fixed, and immunocytochemistry on cervical spinal cord sections was used to map and quantify the distribution of PRV labeled spinal interneurons connected to the injured phrenic network. Preliminary data suggests increased interneuronal connectivity after SCI. The long-term goal of this research study is to understand the neuroplastic potential of these spinal interneurons in contributing to motor recovery after traumatic spinal cord injury.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 461.16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life

Title: Cellular reprogramming for spinal cord repair

Authors: *A. NICEFORO¹, Y. SHAH², L. V. ZHOLUDEVA³, T. FORTINO⁴, L. QIANG⁴, M. LANE⁴;

¹Drexel University-College of Med., Philadelphia, PA; ²Drexel university, Philadelphia, PA;

³Gladstone Inst., San Francisco, CA; ⁴Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Traumatic spinal cord injury (SCI) at cervical levels results in an array of deficits including life-threatening impairments in breathing. This is due primarily to disruption of phrenic motor networks located in the cervical spinal cord. Transplantation of neural precursor cells has been shown to promote repair of damaged spinal tissues and facilitate partial functional recovery. One component - the glial restricted progenitors - has been shown to repair damaged phrenic pathways, but these donor cells lack important neuronal components. The present work builds on this promising strategy using a bi-phasic approach to neural repair, harnessing the strengths of cellular reprogramming. Parallel *in vitro* and *in vivo* experiments were used to generate genetically modified glial restricted progenitors, reprogrammable via doxycycline induction of neuronal differentiation. Primary astrocyte cultures derived from postnatal day 2-3

rat cortex and spinal cord, and embryonic spinal cord, were used for morphological and electrophysiological (multielectrode arrays) characterization of cellular conversion. Notable differences were observed between conversion of cortically-derived and spinally-derived glia. Genetically modified glial restricted progenitors were transplanted into the cervical spinal cord, sub acutely (1 week) after contusive injury. One month after transplantation, animals were given doxycycline (daily subcutaneous injections) to promote expression of transcription factors (*Ascl1* or combination of *miR124*, *miR9/9** and *NeuroD1*) specific to neuronal differentiation. Repair of injured spinal networks was assessed using transsynaptic neuroanatomical tracing (pseudorabies virus) and immunohistochemistry. Phrenic motor function was assessed using terminal diaphragm electromyography in anesthetized animals. These studies are among the first to test this unique bi-phasic approach to cellular repair of the traumatically injured spinal cord.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 461.17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 2021FI_B2 00069
TV3-201736-30-31

Title: Aberrant perineuronal nets alter spinal circuits, impair motor function, and increase plasticity

Authors: *J. SANCHEZ VENTURA¹, C. CANAL¹, J. HIDALGO¹, C. PENAS¹, X. NAVARRO¹, A. TORRES-ESPÍN², K. FOUAD³, E. UDINA¹;
¹Inst. of Neuroscience, Univ. Autonoma de Barcelona, Bellaterra, Spain; ²Dept. of Neurolog. Surgery, Brain and Spinal Cord Injury Center, Univ. of California, San Francisco, CA; ³Physical Therapy, Univ. of Alberta, Edmonton, AB, Canada

Abstract: Perineuronal nets (PNN) are a specialized extracellular matrix that have been extensively studied in the brain. Cortical PNN are implicated in synaptic stabilization, plasticity inhibition, neuroprotection, and ionic buffering. However, the role of spinal PNN, mainly found around motoneurons (MNs), is still unclear. Besides, while cortical PNN reduction is linked to several neurological pathologies, spinal PNN removal by the enzyme Chondroitinase ABC (ChABC) promotes regeneration after a spinal cord injury (SCI). Considering this apparent contradiction and the reduced information on spinal PNN, the goal of our study is to elucidate the role of spinal PNN on motor function and plasticity in both intact and spinal cord injured mice. We used transgenic mice lacking the link protein 1 (cartilage link protein 1 (*Crtl1*) KO mice), which is implicated in PNN assembly. *Crtl1* KO mice showed disorganized PNN with an

altered proportion of their components in both motor cortex and spinal cord. Behavioral and electrophysiological tests revealed motor impairments and hyperexcitability of spinal reflexes in *Crtll* KO compared to WT mice. These functional outcomes were accompanied by an increase in excitatory synapses around spinal MNs. Moreover, *Crtll* KO mice presented a shift in the lumbar MN pool observed as an increase in slow motor units and decrease in fast one. This shift generated a decreased in the number of muscle fibers and muscle weight without a functional repercussion in muscle force assessment probably because slow MN compensate the loss of fast MN innervating more muscle fibers compared to WT mice. Following spinal lesions of the corticospinal tract, *Crtll* KO mice showed increased contralateral sprouting compared to WT mice. Altogether, the lack of *Crtll* generates aberrant PNN that alter excitatory synapses and change the physiological properties of MNs, overall altering spinal circuits and producing motor impairment. This disorganization generates a permissive scenario for contralateral axons to sprout after injury. Thus, these data highlight the importance of spinal PNN on neural function as well as the importance of a precise modulation after injury for an adequate balance between stability and plasticity.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 461.18

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Canadian Institutes of Health Research
Craig H Neilsen Foundation
Alberta Innovates
Canada Foundation for Innovation

Title: Paired transcutaneous spinal cord stimulation with arm and leg cycling to improve walking after incomplete spinal cord injury

Authors: *T. S. BARSS^{1,2,3}, J. PORTER^{1,2,3}, B. PARHIZI^{1,2,3}, Z. KARAMZADEH^{1,2,3}, J. LEE^{1,2,3}, D. OKUSANYA^{1,2,3}, C. CUNNINGHAM^{1,2,3}, A. LOYOLA-SANCHEZ^{1,2,3}, V. K. MUSHAHWAR^{1,2,3};

¹Neurosci. and Mental Hlth. Inst., ²Div. of Physical Med. and Rehabilitation, Dept. of Med., ³Sensory Motor Adaptive Rehabil. Technol. (SMART) Network, Univ. of Alberta, Edmonton, AB, Canada

Abstract: The overall goal of this work is to determine whether non-invasive transcutaneous spinal cord stimulation (tSCS) paired with functional electrical stimulation (FES) assisted arm and leg (A&L) cycling can improve walking after incomplete spinal cord injury (iSCI). When we

walk, we swing our arms in rhythm with our legs due to neural connections in the spinal cord. This coordination between our arms and legs is vital to maintain balance and walk efficiently. Current rehabilitation interventions focus on restoring leg movements through intensive training on a treadmill or with a robotic device, but do not systematically engage the arms in a coordinated fashion with the legs. Previous work has demonstrated that simultaneous A&L cycling assisted with FES doubles the improvements in over-ground walking for individuals with iSCI compared to legs-only cycling and gait-specific training paradigms. Furthermore, neuromodulation of spinal pathways using tSCS has the potential to facilitate improved sensorimotor rehabilitation by modulating circuitry of the spinal cord. Therefore, it was hypothesized that the addition of spinal stimulation would further improve the connectivity of neural networks between the brain and spinal cord, better improve connectivity of spinal cord networks, and better improve the recovery of walking than is currently possible with available rehabilitation strategies. Another component to this work was determining the potential contribution of cutaneous mechanoreceptors to the functional improvements seen with paired tSCS. All participants performed A&L cycling training assisted by FES with half the participants also receiving simultaneous tSCS to the cervical and lumbar regions of the spinal cord. Training included 1 hour of A&L cycling 5 days per week for 12 weeks. Assessments of changes in walking capacity were conducted every 3 weeks throughout the duration of training. Preliminary data indicate that improvements in balance, walking speed, and walking distance occur with the inclusion of tSCS during A&L cycling. Excitingly, this training strategy can be directly translated to the clinic and is not taxing on the therapists. Therefore, if current trends continue, more people with SCI and other neural impairments can improve walking in a cost effective, accessible, and efficacious manner.

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Poster

461. Spinal Cord Repair: From Electrical Stimulation to Single Cells Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 461.19

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Canada Foundation for Innovation
Canadian Institutes of Health Research
Craig H. Nielsen Foundation
University of Alberta Hospital Foundation

Title: Spinal distribution of cFOS positive neurons post epidural stimulation and intraspinal microstimulation

Authors: *C. L. O'SULLIVAN¹, J. R. KRAUTH⁴, N. TYREMAN², W. A. O'STEEN⁴, M. BOAKYE⁵, D. R. HOWLAND⁴, V. K. MUSHAHWAR³;

¹Neurosci. & Mental Hlth. Institute; Sensory Motor Adaptive Rehabil. Technol. Network,

²Neurosci. & Mental Hlth. Institute; Div. of Physical Med. and Rehab, Dept of Med., ³Neurosci and Mental Hlth. Inst; SMART Network; Div. of Physical Med. and Rehab, Dept of Med., Univ. of Alberta, Edmonton, AB, Canada; ⁴Kentucky Spinal Cord Injury Res. Center, Depts of Neurolog. Surgery, Anat, ⁵Neurosurg., Univ. of Louisville, Louisville, KY

Abstract: Spinal cord injury (SCI) is a devastating injury that drastically disrupts functions including the ability to walk. Two methods of spinal cord (SC) neuromodulation show promise to overcome this obstacle, epidural spinal cord stimulation (ESCS) and intraspinal microstimulation (ISMS). ESCS involves placing an array over the dura mater of the SC and indirectly activating the locomotor networks in the ventral horn. ISMS involves inserting ultrafine electrodes into the ventral horn of the SC where it more directly activates the locomotor-related networks. The goal of this project is to determine the type and distribution of neurons activated during ESCS and ISMS to understand their mechanisms of action and functional outcomes.

Neurologically-intact female domestic pigs (DP) were divided into 6 treatment groups: ESCS, sham ESCS, ISMS, sham ISMS, laminectomy control, naïve control. A laminectomy was performed under general anesthesia to expose the lumbosacral enlargement, and depending on the experimental group, an ESCS or ISMS array was implanted. Stimulation lasted for 5 hours at a level just above motor threshold. The DPs with sham ESCS, sham ISMS, or laminectomy only remained anesthetized for 5 hours. No surgery was performed in the naïve control group, and anesthesia was maintained for 5 hours. At the end of the experiments, DPs were euthanized, perfused and tissue preserved for immunohistochemistry (IHC). The distribution of neurons activated by stimulation were determined using antibodies against c-Fos, a marker of activity, and NeuN, a marker of neurons.

Quantitative analysis blinded to group allocation is underway to determine the distributions of neurons activated by ESCS and ISMS. In preliminary IHC findings, positive c-Fos staining is apparent in the ventral horn and intermediate gray matter regions after ISMS, and in the dorsal horn and intermediate gray matter regions after ESCS.

To our knowledge, this is the first time the distribution of neuronal activation by ESCS and ISMS is investigated and compared in an animal model with spinal cord size and morphology that resemble those of humans. These data will provide the normative distribution of neurons activated by ESCS and ISMS in intact DPs and will set baselines for understanding how this distribution may be altered after SCI and chronic stimulation. IHC will be used to assess the types of neurons activated by the two stimulation modalities using antibodies against ChAT (cholinergic neurons), calretinin (excitatory interneurons), calbindin (inhibitory interneurons), parvalbumin (inhibitory interneurons), and GAD67 (inhibitory interneurons).

Disclosures: **C.L. O'Sullivan:** A. Employment/Salary (full or part-time);; University of Alberta. **J.R. Krauth:** A. Employment/Salary (full or part-time);; University of Louisville. **N. Tyreman:** A. Employment/Salary (full or part-time);; University of Alberta. **W.A. O'Steen:** A. Employment/Salary (full or part-time);; University of Louisville. **M. Boakye:** A. Employment/Salary (full or part-time);; University of Louisville. **D.R. Howland:** A. Employment/Salary (full or part-time);; University of Louisville. **V.K. Mushahwar:** A. Employment/Salary (full or part-time);; University of Alberta.

Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 462.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS
VA
Craig H. Neilsen Foundation

Title: Paired Corticospinal-Motoneuronal Stimulation Targeting Multiple Spinal Cord Levels Restores Grasping and Walking in Humans with Tetraplegia

Authors: H. JO^{1,2}, S. SANGARI^{1,2}, S. GAIKWAD^{1,4}, B. CHEN¹, M. OUDEGA^{1,3,4}, *M. PEREZ^{1,2,4};

¹Shirley Ryan AbilityLab, Chicago, IL; ²Dept. of Physical Med. and Rehabilitation,, ³Dept. of Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL; ⁴Edward Hines Jr. VA Hosp., Hines, IL

Abstract: Traumatic cervical spinal cord injury disconnects corticospinal axons from their motor neuron targets, resulting in devastating tetraplegia. Synapses, including those that transmit spinal motor commands, can be modified by Hebbian plasticity (i.e., “neurons that fire together, wire together”) throughout life, suggesting that such modification could be used to rebuild damaged connections. We developed a noninvasive paired corticospinal-motoneuronal stimulation (PCMS) protocol that targets multiple upper- and lower-limb muscles in parallel by activating spinal motor neurons a few milliseconds after the arrival of corticospinal action potentials. Tetraplegic patients underwent forty thirty-minute sessions combined with standard physical rehabilitation over eight weeks. All patients exhibited twofold improvements in grasping, overground walking, and quality of life, durable at nine months after therapy. In all targeted muscle groups, electrophysiological responses elicited by corticospinal pathway stimulation were potentiated. These results demonstrate that a brief, non-invasive intervention aimed at engaging endogenous plasticity can achieve long-lasting functional restoration from tetraplegia.

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Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 462.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS
VA

Title: Enhancing Corticospinal-Motoneuronal Plasticity by an Acoustic Stimuli in Intact Humans

Authors: *S. GAIKWAD^{1,2}, M. A. PEREZ^{1,2,3};

¹Shirley Ryan AbilityLab, Chicago, IL; ²Dept. of Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; ³Edward Hines Jr., VA Hosp., Hines, IL

Abstract: Paired corticospinal-motoneuronal stimulation (PCMS) has been used to enhance voluntary motor output and corticospinal excitability in humans with and without central nervous system injury. To date the extent to which we can potentiate PCMS-induced plasticity remains poorly understood. PCMS increases plasticity by strengthening spinal synapses, thus, it is possible that activation of other descending motor tracts converging on motor neurons can further enhance this plasticity. The reticulospinal tract projects directly and indirectly motor neurons and controls proximal and axial muscles. In humans, an acoustic startle cue, which engages the reticular system, enhances motor neuron excitability (Tazoe and Perez, 2016). We hypothesize that recruitment of reticulospinal inputs during PCMS will enhance spinal plasticity. During PCMS, corticospinal volleys evoked by transcranial magnetic stimulation (TMS) over the arm representation of the primary motor cortex were timed to arrive at corticospinal-motoneuronal synapses of the bicep brachii muscle ~1-2 ms before the arrival of antidromic potentials elicited in motoneurons by electrical stimulation of the brachial plexus. Subjects participated in the following two randomized sessions on separate days: PCMS and PCMS+startle; they received 180 pairs of central and peripheral stimulation every five seconds with and without a preceding loud acoustic stimuli (120 db) given 80 ms before TMS through headphones. MEPs evoked by cervicomedullary electrical stimulation (CMEPs) were tested before and after each session. Latencies of responses in the biceps brachii among participants used to determine central and peripheral stimulation during either PCMS and PCMS+startle protocol, respectively, were as follows: maximal motor response (M-max)=4.7±0.4 ms and 4.8±0.4 ms, cervical root (c-root)=5.9±0.6 ms and 5.9±0.3 ms, and motor evoked potential (MEPs) elicited by TMS=11.2±0.9 ms 11.5±1.2 ms. We found that CMEPs size in the biceps brachii increased by 223.6±94.9% after PCMS and by 304.5±118.2 after PCMS+startle compared with baseline. Baseline CMEPs latencies were 7.9±0.4 ms and 8.0±0.3 ms during PCMS and PCMS+startle sessions, respectively. CMEPs latencies did not change following 180 paired pulses of PCMS or PCMS+startle. Our findings suggest that a startle stimuli can represent an avenue to potentiate corticospinal-motoneuronal plasticity. Thus, engaging reticulospinal inputs to motor neurons, may represent a strategy to boost after-effects of PCMS in humans with spinal cord injury and other disorders affecting the corticospinal pathway.

Disclosures: S. Gaikwad: A. Employment/Salary (full or part-time):: Shirley Ryan AbilityLab, Chicago, IL, Edward Hines Jr.VA Hospital, Hines, IL, USA. M.A. Perez: A. Employment/Salary (full or part-time):: Shirley Ryan AbilityLab, Chicago, USA, Department of Physical Medicine and Rehabilitation, Northwestern University, Chicago, USA, Department of Physical Therapy and Human Movement Sciences, Northwestern University, Chicago, USA, Edward Hines Jr.VA Hospital, Hines, IL, USA.

Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 462.03

Topic: C.11. Spinal Cord Injury and Plasticity

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Title: Effectiveness of ventrolateral periaqueductal gray (vlPAG) stimulation with very low frequency in the spared nerve injury model

Authors: *H. CHANG¹, M. PARK^{1,2}, C. KOH¹, H. JUNG¹;
¹Dept. of Neurosurg., Yonsei Univ. Col. of Medine, Seoul, Korea, Republic of; ²Yonsei Univ. Col. of Med., 2Brain Korea 21 PLUS Project for Med. Sci. and Brain Res. Inst., Seoul, Korea, Republic of

Abstract: The periaqueductal gray (PAG) is one of the major centers of the descending pain inhibitory system. It has been known that the of PAG alleviates mechanical allodynia and the activity of nociceptive neurons in the spinal cord. This study aimed to understand whether long-term PAG stimulation in neuropathic pain model may attenuate mechanical allodynia. Male Sprague-Dawley rats were used. Rats were assigned into 3 groups: Normal, Sham, Stimulation. Rats with spared nerve injury were implanted with customized microelectrodes in the vlPAG (AP:-7.8mm, ML: 0.5mm, DV: 5.2mm). Behavioral responsiveness was evaluated using von Frey test before modeling SNI, on post-operative day (POD) 14 and every 1 hour for five days. Stimulation was maintained for 9 hours throughout 5 days (Stimulation parameter: pulse with duration 200us, amplitude 200uA during 200ms at 5Hz). At the end of the 9 hours of stimulation, it was turned off and resumed stimulation next day. On day 5, rats were sacrificed immediately after the end of the stimulation. Brain and spinal cord were harvested for immunohistochemistry, and electrode tract was confirmed using hematoxylin and eosin (H&E) staining. Based on the H&E staining results, we confirmed the electrode was properly implanted in the vlPAG region. Mechanical allodynia was attenuated in the stimulation group compared to sham group, and the effects were more statistically significant, especially 6 hours after the stimulation (PreStim: 1.584 ± 0.35 , 6hr Stim: 6.665 ± 0.82 , * $p < 0.05$). Immunohistochemical analysis showed decreased glial activation in the spinal dorsal horn. This study demonstrated that long-term vlPAG stimulation in the SNI model effectively attenuated behavioral hypersensitivity. These results suggest that neuropathic pain could be modulated with very low frequency. Overall, the effectiveness of PAG stimulation may be used as a novel neuromodulation method for the treatment of neuropathic pain.

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Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

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Topic: C.11. Spinal Cord Injury and Plasticity

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Title: Brain-machine-interface based on gamma oscillation of neural signals in somatosensory cortex with virtual reward

Authors: ***T. KIM**¹, **Y. CHO**³, **C. KOH**¹, **M. PARK**^{1,2}, **S. JUN**³, **H. JUNG**¹, **J. CHANG**^{1,2};
¹Dept. of Neurosurg., ²Brain Korea 21 PLUS Project for Med. Sci. and Brain Res. Inst., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; ³Dept. of Electronic and Electrical Engin., Ewha Womans Univ., Seoul, Korea, Republic of

Abstract: Brain-Machine-Interface (BMI) technology can control external machines or computers by detected neural signal changes. We Provided visual feedback followed by stimulation of medial forebrain bundle (MFB). MFB is the main area of virtual reward, enables the animal to learn how to control the mechanical unit, a cursor. In this experiment, we analyzed neural signals from the somatosensory area of the rats in real-time and used as a control signal for external device. This study implemented a design of BMI that can be controlled by visual feedback and analyze neural signals from somatosensory cortex in the absence of signals from motor cortex. For chronic neural recording and electrical stimulation, customized electrodes are implanted into the somatosensory barrel cortex and the MFB of Sprague Dawley rats. After surgery, rats were trained for 6 days in the skinner box for self-stimulation training. When addiction process is finished, the machine control session starts with getting used to the monitor setting for a day, then training lasted 7 days. Total 10 sessions for each rat were recorded for further analysis to validate the system. The gamma power between right and left barrel cortex showed differences in trials and it was used for control signal. The success rates were gradually increased from day 1 to day 7 ($62.0 \pm 6.49\%$, $89.0 \pm 4.69\%$, respectively; $p^* < 0.05$). This shows that the animal effectively learned how to control the object (circular figure) on the monitor. This study showed the possibility that somatosensory cortex can be an effective alternative region for BMI when the motor region is impaired. It has been demonstrated that rats can move the cursor without any physical movements with stimulation-based visual feedback.

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Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 462.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: KDDF Grant HN22C0307
NRF Grant 2021R1I1A1A01047750
NRF Grant 2020R1F1A1074104

Title: Liquid metal based neural electrodes for deep brain stimulation and recording

Authors: *C. KOH¹, T. KIM¹, D. LEE³, Y. KWON³, Y.-G. PARK³, J.-U. PARK³, J. CHANG^{1,2}, H. JUNG¹;

¹Neurosurg., ²Brain Korea 21 PLUS Project for Med. Sci. and Brain Res. Inst., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; ³Materials Sci. and Engin., Yonsei Univ., Seoul, Korea, Republic of

Abstract: Micro-electrical stimulation of neurons and monitoring of neural activities in the brain are the essential technology to diagnose and treat neurological disorders such as Parkinson's disease, essential tremor, dystonia, and neuropathic pain. Recently, various neural probes have been developed to treat those brain disorders. However, the use of solid metals in electrodes have limitations in biocompatibilities. Metal based electrodes are rigid and stiff, which enables to reach the target region in implanting surgery. However, brains are normally submerged in cerebrospinal fluid so highly rigid electrodes could damage the adjacent soft brain tissues. This can cause immune responses and inflammations resulting in glial scars, which hinders the long-term and stable stimulation and recording processes. To overcome the conventional limitation of electrodes, we presented facile Gallium-Indium eutectic (EGAI_n) based liquid metal electrodes to detect neural activities and deliver micro-electrical stimulation in the deep brain region. Liquid metal electrode was made using EGAI_n and polyimide tubing. The tip of the electrodes was coated with platinum black for higher electrochemical properties and biocompatibility. Its neural signal recording capability and implantation stability were verified through *in vivo* animal study by recording the nucleus accumbens (NAcc) and long term stimulation of ventral posterolateral nucleus of thalamus (VPL). EGAI_n has comparable Young's modulus to the neural tissues, which minimizes the formation of inflammation and glial scars compared to conventional rigid metals. We demonstrated the *in-vivo* tests for electrical stimulation and *in-situ* recording of neuronal signals from the rat brain. *In vivo* recording of NAcc, the firing rate of neurons were increased following medial forebrain bundle stimulation (pre stimulation: 21.8 ± 3.6 Hz, post stimulation: 36.45 ± 4.4 Hz, respectively; $p^* < 0.05$). In chronic implant test, stimulating the VPL with liquid metal electrode successively induced turning behavior in awake rats. Collectively, this liquid electrode can be advantageous to long-term, stable recording and stimulation to treat neurological disorders.

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Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 462.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: R03 AG073929
R01 NS121191
F32 HD107806

Title: Intravital evaluation of microvascular structure and reactivity in the aged rodent spinal cord

Authors: *A. CHANDRASEKARAN¹, J. S. HARMON¹, J. E. HYDE¹, M. J. REED², C. P. HOFSTETTER¹, M. F. BRUCE³, Z. Z. KHAING¹;

¹Neurolog. Surgery, ²Geriatric Med., ³Applied Physics Lab., Univ. of Washington, Seattle, WA

Abstract: Significant reductions in microvascular density and function have been observed in the brain with normal aging; it has been hypothesized that this underlies the increased susceptibility of the older population to ischemic injuries. Prior studies in preclinical models of spinal cord injury (SCI) have shown that aged rodents sustain larger injury volumes and greater locomotor deficits compared to young rodents, suggesting that similar age-related microvascular changes are present in the spinal cord. However, there is a dearth of evidence, particularly in vivo, that elucidates alterations in spinal hemodynamics and microvascular reactivity in response to a vascular challenge in older animals. To investigate these changes, we developed a novel ultrafast contrast-enhanced ultrasound imaging modality (CEUS) to quantify hemodynamic parameters at baseline and following intermittent hypoxia (IH) challenges. Male rats acquired from the National Institute of Aging (F344xBN F1; 6 and 24 months) were subjected to epidural imaging following a three-level laminectomy (C4-C6). CEUS imaging was conducted both longitudinally and axially at baseline and axially only during IH (3 min on, 8 min off, 10% O₂ balanced with N₂). Rise time and area under the curve were quantified following a bolus injection of contrast agent (Definity®, 20 µl per 100 g body weight) to assess relative vascular resistance and blood volume. At baseline, aged rats exhibited a significantly higher rise time than young rats (1.63±0.095 vs 1.33±0.089 sec, N=4, p < 0.05), indicating increased vascular resistance in the aged animals. Additionally, aged rats exhibited substantially increased vascular tortuosity, especially in the ventral vertebral artery. Interestingly, at baseline aged rats did not differ in area under the curve (i.e., total blood volume). Histological analysis revealed no detectable differences in the microvessel density between the spinal cords of young and aged rats. Perhaps more critically, aged rats were less able to respond to the vascular challenge of IH. Young and aged rats exhibited opposite responses to IH exposure; whereas young rats showed increased blood volume within the spinal cord during IH (+21.8% from baseline; N=4), aged rats showed decreased blood volume (-21.2% from baseline; N=4), suggesting an inability to adequately respond to increased oxygen demand. Ongoing experiments will: 1) determine if these observed age-related changes occur progressively or in a stepwise manner, by examining

middle-aged rats, and 2) elucidate age-related differences in the dynamics of injury expansion in the acute phase following an experimentally induced contusion model of SCI.

Disclosures: A. Chandrasekaran: None. J.S. Harmon: None. J.E. Hyde: None. M.J. Reed: None. C.P. Hofstetter: None. M.F. Bruce: None. Z.Z. Khaing: None.

Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 462.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CONACyT doctoral fellowship 893213

Title: Changes in c-Fos expression in the cerebellar hemispheres of male rats with thoracic spinal cord injury

Authors: S. Y. LARA-APARICIO¹, A. J. LAUREANI-FIERRO¹, L. BELTRAN-PARRAZAL², C. MORGADO-VALLE², L. I. GARCIA², F. ROJAS-DURAN², A. J. MARTINEZ-CHACON³, R. TOLEDO-CARDENAS², P. CARRILLO³, M. E. HERNANDEZ², J. MANZO², *C. A. PEREZ²;

¹Doctorado en Investigaciones Cerebrales, ²Inst. de Investigaciones Cerebrales, ³Inst. de Neuroetologia, Univ. Veracruzana, Xalapa, Mexico

Abstract: Spinal cord injury (SCI), depending on its location and severity, can result in temporary or permanent changes in the spinal cord's motor, sensory, or autonomic function. Previous studies have shown that, in addition to increasing cell activity at the site of injury, cell activity in regions distant from the injury is also modified, such as the hypothalamus, the autonomic nuclei of the brainstem, and the cerebellar vermis. However, no studies are focusing on the hemispheres of the cerebellum. Therefore, the present work investigated whether their cell activity is altered 6 and 24 hours after complete spinal cord transection in rats. To achieve this objective, adult male Wistar rats (300-400 g) were used and divided into three groups: intact, sham, and SCI (T8 level). After the experimental manipulation, locomotion was evaluated with the Tarlov/Bohlman scale to assess whether the spinal injury was well done, and the cerebellum was processed to perform immunocytochemistry against c-Fos. A Generalized Linear Model test was performed for a unifactorial ANOVA design with a Poisson type error distribution and multiple contrasts adjustment to determine the significant differences in the number of c-Fos immunoreactive cells of the different groups analyzed. Our results showed that the intact and sham groups presented locomotion grade 4, normal; while the SCI group presented grade 0, paraplegia. Six hours after injury, a significant increase in c-Fos immunoreactivity was detected in all three deep lateral nuclei and all lobes of the granular and Purkinje layers except lobe 10 of the injured rats. The increase in c-Fos immunoreactivity was greater in the SCI group compared to the sham and intact groups. On the other hand, 24 h after the injury, a significant increase in c-

Fos immunoreactivity was observed in all the lobes of the granular and Purkinje layers, as well as in the three deep lateral nuclei. Furthermore, the increase in the number of positive cells at 24 h was significantly greater than the data observed at 6 h. We suggest that the cellular activity that increases at 6 h is related to the activation of protective mechanisms. While those observed at 24 h would be related to neurodegenerative mechanisms. We speculate that the c-Fos immunoreactivity increased in the cerebellum could be related to the activation of proinflammatory mechanisms that would lead to neuronal death.

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Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 462.08

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Canadian Institutes of Health Research Grant PJT 165823

Title: Spontaneous GABA_A receptor activity in sensory axons contributes to exaggerated sensory transmission to motoneurons and spasms after spinal cord injury

Authors: K. HARI¹, A. KHATMI², L. SANELLI³, S. LIN³, K. K. FENRICH⁴, A. M. LUCAS-OSMA³, *D. J. BENNETT²;

¹Neurosci. and Mental Hlth. Inst., UNIVERSITY OF ALBERTA, EDMONTON, AB, Canada;

²Neurosci. and Mental Hlth. Inst., ³Univ. of Alberta, ⁴Univ. of Alberta, Edmonton, AB, Canada

Abstract: GABA has recently been shown to have a paradoxical excitatory action on sensory axons, by activating GABA_A receptors near nodes of Ranvier in myelinated branches of proprioceptive Ia afferents in the spinal cord. These receptors cause a depolarization (primary afferent depolarization, PAD) that helps facilitate spike propagation through the many nodes and complex branch points in these afferents (termed nodal facilitation), without which spike propagation failure is common. This nodal facilitation is caused by both phasic PAD produced by synaptic activation of GABAergic neurons and tonic PAD produced by extrasynaptic GABA_A receptors containing the $\alpha 5$ subunit. Considering that sensory transmission to motoneurons is tonically increased with spinal cord injury (SCI) and this leads to muscle spasms, we wondered whether this increased transmission is due to exaggerated axonal GABA receptor activity in Ia afferents, leading to an exaggerated tonic PAD and nodal facilitation. We explored this in the sacrocaudal spinal cord, maintained in vitro, from adult mice following a chronic S2 spinal transection (1 - 2 months prior), as well as with confocal imaging of receptors. As expected, we found that there was more tonic PAD after SCI, since blocking $\alpha 5$ GABA_A receptors with

L655708 caused a larger hyperpolarization of afferents than in uninjured mice. This exaggerated tonic PAD with SCI led to a greater conduction of afferents (both antidromic and orthodromic conduction), increased monosynaptic reflexes and ultimately spasms, since these changes were reversed by L655708. This left no headroom for further nodal facilitation, since optogenetic activation of GABAergic neurons produced little if any increase in spike conduction or transmission to motoneurons, unlike in uninjured mice. However, we unexpectedly found that this tonic PAD after SCI was resistant to inhibiting spinal neuronal activity with glutamate blockers or silencing synaptic transmission with TTX, implying that the tonic PAD that enables spike conduction after SCI is not produced by neuronal circuit activity from GABAergic neurons. Interestingly, GABA_A receptor blockers that act as inverse agonists at the GABA_A receptor, including L655708 and bicuculline, reduced tonic PAD after SCI (in TTX), whereas those that are neutral antagonists, like gabazine, did not, suggesting that the tonic PAD and associated GABA_A receptor activity after SCI results partly from constitutive GABA_A receptor activity. Overall, these results imply that, unlike in clinical practice, blocking GABA receptors, rather than activating them, might be useful in reducing spasms and restoring function after SCI.

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Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

Location: SDCC Halls B-H

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Program #/Poster #: 462.09

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Swedish Research Council (2015-03359)
Swedish Research Council (2020-00943)
Swedish Brain Foundation (FO2019-0011)
Swedish Brain Foundation (FO2020-0003)

Title: Fighting the spinal cord injury: Neuroprotective plasticity in spinal cord motoneurons

Authors: ***A. PEDRONI**¹, **Y.-W. DAI**², **L. LAFAOUSSE**¹, **I. SRIVASTAVA**¹, **W. CHANG**¹, **K. AMPATZIS**¹;

¹Karolinka Institutet, Karolinka Institutet, Stockholm, Sweden; ²Karolinska Institutet, Stockholm, Sweden

Abstract: The spinal cord injury (SCI) triggers a cascade of degenerative and self-propagating aberrant biological events, including a massive release of glutamate that leads to the elevations of intracellular calcium to toxic levels, resulting in an even more extensive loss of neuronal function and cell death (excitotoxic-mediated secondary injury). Spinal motoneurons are vulnerable to excitotoxic damage in progressive motor neuron disease (MND) and spinal lesions. Despite numerous preclinical studies and clinical trials, there are no effective neuroprotective

strategies to prevent neuronal death or favor the recovery and restoration of network functionality after the trauma. In contrast to mammals, adult zebrafish are capable of sustaining throughout the whole life neuronal proliferation, regeneration, and functional restoration of motor function after a complete spinal cord transection. By combining different approaches, we aimed to investigate whether excitotoxic-mediated degeneration similarly occurs in zebrafish motoneurons following the SCI. Here, we present a body of evidence revealing the existence of a complex of dynamic and adaptive neuroprotective mechanisms, acting at the physiological, morphological, synaptic, and cellular levels, conferring resilience to motoneuron to excitotoxic stress. Our general assumption, that will be further investigated, is the existence of a causal link between the suggested neuroprotective machinery and the remarkable regenerative capability of the zebrafish spinal cord.

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Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

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Program #/Poster #: 462.10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: VA Grant 1I01BX005287

Title: Cervical excitatory interneuron mediated response to respiratory stress and spinal cord injury

Authors: *A. BREZINSKI^{1,2,3}, C. DOLICK^{1,3}, S. KURPAD^{1,3}, K. SATKUNENDRARAJAH^{1,2,3};

¹Neurosurg., ²Physiol., Med. Col. of Wisconsin, Milwaukee, WI; ³Clement J. Zablocki Veterans Affairs Med. Ctr., Milwaukee, WI

Abstract: Cervical spinal cord injury (cSCI) is a lifelong affliction in which respiratory dysfunction is the most significant cause of morbidity and mortality. Bulbospinal input to the diaphragm from the respiratory premotor center in the brainstem is damaged in cSCI, resulting in diaphragm paralysis and other related respiratory deficits. Neuromodulation of cervical eINs has been shown to restore function to the paralyzed hemidiaphragm immediately after hemisection injury at the C2 spinal level (C2Hx). Furthermore, our lab has previously demonstrated that respiratory-related cervical excitatory eINs, while not essential for eupneic breathing, are critical for sustaining ventilation in the chronic phase of cSCI. Despite this, critical gaps remain in our knowledge regarding the functional and anatomical integration of eINs into the brainstem respiratory network in uninjured and C2Hx conditions. Using monosynaptic viral tracing strategies, we report that the anatomical integration of cervical eINs into the respiratory neural circuitry is progressively increased during the chronic phase of cSCI. In particular, we

demonstrate for the first time, direct monosynaptic input from brainstem raphe serotonergic neurons to cervical eINs. Subsequently, we used a genetic and viral approach to induce Pharmacologically Selective Actuator Module (PSAM) expression in a Cre-recombinase-dependent manner in cervical eINs. These PSAM expressing eINs were then acutely silenced via Pharmacologically Selective Effector Molecules (PSEM) during normoxic and hypercapnic conditions. When compared to saline controls, silencing of cervical eINs in healthy mice resulted in significantly decreased ventilatory response to hypercapnia, as measured by tidal volume. Conversely, silencing cervical eINs did not significantly alter the ventilatory response to hypercapnia in C2Hx mice. Together these findings demonstrate that brainstem-spinal interneuronal circuitry modulates ventilation in response to acute respiratory demands.

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Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

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Program #/Poster #: 462.11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Postdoctoral Fellowship #651019 (FMM)
SCoBIRC Chair Endowment (AGR)

Title: Reversible chemogenetic silencing of ascending propriospinal neurons modulates hemodynamic changes associated with autonomic dysreflexia in response to noxious stimuli following spinal cord injury

Authors: *F. M. MICHAEL, S. P. PATEL, H. M. VAUGHT, J. C. THARAPPEL, A. G. RABCHEVSKY;

Spinal cord injury & brain research Ctr. (SCoBIRC) and Dept. of Physiol., Univ. of Kentucky, Lexington, KY

Abstract: Autonomic dysreflexia (AD) is a condition that develops following high thoracic spinal cord injury (SCI) which is characterized by acute episodic hypertension and baroreflex-mediated bradycardia in response to noxious stimuli below the injury. Thoracolumbar sympathetic preganglionic neurons (SPN) localized in the intermediolateral cell column (IML) discharge sympathetic responses, however their dysregulation below the injury level due to loss of supraspinal control contributes to development of AD. The neural circuitry underlying AD includes 1) nociceptive afferent C-fibers that perceive stimuli below injury level, 2) SPN that trigger the sympathetic response, and 3) ascending propriospinal neurons (APN) that relay information from afferent terminals to the IML. While maladaptive plasticity of lumbosacral C-fibers following SCI is correlated with the onset and development of AD, we aimed to document the contribution of APN terminal sprouting into the IML by using chemogenetic tools to modify

hemodynamic responses during experimental AD in the adult rat. Putative APN residing in the lumbosacral (L6/S1) spinal cord with terminals projecting rostrally to the IML were selectively and reversibly silenced by injecting at the L6/S1 spinal level a cre recombinase-dependent adeno-associated viral vector (AAV) under a neuron-specific promoter (i.e. hsyn) expressing HM4Di, the inhibitory designer receptors exclusively activated by designer drugs (DREADD). Cre recombinase was delivered via AAV-retro-cre injections bilaterally at APN terminals projecting to T7 or T13 spinal levels and retrogradely transported to cell bodies in the L6/S1 level. We injected vectors either immediately after complete T4 spinal cord transection versus delayed 2-weeks to compare transfection efficiency before and during injury-induced sprouting (e.g. APN mCherry labeling in L6/S1). Then, beginning two-four weeks after injections (n=4 immediate, n=5 delayed), we evaluated the severity of colorectal distension (CRD)-induced AD before, during and after DREADD-mediated silencing of APN, using CNO ligand. Our results demonstrate that reduced AD severity during CRD, which was reversible, was associated with greater numbers of silenced APN (e.g. mCherry labeling), irrespective of immediate vs delayed viral injections. We are further quantifying the expression of the immediate early gene, c-Fos, in the IML following terminal intermittent CRD to evaluate the extent of propriospinal neuronal activation, with or without DREADD-mediated silencing of APN.

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Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 462.12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Mary Tucker Currie Professorship, Awarded to J.W. Grau, TAMU

Title: Positivity in the spinal cord: Proprioceptive gating and learning promote adaptive plasticity in the spinal cord

Authors: *K. E. HUDSON, M. M. TARBET, J. W. GRAU;
Texas A&M Univ., Bryan, TX

Abstract: Prior studies have shown that neurons within the spinal cord can support instrumental learning and that this adaptive plasticity can be disrupted by uncontrollable nociceptive input. Rats undergo a thoracic (T2) transection and are subsequently tested. Over time rats shocked whenever the leg is extended (controllable stimulation) learn to maintain the stimulated leg in a flexed position that minimizes net shock exposure. This capacity to learn is prohibited by as little as 6 mins of uncontrollable shock. The learning deficit can be prevented by exposure to controllable and fixed time stimulation prior to uncontrollable nociceptive signals. Recently we developed a framework for characterizing how spinal cord function is affected by behavioral

control and temporal regularity (see abstract: “A Framework for Characterizing How Behavioral Control and Temporal Regularity Affect Spinal Cord Function”, Grau et al). To identify ways to maximize adaptive plasticity while minimizing maladaptive impacts the present study assessed the role of adaptive plasticity thresholds, proprioceptive signals, and priming of the cord with controllable stimulation. In Exp 1 rats were trained for either 6’ or 30’ with response contingent shock. After training, maintenance was assessed. Animals were then challenged with 6’ uncontrollable shock. In Exp 2 rats received 6’ of uncontrollable shock to the hind limb while it was held in a flexed position or allowed to hang freely. Exp 3 mirrored Exp 2 using a clinically relevant source of nociceptive input, capsaicin. Exp 4 assessed whether the effects of proprioceptive signaling were dependent upon the stimulation occurring on the limb in question. 6 mins of uncontrollable shock to the tail was administered while both hind limbs were held in a flexed position or allowed to hang freely. Rats that were exposed to nociceptive stimulation while the hindlimbs were flexed were able to learn, whereas those that received pain input with the legs hanging freely expressed a learning deficit. Interestingly, administration of uncontrollable shock after training (Exp 1) appeared to drive learning. Given this, we examined if the spinal cord interprets stimulation differently after controllable stimulation in Exp 5. Spinalized rats received either 30’ of training or nothing followed by 30’ yoked shock to the contralateral leg. Surprisingly, pre-trained rats learned with yoked shock. Together these results suggest that proprioceptive signals gate the effects of nociceptive input after spinal cord injury (SCI) and that initial stimulation can prime the spinal cord to interpret stimulation as adaptive, both of which may have therapeutic implications in SCI.

Disclosures: **K.E. Hudson:** None. **M.M. Tarbet:** None. **J.W. Grau:** None.

Poster

462. Probing and Harnessing Neural Plasticity After Spinal Cord Injury

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 462.13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Mary Tucker Currie Professorship

Title: A framework for characterizing how behavioral control and temporal regularity affect spinal cord function

Authors: ***J. W. GRAU**¹, **K. E. HUDSON**², **M. M. TARBET**³;

¹Psychological and Brain Sci., Texas A&M Univ., College Station, TX; ²Texas A&M Univ., Bryan, TX; ³McGovern Med. Sch., Houston, TX

Abstract: Prior work has shown that neurons within the lower (lumbosacral) spinal cord can learn about environmental relations after communication with the brain has been severed by means of a rostral (T2) transection. Spinally transected rats given shock whenever a hind leg is extended quickly learn to maintain the leg in a flexed position, implying a sensitivity to

response-outcome (instrumental) relations. This behavioral modification is not observed when intermittent shock is given independent of leg position (uncontrollable). Further, just 6 min of uncontrollable stimulation (Figure 1) impairs the capacity to learn. If intermittent stimulation is given in a regular (predictable) manner, further exposure (24 min) induces a restorative effect that enables learning. This sensitivity to regularity has been linked to the engagement of an oscillator (central pattern generator). Further analysis has revealed that exposure to controllable stimulation enables instrumental learning and induces a peripheral modification at the neuromuscular junction that fosters the maintenance of a flexion response. Here we present a framework to characterize how these environmental relations affect spinal cord function (Figure 2; Grau, 2022, J Exp Psychol: Animal Learn Cog).

Figure 1. Emergence of learning effects as a function of training

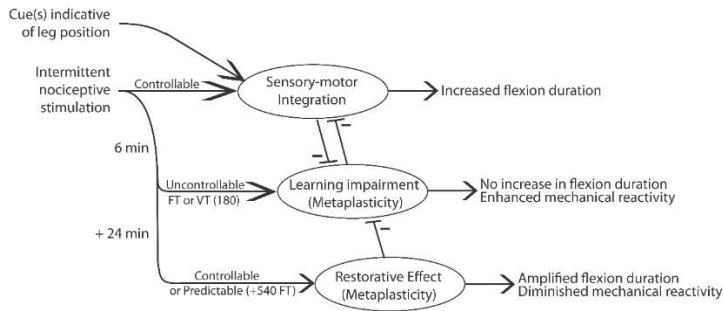
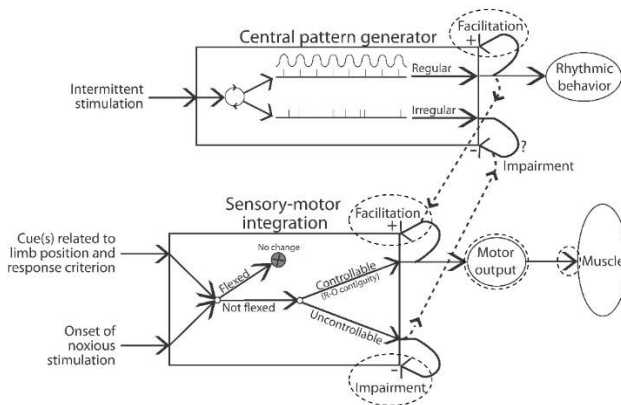


Figure 2. Hierarchical regulation of the consequences of stimulation



Disclosures: J.W. Grau: None. K.E. Hudson: None. M.M. Tarbet: None.

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.01

Topic: D.02. Somatosensation – Pain

Support: Department of Defense OR170276

Title: Recombinant gene therapy using serine-histogranin and endomorphin-1 in a model of phantom limb pain in male and female rats

Authors: *K. PERRUCCI, A. EESWARA, A. PACHECO-SPIEWAK, S. JERGOVA, J. SAGEN;
Univ. of Miami, Miami, FL

Abstract: Phantom limb pain (PLP), the emergence of pain seeming to originate from a missing limb, is a common consequence of limb amputation. Pharmacologic treatments including opioids are modestly effective at best and accompanied with numerous side effects including tolerance and addiction. Therapy targeting multiple pain pathomechanisms may be most beneficial for management of severe neuropathic pain syndromes. A key mechanism thought to contribute to neuropathic pain is enhanced signaling via glutamate NMDA receptors. Previously characterized peptide serine-histogranin (SHG) demonstrates inhibitory properties at the NMDA receptor and can reduce pain in other models. Recent studies in our lab have shown that direct intraspinal injection of viral vectors producing SHG can reduce pain symptoms following peripheral and CNS injury. The addition of a potent mu-opioid peptide, endomorphin-1 (EM-1), delivered via gene therapy, may provide additional synergistic antinociception. The current study explored management of PLP-like behavior by developing a targeted gene therapy which could provide a sustained source of pain-reducing peptides. To induce PLP, male and female Sprague-Dawley rats underwent chronic constriction injury (CCI) on day 0, followed by complete axotomy of the sciatic nerve at either day 1 or day 7 following the CCI. Several scenarios were selected to evaluate optimal timing of the intervention and to characterize sex-related differences. AAV2/8 particles encoding SHG, SHG+EM-1 or control GFP were injected intraspinally, intrathecally or into the dorsal root ganglia. Animals were observed and scored daily for the onset and severity of PLP autotomy behavior. The overall development of autotomy behavior was slightly less severe in females compared to males. The reduced severity, onset or even prevention of this PLP behavior was observed in animals treated by SHG or SHG/EM1, with the latter showing significantly better outcome compared to SHG alone in some cases, especially in females. Intraganglionic injections lead to the most potent effect when administered on the day of axotomy, while intrathecal injection showed the most consistent beneficial outcomes throughout the various scenarios. FLISA analysis confirmed the presence of SHG and/or EM1 in the tissue or CSF of treated animals. The levels of pain-related biomarkers were reduced in treated animals,

with significantly lower levels observed in animals treated with both SHG and EM1, especially in females. Our data suggests combined targeting of glutamate and opioid signaling pathways using a gene therapy approach can reduce or prevent the severity and development of PLP behavior.

Disclosures: **K. Perrucci:** None. **A. Eeswara:** None. **A. Pacheco-Spiewak:** None. **S. Jergova:** None. **J. Sagen:** None.

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.02

Title: WITHDRAWN

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.03

Topic: D.02. Somatosensation – Pain

Support: 2021R1H1A1A01058139
2022R1F1A107365

Title: Rapamycin, an mTOR inhibitor suppresses orofacial neuropathic pain and p-mkk4/p-p38 MAPK-mediated microglial activation in TNC in trigeminal nerve injured mice

Authors: **J. YEO**, *D. ROH;
Kyunghee Univ., Seoul, Korea, Republic of

Abstract: **Rapamycin, an mTOR inhibitor suppresses orofacial neuropathic pain and p-mkk4/p-p38 MAPK-mediated microglial activation in TNC in trigeminal nerve injured mice**

Ji-Hee Yeo¹, Dae-Hyun Roh¹

¹Department of Oral Physiology, School of Dentistry, Kyung Hee University, Seoul, Republic of Korea

Neuropathic pain caused by trigeminal nerve injury is a typical refractory orofacial chronic pain accompanied by the formation of hyperalgesia and allodynia. We previously demonstrated that rapamycin, an mTOR inhibitor suppressed orofacial formalin-induced nociception. However, it is unclear whether rapamycin can reduce trigeminal neuropathic pain and which mechanism is

involved. In mice, infraorbital nerve was exposed, and partial nerve ligation (ION-PL) was performed using silk suture (8-0). At 14 days after surgery, neuropathic pain behaviors were examined on the whisper pad, and rapamycin (1 mg/kg) was intraperitoneally treated. The mechanical and cold sensitivity in left orofacial region was quantified using von-Frey filaments and acetone solution, respectively. The changes of mTOR and related proteins, p-mkk4 and p-p38 MAPK, GFAP and Iba-1 in TNC tissue were examined using western blot assay or immunohistochemistry. In addition, the cytokine assay was performed to verify the underlying mechanism for the anti-allodynic effect of rapamycin. Mice showed significant mechanical and cold allodynia at 2 weeks after ION-PL injury. Both mechanical and cold allodynia were significantly reduced 1 hour after rapamycin injection. In TNC tissue, ION-PL surgery or rapamycin treatment did not alter the p-mTOR, p-S6 and p-4EBP1 expression, whereas rapamycin significantly decreased the increased expression of GFAP and Iba-1 in ION-PL mice. In addition, rapamycin suppressed the increase of p-p38 MAPK expression, which was related to decreased p-mkk4, but not p-mkk3/6 expression. In particular, the p-p38 MAPK positive cells were co-localized with the increased Iba-1, the microglia marker. Furthermore, rapamycin potently reduced cytokines and chemokines such as CXCL10, CXCL13, C5/C5a, IL-3, M-CSF and TNF-alpha in ION-PL mice. These findings demonstrated that rapamycin treatment reduced both facial mechanical and cold allodynia in trigeminal neuropathic mice, which was closely associated with the modulation of p-mkk4/p-p38 MAPK-mediated microglial activation in TNC. Moreover, the regulation of several inflammatory cytokines and chemokines were involved in these effects of rapamycin.

Disclosures: J. Yeo: None. D. Roh: None.

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.04

Topic: D.02. Somatosensation – Pain

Support: MOST 109-2314-B-004 -001

Title: The analgesic mechanisms of trimethylglycine on cisplatin-induced neuropathic pain in mice

Authors: *M.-H. CHAN¹, Y.-Z. LUO¹, Y.-T. HUNG², H.-H. CHEN²;

¹Natl. Chengchi Univ., Taipei, Taiwan; ²Natl. Hlth. Res. Inst., Miaoli, Taiwan

Abstract: N-methyl-D-aspartate (NMDA) receptor modulators can relieve neuropathic pain. This study aimed to investigate the analgesic efficacy and mechanisms of trimethylglycine that could regulate NMDA receptor in a mouse model of cisplatin-induced neuropathic pain by von Frey test to examine animal paw withdrawal threshold. Our results showed that cisplatin-elicited neuropathic pain was alleviated by trimethylglycine via intraperitoneal (i.p.), intrathecal (i.t.),

intraventricular (i.c.v.), and microinjection into medial prefrontal cortex (mPFC). The anti-allodynic effect of trimethylglycine (i.p.) was reversed by an NMDA receptor inhibitor 7-chlorokynurenic acid (7-CK) via i.c.v. injection but not i.t. injection. Microinjection of 7-CK into mPFC could block the pain relief effect of trimethylglycine microinjection into mPFC, but could not inhibit the anti-allodynic effect of trimethylglycine (i.p.). An α 2-adrenoceptor antagonist yohimbine (i.p., i.t. or i.c.v. injection) could inhibit the analgesic effect of trimethylglycine. The anti-allodynic effect of trimethylglycine was also reduced by β -adrenoceptor antagonist propranolol. WAY100635, a 5-HT1A receptor antagonist, can diminish the pain relieving effect of trimethylglycine by i.p., i.t. or i.c.v. injection. 5-HT7 receptor antagonist SB269970 blocked the analgesic effect of trimethylglycine by i.t. injection, whereas, i.c.v. injection of SB269970 had no effect. In addition, i.t. and i.c.v. injection of the α 7-nAChR antagonist MLA and the α 4 β 2-nAChR antagonist DH β E could inhibit the analgesic effect of trimethylglycine. These results showed that trimethylglycine could relieve neuropathic pain involved in neuronal regulation including NMDA, adrenergic, serotonergic, and cholinergic receptors. Therefore, activation of descending pain inhibitory pathway and regulation of spinal cord play a role in trimethylglycine alleviating cisplatin-induced neuropathic pain.

Disclosures: M. Chan: None. Y. Luo: None. Y. Hung: None. H. Chen: None.

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.05

Topic: D.02. Somatosensation – Pain

Support: Boston Scientific and Duke University Anesthesiology Research Funds

Title: Combination of 1 kHz and 60 Hz spinal cord stimulation produces analgesia with prolonged wash-out in a rat spare nerve injury model of neuropathic pain

Authors: *Y. D. HUH^{1,2}, J. XU¹, T. ZHANG⁴, R. ESTELLER⁴, B. HERSHEY⁴, R.-R. JI^{1,2,3}; ¹Ctr. for Translational Pain Medicine, Dept. of Anesthesiol., ²Dept. of Cell Biol., ³Dept. of Neurobio., Duke Univ. Med. Ctr., Durham, NC; ⁴Boston Scientific Neuromodulation Res. and Advanced Concepts, Valencia, CA

Abstract: Spinal cord stimulation (SCS) has been used in clinical settings at either high (1 kHz) or low (60 Hz) frequencies to effectively produce analgesia in neuropathic pain conditions. However, the onset and offset of analgesia provided by SCS has been assumed to follow the amount of time that active stimulation is applied. In this study, the combination of 1 kHz and 60 Hz spinal cord stimulation were delivered at 40% of motor threshold to a spared nerve injury (SNI) rat model of neuropathic pain over four 2-hour treatment sessions, each spaced two days apart (days 1, 3, 5, and 7). In addition to statistically significant reversal of mechanical allodynia by SCS during the 2-hour stimulation periods, we observed sustained significant reversal of

mechanical allodynia after stimulation was shut off. This prolonged wash-out effect became evident on day 3 and increased in magnitude following SCS on days 5 and 7. After day 7, statistically significant reversal of mechanical allodynia continued with SCS off until day 15 of this study, 8 days after the final SCS session. Furthermore, cold allodynia behaviors showed a similar progressive statistically significant reduction over the course of the four treatment sessions and extended until day 11 of this study, 4 days after the final SCS session. Significant reductions of nerve injury-induced astroglial (GFAP expression), microglial (Iba1 expression), as well as satellite glial cell (GFAP expression) reactivity were observed in the spinal cord and dorsal root ganglia ipsilateral to SNI injury from rats that received combination SCS, suggesting that anti-inflammatory, non-neuronal mechanisms contribute to the observed sustained analgesia. Thus, the combination of high and low frequency SCS may represent a novel methodology for extending the time course of analgesia for chronic neuropathic pain.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.06

Topic: D.02. Somatosensation – Pain

Support: The J. Willard and Alice S. Marriott Foundation
Marriott Daughters Foundation

Title: Nociceptor-macrophage crosstalk as a driver of endometriosis pain and lesion growth in mice

Authors: *V. FATTORI¹, T. H. ZANINELLI^{1,2}, F. S. RASQUEL-OLIVEIRA¹, O. K. HEINTZ¹, M. L. SESHAN³, A. E. LINDHOLM³, R. M. ANCHAN³, W. A. VERRI, Jr², M. S. ROGERS¹;

¹Vascular Biol. Program, Boston Children's Hosp., Boston, MA; ²Dept. of Pathology, Londrina State Univ., Londrina, Brazil; ³Dept. of Obstetrics, Gynecology and Reproductive Biol., Brigham and Women's Hosp., Boston, MA

Abstract: Endometriosis is a debilitating and painful estrogen-dependent inflammatory disease that affects approximately 10% of reproductive-age women. Approximately 30% of patients continue to experience pain despite the use of currently available treatments, underscoring the need for new medical therapies with long-term benefit. Neuroimmune communication plays a role in several diseases therefore, we sought evidence for a role in endometriosis-associated pain and lesion growth. Endometriosis was induced non surgically in female C57BL/6 mice (8 weeks of age, 10 mice/group). Data was analyzed by one- or two-way ANOVA followed by Tukey's *post hoc* test with $p < 0.05$ for significance. With calcium imaging of mouse DRG neurons we

showed that mouse endometriosis lesions directly activate nociceptors. Moreover, using cytokine and angiogenesis array kits we identified that the lesion components VEGF and PLGF induced neuropeptide release by cultured DRG neurons. We also showed that mouse and human lesions are highly innervated by peptidergic nociceptors and at the DRG level, endometriosis mice showed activation of that same nociceptor subpopulation. In corroboration, targeted ablation of TRPV1⁺ nociceptors (TRPV1creDTA or RTX-treated mice) reduced mechanical (von Frey filaments) and spontaneous pain (abdominal squashing, contortions, and licking) as well as reduced lesion size, suggesting that neuropeptide release contributes to lesion attachment and/or growth. Blocking nociceptor signaling with QX-314 (charged lidocaine derivate that only blocks activated TRPV1 neurons) reduced mechanical and spontaneous pain, and reduced lesion size in mice. In lesion explants, we also observed QX-314 reduced neuropeptide release. In the peritoneal cavity (PerC), RTX-treated mice showed reduced number of F4/80⁺Ly6C⁺ cells both in the PerC and lesions by FACS. In corroboration, by co-culturing mouse PerC macrophages and endometriosis epithelial cells, we observed that nociceptor released factors stimulated macrophage support of mouse endometriosis cell (promoted cell growth) as well as impaired the ability of macrophages to efferocytosis 12z cells (human endometriosis cell line). Finally, in oppose to CCR2 KO mice, resident macrophage depletion with clodronate reduced endometriosis lesion size. Our results suggest that neuroimmune communication mediates monocyte recruitment to the PerC during endometriosis and contributes to lesion growth. Lack of drug efficacy at reducing ongoing pain drives most endometriosis therapy failure, thus, our data with QX-314 blocking nociceptor signaling might result in clinical benefit for endometriosis patients.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.07

Topic: D.02. Somatosensation – Pain

Support: Funding by TrueRelief, Santa Monica
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NIH Grant R01GM132672

Title: High frequency electrical stimulation reduces neuronal high mobility group box 1 (HMGB1) release and ameliorates neuropathic pain

Authors: *H. YANG, T. DATTA-CHAUDHURI, S. J. GEORGE, B. HAIDER, J. WONG, M. BRINES, K. J. TRACEY, S. S. CHAVAN;
Feinstein Inst., Feinstein Inst. for Med. Res., MANHASSET, NY

Abstract: Chronic pain presents a major unmet clinical problem. High-frequency electrical nerve stimulation (HFES) has achieved clinical success as an analgesic modality for pain management, but the underlying mechanism is unknown. We reasoned that HFES may inhibit neuroinflammatory mediator release by sensory neurons to reduce pain. HMGB1, a key mediator of injury- and infection-elicited inflammation, is involved in the pathogenesis of persistent pain. We recently reported that neuronal HMGB1 is required for mediating inflammation and hyperalgesia following nerve injury (Yang H et al. PNAS, 2021). Here we assessed the effects of HFES in modulating HMGB1 release by sensory neurons. Using microelectrode arrays (MEAs) in cultured dorsal root ganglia (DRG) harvested from transgenic mice that express light-sensitive channelrhodopsin in sensory neurons, we observe that light-evoked HMGB1 release from DRGs is significantly reduced with HFES (HMGB1 levels in unstimulated group = 5.3 ± 0.5 ng/ml; in light exposed group = 25.8 ± 6.0 vs. light + HFES = $8.2 \pm 2.1^*$ pg/ml, N=6, *: P<0.01 vs. light group). In agreement, *in vivo* studies showed that HFES (10 min/per day X 3 days) significantly reduces mechanical hyperalgesia and HMGB1 levels in the inflamed paws in C57BL/6 mice that subjected to chronic constriction injury (CCI) of the sciatic nerve (HMGB1 levels in sham surgery group = 10.6 ± 1.1 ng/mg protein, in CCI group = 29.0 ± 3.9 vs. CCI + HFES = $12.8 \pm 1.6^*$ ng/mg protein, N=10 mice per group, *P<0.001 vs. CCI group). Similar findings were observed in Sprague-Dawley rats subjected to sciatic nerve injury. Together, these results support the mechanistic insight that HFES may reset sensory neurons into a less pro-inflammatory state via inhibiting the release of neuroinflammatory mediators such as HMGB1.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.08

Topic: D.02. Somatosensation – Pain

Support: DOD W81XWH-17-PRCRP-TTSA
Thomas Jefferson University MD-PhD Program

Title: Neuropod mediated changes to DRG electrical properties potentially play a role in the pathophysiology of visceral abdominal pain

Authors: *T. D. ALEXANDER¹, J. BARTON², A. LONDREGAN², S. WALDMAN², M. COVARRUBIAS¹;

¹Farber Inst. of Neurosci. Thomas Jefferson Univ., Philadelphia, PA; ²Pharmacol. and Exptl. Therapeut., Philadelphia, PA

Abstract: Chronic visceral pain (CVP) of gastrointestinal (GI) origin results from dysregulated interactions between the GI and nervous systems and is a significant health burden in the US.

Guanylyl cyclase C (GUCY2C) is an intestinal receptor highly expressed in a subpopulation of enteroendocrine cells (EECs) that interact with dorsal root ganglion (DRG) neurons. These EECs are known as neuropod cells because they exhibit neuron-like properties, and may transmit nociceptive information to DRG neurons. Supporting this role, the synthetic GUCY2C agonist linaclotide has significant analgesic effects in CVP. Yet the mechanism by which GUCY2C activation leads to CVP analgesia remains unclear. To determine how GUCY2C-high neuropod EECs regulate visceral pain signaling, we generated cocultures of these cells with embryonic mouse DRG neurons and performed whole-cell current-clamp recordings from DRG neurons that were near neuropod EECs. DRG neurons that were not near neuropod EECs were recorded as controls. GUCY2C-high neuropod EECs and surrounding crypt cells from small intestine were obtained from fluorescent mouse reporter lines, and DRG neurons were obtained from E15 mouse embryos. To characterize the intrinsic excitability of the DRG neurons, we measured the resting membrane potential (RMP), input resistance (Ri), rheobase, and number of action potentials (APs) fired upon current stimulation at 3x rheobase. We found that, in the absence of a GUCY2C ligand, DRG neurons that are potentially interacting with GUCY2C-high neuropod EECs are hyperexcitable, as demonstrated by a more depolarized RMP, a lower rheobase and an increased number of APs fired at 3x rheobase, compared to DRG neurons not interacting with neuropod cells ($p < 0.001$). These effects were eliminated upon exposure to 1 μM linaclotide ($p = 0.01$). In contrast, 10 μM extracellular cGMP (the product of GUCY2C) had no effect on the electrophysiological phenotype of the hyperexcitable DRG neurons. These results strongly suggest a new pathway of visceral pain signaling involving GUCY2C-high neuropod EECs that communicate with DRG neurons to regulate their intrinsic excitability and thereby nociceptive signaling. Therefore, GUCY2C-high neuropod EECs emerge as therapeutic targets to develop new interventions to treat CVP.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.09

Title: WITHDRAWN

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.10

Topic: D.02. Somatosensation – Pain

Support: NIH Grant RO1CA208371
NIH Grant RO1CA227064

Title: Nasally administered mesenchymal stem cells need B cells to resolve neuropathy and chemobrain

Authors: *N. BOUKELMOUNE, A. K. SINGH, J. ZHANG, A. KAVELAARS, C. J. HEIJNEN;
Symptom Res., The Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Background: Chemotherapy-induced peripheral neuropathy (CIPN) and cognitive deficits (chemobrain) are common, often severe side effects that occur in ~ 75% of cancer patients undergoing chemotherapy and persist in over 30% of survivors, considerably affecting quality of life. Neuronal mitochondrial dysfunction is a key pathogenic mechanism involved in neuropathic pain and chemobrain. Currently, there are no effective FDA-approved drugs to prevent or cure these conditions. We previously showed that nasal administration of mesenchymal stem cells (MSC) reverses CIPN and cognitive impairment in mice (PMID 33316379 and 30473752). Here, we examined the mechanisms that mediate the beneficial effects of MSC after chemotherapy. **Methods-** 8 weeks old mice received cisplatin or saline for 2 cycles of 5 daily injections (2.3 mg/kg) with 5 days of rest in between. Adoptive transfer of T cells or B cells to mice deficient in T cells and/or B cells was performed before chemotherapy. MSC were administered nasally at 48 and 96 h after the last dose of cisplatin. Macrophages were depleted via nasal or intrathecal delivery of clodronate-containing liposomes prior to MSC administration. CIPN was assessed prior to cisplatin and nasal MSC treatment and monitored over time after the last dose of MSC. Mechanical allodynia was measured using von Frey hairs; spontaneous pain was tested using a conditioned place preference test. Mitochondrial function of dorsal root ganglia (DRG) and brain synaptosomes was determined by Seahorse Flux analysis. Cognitive functioning of the mice was evaluated using puzzle box test and novel object and place recognition test. **Results-** Nasal MSC administration resolved cisplatin-induced mechanical allodynia, spontaneous pain and corrected perturbed DRG mitochondrial bioenergetics in WT mice as opposed to mice deficient in T cells and B cells (Rag2^{-/-}). Adoptive transfer of T cells to Rag2^{-/-} mice prior to cisplatin treatment only partially attenuated mechanical allodynia but failed to restore DRG mitochondrial dysfunction. Depletion of anti-inflammatory macrophages in WT mice minimized the positive effect of MSC on mechanical allodynia caused by cisplatin treatment. Interestingly, reconstitution of B cell-deficient mice (muMt⁻) with splenic B cells before cisplatin and MSC treatment completely resolved CIPN and chemobrain symptoms. **Conclusion-** B cells are primarily required for MSC to resolve CIPN and chemobrain possibly in interaction with T cells and macrophages.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.11

Topic: D.02. Somatosensation – Pain

Title: Frequency-dependent loss of conduction fidelity in dorsal column axons during epidural spinal cord stimulation (SCS)

Authors: *K. J. MOHSENIAN, N. D. TITUS, W. M. GRILL;
Biomed. Engin., Duke Univ., Durham, NC

Abstract: Epidural SCS is an effective therapy for chronic pain, but the lack of understanding of the mechanisms of action has limited optimization of this promising treatment modality. Conventional paresthesia-producing SCS delivers pulse repetition frequencies from 50-150 Hz, and the generally accepted gate-control mechanism is mediated by activation of large dorsal column (DC) axons that antidromically activate inhibitory interneurons, which, in turn, inhibit projection neurons and block the pain signal. This understanding and current research presume that DC axons fire faithfully every time a stimulation pulse is delivered. However, recent computational studies suggested that there may be a loss of conduction fidelity, or faithful generation of an action potential for each stimulation pulse, depending on the stimulation frequency. We measured the changes in conduction fidelity in single DC axons during and after epidural SCS in healthy, urethane-anesthetized Sprague-Dawley rats. Thoracic laminectomy was performed, and a bipolar tungsten electrode was used to conduct single axon recordings. Different stimulation frequencies (10, 20, 50, 100, 200, and 500 Hz) were randomly delivered during 5- and 15-minute trials of SCS with 200 μ s biphasic pulses at an amplitude equal to twice the axon's activation threshold. Lipski's criteria for antidromic identification were used to determine that data were recorded from individual DC axons. There was little change in firing fidelity (< 7% loss of fidelity) during 10 Hz & 20 Hz SCS, but SCS between 50-500 Hz generated a significant decrease in axon fidelity, with a greater loss in fidelity at higher frequencies (50 Hz: $56 \pm 25\%$ fidelity, 100 Hz: $28 \pm 12\%$ fidelity, 200 Hz: $22 \pm 15\%$ fidelity, 500 Hz: $23 \pm 24\%$ fidelity), quantified from 17 recordings across 6 animals. At stimulation frequencies ≥ 50 Hz, the firing fidelity decreased to reach a steady-state, and the time to reach the plateau decreased with frequency (50 Hz: 2.7 min, 100 Hz: 1.8 min, 200 Hz: 1.0 min, 500 Hz: 0.9 min). The recovery time for the axon to respond consistently and with the same action potential shape was measured by stimulating the axons at 1 Hz for 3 minutes following each SCS trial. Axons recovered more slowly after higher stimulation frequencies, and some axons took longer than 2 min to recover after 500 Hz SCS. The discovery of a loss of fidelity in DC axon responses during epidural SCS contradicts the widespread assumption that DC axons follow the rate or pattern of SCS. This loss of fidelity should be factored into future SCS investigations and modeling, and more specifically, used to design stimulation patterns that activate the DC axons without a reduction in fidelity.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.12

Topic: D.02. Somatosensation – Pain

Title: 10kHz scs normalizes central sensitization-based receptive field size in a rodent model of neuropathy.

Authors: *K. LEE¹, D. LEE², D. WANG², Z. KAGAN², K. BRADLEY²;

¹Nevro Corp, Redwood City, CA; ²Nevro Corp, Poway, CA

Abstract: It has been shown that nerve injury results in the reduction of inhibitory tone in spinal dorsal horn. Since a primary function of inhibition in sensory processing is to restrict receptive field size, disinhibition after nerve injuries can cause not only abnormal pain but also distinct enlargement of somatic receptive fields, thus reducing sensory acuity. Clinically, reduced two-point discrimination in patients with neuropathic pain is consistent with increased RF overlap that could occur following their disinhibition-induced expansion. Previously, we found that low intensity 10kHz spinal cord stimulation preferentially activated inhibitory interneurons in the DH. Here we explored the effect of 10kHzSCS on the receptive field of the hindpaw of naïve and spinal nerve ligation model rats. SNL model rats were made by ligating the L5 spinal nerve under deep isoflurane anesthesia. Rats showing allodynic responses to the von Fey stimulation until 7 days after surgery were used. Rats were anesthetized with urethane and a spinal-segmental L1-L4 laminectomy was performed. A cylindrical 3-contact mini-SCS lead was advanced rostrally in the epidural space so that the stimulating contacts were dorsally positioned over the L5-L6 spinal segments. 10kHzSCS at 30% of motor threshold was administered via a modified clinical trial stimulator module. A 16-channel microelectrode was implanted at the L5 level to record from neurons in the DH with a receptive field of the left hind paw. RFs were defined on the basis of spiking of wide dynamic range neurons evoked by mechanical stimuli to of the left hindpaw. The RF zone eliciting the largest response was defined as the RF Center, and the zones with the smallest or no responses as RF Surround. First, we measured the RF zone size in naïve and SNL rats. Next, we tested the functional consequences of the inhibitory surround, by comparing responses to afferent probing in the RF center with responses to afferent probing simultaneously in the RF center *and* RF surround. The expansion of the RF size were confirmed in all SNL rats. In naïve rats, afferent probing in the RF surround significantly reduced the response to afferent probing in the RF center, while, in SNL rats, afferent probing in the RF surround significantly enhanced the response to afferent probing in the RF center. However, in SNL rats receiving 10kHzSCS, afferent probing in the RF surround was able to reduce the response to afferent probing in the RF center to the naïve level. These data suggest that 10kHzSCS, by reducing central sensitization via selective inhibitory dorsal horn activity, may help recover sensory acuity by restoring somatic surround inhibition.

Disclosures: **K. Lee:** A. Employment/Salary (full or part-time);; NEVRO CORP. **D. Lee:** A. Employment/Salary (full or part-time);; NEVRO CORP. **D. Wang:** A. Employment/Salary (full or part-time);; NEVRO CORP. **Z. Kagan:** A. Employment/Salary (full or part-time);; NEVRO CORP. **K. Bradley:** A. Employment/Salary (full or part-time);; NEVRO CORP.

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.13

Topic: D.02. Somatosensation – Pain

Support: DoD grant W81XWH2110885

Title: Npd1 protects against chemotherapy-induced neuronal degeneration and neuropathic pain through gpr37 activation in drg neurons

Authors: *J. XU;

Duke university, Duke Univ., Durham, NC

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) limits cancer treatment and impairs the quality of life of patients. CIPN is characterized by acute pain, paraesthesias, sensory ataxia, mechanical and cold allodynia, and there are currently no effective treatments for these side effects. Previous studies from our lab have shown that neuroprotectin D1 (NPD1), a lipid mediator derived from omega-3 polyunsaturated fatty acids (DHA) has potent analgesic effects in animal models of inflammatory pain and neuropathic pain. We recently also identified GPR37 as a novel receptor for NPD1, and NPD1 activates GPR37 in macrophages to resolve inflammatory pain and infection-induced pain. However, it is unknown whether NPD1 can protect CIPN via GPR37 expression in neurons. We found that intrathecal injection of NPD1 can reduce the mechanical allodynia induced by paclitaxel. In primary neuronal cultures of mouse dorsal root ganglia (DRG), the chemotherapy agent paclitaxel (PTX, 1 μ g/ml) substantially suppressed neurite outgrowth; but NPD1 treatment (10 ng/ml) can significantly prevent the inhibition of neurite outgrowth by PTX in DRG neurons of wild-type mice. Notably, this protective effect of NPD1 is lost in DRG neurons of *Gpr37* knockout mice. In situ hybridization by RNAscope shows that *Gpr37* is expressed in DRG neurons but not satellite glial cells. Compared to WT mice, paclitaxel-induced mechanical allodynia is prolonged in KO mice, suggesting a critical role of GPR37 in the resolution of neuropathic pain. Finally, knockdown of GPR37 expression by intra-DRG microinjection of *Gpr37*-targeting siRNA in the L4 and L5 DRG was sufficient to produce mechanical hypersensitivity in naïve mice, and the siRNA-treated mice exhibited increased frequency response to a subthreshold 0.16 gram Von Frey stimulation in the ipsilateral hind paw. Our findings suggest that NPD1 can prevent chemotherapy-induced neuronal degeneration and chronic pain through activation of GPR37 in DRG neurons.

Disclosures: J. Xu: None.

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.14

Topic: D.02. Somatosensation – Pain

Support: Nevro Corp. Funding

Title: Profound reduction of device battery recharge duration using ultra-low duty cycled 10 kHz spinal cord stimulation

Authors: M. GUPTA¹, *Z. KAGAN², K. BRADLEY²;

¹Neurosci. Res. Center, LLC., Overland Park, KS; ²Nevro Corp., Redwood City, CA

Abstract: Spinal cord stimulation (SCS) is a well-established, evidence-based treatment for selected chronic pain patients. In the last decade, 10 kHz SCS has been shown to be statistically and clinically superior to low frequency SCS in the treatment of chronic back and leg pain. Typically, paresthesia-free 10 kHz SCS is delivered continuously, which requires the implantable pulse generator (IPG) battery to be charged daily for approximately 30 minutes. In a recent clinical study, patients were offered a variety of duty-cycle settings for 10 kHz SCS in which the stimulation was cycled between ON and OFF states, e.g., in 14% duty-cycle mode, stimulation was delivered for 20 seconds ('ON') then stimulation was not delivered for 2 minutes ('OFF'). A plurality of these patients responded to 3% duty-cycled mode, the lowest setting tested, while maintaining the same pain relief obtained with continuous stimulation. Additionally, these patients had a significant reduction in the time they spent charging the IPG. From these results, we hypothesized that patients may achieve 50% pain relief and further reduced recharging time at even lower duty-cycle settings.

Patients (n = 11) already implanted for at least 3 months with a 10 kHz SCS system were enrolled in this study. Enrolled subjects then had their preferred SCS program duty-cycled at 3% for 7-10 days, during which time they tracked their pain via a numeric rating scale (NRS). If the patient achieved at least 50% pain relief, the program's duty cycle was reduced to 0.6%, otherwise they exited the study. This 'waterfall' pattern of '7-10 day test then decision' was continued for duty cycle settings of 0.3%, 0.14%, and 0.06%. If at any decision point, the subject preferred the prior higher duty cycle program, the system was reprogrammed with that duty cycle, no further duty cycles were tested, and the subject entered a 3-month observational period, after which they exited the study. If the subject most preferred the 0.06% program, they entered the observational study with that.

At the time of most recent data collection, 5 subjects are in the observational period. These subjects reduced their daily charging from 24.3 +/- 12.3 (mean +/- SD) min/day before enrolling to 7.5 +/- 2.8 min/day with their chosen duty cycle. These results suggest that 10 kHz SCS may be successfully delivered using very brief boluses. The mechanism by which such a short duration of stimulation is able to produce long-lasting effects is still under investigation.

Disclosures: **M. Gupta:** F. Consulting Fees (e.g., advisory boards); Nevro Corp. **Z. Kagan:** A. Employment/Salary (full or part-time); Nevro Corp.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nevro Corp. **K. Bradley:** A. Employment/Salary (full or part-time); Nevro Corp.. E.

Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nevro Corp..

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.15

Topic: D.02. Somatosensation – Pain

Support: CIFAR-Temery Innovation Catalyst Grant
Digital Research Alliance of Canada (Resource allocation)

Title: Machine learning-driven system of grading trigeminal neuralgia and prediction of surgical outcome

Authors: *T. LATYPOV^{1,4}, R. YAKUBOV^{4,5}, P. TSAI^{1,4}, M. R. WALKER⁴, P. S.-P. HUNG⁴, W. WANG⁴, M. TAWFIK^{2,6}, F. RUDZICZ^{2,6,7}, M. HODAIE^{3,4};

¹Inst. of Med. Sci., ²Dept. of Computer Sci., ³Neurosurgery, Toronto Western Hosp., Univ. of Toronto, Toronto, ON, Canada; ⁴Krembil Res. Inst., Univ. Hlth. Network, Toronto, ON, Canada; ⁵Fac. of Hlth. Sci., McMaster Univ., Hamilton, ON, Canada; ⁶Vector Inst. for Artificial Intelligence, Toronto, ON, Canada; ⁷Li Ka Shing Knowledge Institute, St. Michael's Hosp., Unity Hlth., Toronto, ON, Canada

Abstract: Objectives: Patients with trigeminal neuralgia (TN) typically experience shock-like episodes of pain. Over time, their pain character may change in both frequency and severity. Many patients eventually develop dull or burning, as opposed to shock-like overtones of pain. Previously, TN has been classified into subtypes according to the nature of patients' pain. Here, we hypothesize that TN is a single pain syndrome with variable expression based on a spectrum of grades, each with unique brain imaging correlates, pain characteristics, and treatment outcomes (such as the likelihood and duration of surgical response). We propose a novel machine learning(ML)-derived grading system for TN based on brain imaging and clinical data. This framework facilitates a combined assessment of TN progression in accordance with surgical response duration.

Methods: We included 95 classical TN patients with 3T T1-imaging data treated with either Gamma Knife radiosurgery (GKRS) or microvascular decompression (MVD) and followed up subsequently for 5 years. Pre-surgical clinical data, including 25 metrics such as reports of pain severity, frequency, medical history, and co-morbidities, was used with Shapley values feature selection and principal component analysis (PCA) for the computation of 'pain grade'. Correlation of principal components (PCs) and duration of surgical response was assessed using Spearman correlation. Separately, we combined pre-surgical imaging data with convolutional neural networks to distinguish surgical responders from non-responders (< 5 years pain relief vs. ≥ 5 years pain relief) and to predict surgical response (non-responder vs. responder)) from T1 data.

Results: The PCA of raw and feature selected data yielded 19 and 15 PCs, respectively. In both trials, PC1, largely representing TN-related variables, significantly correlated with duration of surgical response ($r=-0.48$ and $r=-0.51$ ($p<0.001$)). Thus, PC1 can be defined as both a novel measure for TN disease severity and an estimate of surgical response duration. The convolutional neural networks based on T1 data distinguished responders from non-responders with 86% accuracy, and predicted pain relief duration with 78% accuracy.

Conclusions: In this study, we demonstrated an ML-driven approach to deriving a TN grading system using objective brain imaging data and individual clinical characteristics. Our grading framework highlights key clinical prognosticators of effective surgical treatment and estimates the duration of surgical response.

Disclosures: T. Latypov: None. R. Yakubov: None. P. Tsai: None. M.R. Walker: None. P.S. Hung: None. W. Wang: None. M. Tawfik: None. F. Rudzicz: None. M. Hodaie: None.

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.16

Topic: D.02. Somatosensation – Pain

Title: The HMGB1/TLR4 pathway and TRPV1 are involved in the REM sleep deprivation-induced mechanical allodynia in rats

Authors: *C. MARTÍNEZ MAGAÑA, J. MURBARTIAN;
Ctr. de Investigacion y de Estudios Avanzados del Inst. Politécnico Nacional, Ctr. de Investigación y Estudios Avanzados, Ciudad de México, Mexico

Abstract: The Toll Like Receptor 4 (TLR4) plays an important role in initiating physiological immune responses, however, its enhanced activity has been linked to several chronic inflammatory diseases and pathological pain. Moreover, HMGB1, one of the main endogenous ligands of TLR4, has been demonstrated that induce nociceptive responses. Interestingly, some in vitro studies have proposed a possible interaction between TLR4 receptor signaling and a facilitation of TRPV1 channel activity, an important transducer of thermal and chemical stimuli in nociceptors. In the other hand, several studies have linked sleep deprivation to the development of pain hypersensitivity in humans and rodents. Specifically, Rapid Eye Movement Sleep Deprivation (REMSD) induces a state of painful hypersensitivity to thermal and mechanical stimuli, but the mechanisms involved in the pathophysiology of this phenomenon remains unclear. The aim of this study was to explore the participation of HMGB1/TLR4 and TRPV1 on REMSD-induced pain in rats. Young adult female and male Wistar rats were subjected to two-days of REMSD using the flowerpot multi-platform method. Von Frey filaments were used to characterize model-induced paw withdrawal threshold changes and the effects of tested drugs. Protein expression in the lumbar spinal cord and dorsal root ganglia (L4 and L5) was determined by Western blotting. Our data confirms that REMSD during 48 h

induces a generalized mechanical allodynia in rats of both sexes. The mechanical allodynia was transiently reversed after intrathecal administration of A784168 (10 -1000 ng) a selective TRPV1 antagonist, glycyrrhizin (25 ng - 2.5 µg) a drug that prevents the binding of HMGB1 to receptors, LPS-RS (0.1 - 10 µg) a TLR4 antagonist, and TAK-242 (0.3 - 30 µg) an intracellular TLR4 signaling inhibitor. No differences in the effect of drugs between sexes were identified. Female rats subjected to REMSD up-regulate protein expression of HMGB1 and TRPV1 in spinal cord and dorsal root ganglia, but not changed TLR4 expression. The results of this study suggest that spinal TRPV1 and HMGB1/TLR4 signaling pathway have a pronociceptive role and participate in the maintenance of REMSD -induced allodynia. Thus, they could be considered therapeutic targets for the development of new drugs that combat the pain hypersensitivity related to sleep disorders.

Disclosures: C. Martínez Magaña: None. J. Murbartian: None.

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.17

Topic: D.02. Somatosensation – Pain

Support: NIH/NIGMS R35GM138168

Title: Does the lack of preoperative diffuse noxious inhibitory controls predict who will develop chronic postoperative pain? A consomic rat approach

Authors: L. F. FERRARI, *A. WILKINSON, A. RAMIREZ, C. REY, G. DONALDSON, N. E. TAYLOR;
Anesthesiol., Univ. of Utah, Salt Lake City, UT

Abstract: Chronic postoperative pain (CPOP) is a poorly recognized outcome of surgery where patients experience pain long after healing from the surgical insult. Diffuse noxious inhibitory controls (DNIC), a phenomenon whereby application of a strong nociceptive stimulus to one part of the body inhibits pain in remote body regions, offers one strategy to identify patients prone to develop CPOP. Reduced DNIC efficiency predicted patients with persistent pain following thoracotomy and cesarean surgeries. We therefore measured preoperative DNIC and the pain response to a paw incision in a consomic rat panel to determine if they were mechanistically linked. The consomic strains were developed by introgressing individual Brown Norway (BN) rat chromosomes on the Dahl S (SS) rat genetic background, as SS rats lack preoperative DNIC. DNIC was assessed in male and female rats (n=8) of the 2 parental strains along with 8 available SSBN consomic strains by performing a subdermal injection of capsaicin (125µg) into a single forepaw, followed by measurement of hind paw withdrawal thresholds (Randall-Selitto method) at 15min intervals for 1 hour post-injection. To assess CPOP, a plantar incision was made on one hind paw of each rat and paw withdrawal thresholds tested (von Frey method) in the peri-

incision area 2 hours following surgery, then daily for 7 days. The results were plotted and a mixed effects linear model was used to determine regression statistics. The overall regression of DNIC on CPOP was not significant ($p=.261$) and only explained 1.3% of the variation. Maximum likelihood estimates revealed that the strain-by-DNIC interaction was significant ($p=.022$), meaning the regressions differed across strains. Formal analyses indicate that the SSBN13 and SSBN15 strains have a negative DNIC relationship with CPOP ($p<.001$ and $p=.010$, respectively) in contrast with the insignificant relationships of the other strains. Standardized coefficients, -0.613 and -0.614 , represent large effects and imply that SSBN13 and SSBN15 account for 37.6% and 37.7% of the pain variance. While an overall correlation between DNIC and CPOP was not observed, variation in the strain-by-strain results is analogous to, and may help explain, the conflicting conclusions of clinical studies where lower preoperative pain thresholds and DNIC are predictive of who will develop CPOP in one patient cohort but fail to predict it in a different cohort. Results also identify two consomic strains that display a strong negative correlation between these two phenotypes, thus offering the best candidates for future experiments seeking to identify genes and neural circuits responsible for the link between DNIC and CPOP.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.18

Topic: D.02. Somatosensation – Pain

Support: CONACYT-FOP16-2021-01/319379
SIP 20220300

Title: Evaluation of the antiallodynic and antihyperalgesic effect of LMH-2, a sigma-1 receptor antagonist, in a model of neuropathic pain induced by chronic hyperglycemia.

Authors: *T. DOMÍNGUEZ PÁEZ¹, R. VENTURA-MARTINEZ², D. GONZALEZ-UGALDE¹, G. E. ANGELES-LOPEZ², G. NAVARRETE-VAZQUEZ³, M. DÉCIGA-CAMPOS¹;

¹Sección de Estudios de Posgrado e Investigación. Esc. Sup. de Med., Inst. Politécnico Nacional, Ciudad de México, Mexico; ²Fac Med, UNAM, Fac Med, UNAM, Mexico, Mexico; ³Facultad de Farmacia, Univ. Autónoma del Estado de Morelos, Morelos, Mexico

Abstract: This study evaluated the antiallodynic and antihyperalgesic effects of LMH-2, a $\sigma 1R$ antagonist and haloperidol analog. We use an experimental model of neuropathic pain induced by chronic hyperglycemia. Hyperglycemia was induced in CD1 male mice with a single dose of streptozotocin and nicotinamide (130-50 mg/kg, i.p.). These animals developed allodynia and

hyperalgesia after four weeks; at this time, allodynia and hyperalgesia were evaluated by the different treatments. Mechanical allodynia was evaluated with the up & down method and the hyperalgesia was evoked with formalin 0.5%. Curve dose-response were built for LMH-2 (5.6-56.2 mg/kg, s.c.), haloperidol (HAL, 0.017-0.178 mg/kg, s.c.) and gabapentin (GBP, 5.6-56.2 mg/kg, s.c.) as a positive control. Results showed that LMH-2 induced antiallodynic and antihyperalgesic effects dose-dependent on hyperglycemic mice. The analysis of the DRCs of all drugs showed that LMH-2 had greater efficacy antiallodynic ($E_{max}=90.4\pm 8.7\%$) than HAL ($E_{max}=75.1\pm 3.1\%$) and GBP ($E_{max}=41.9\pm 2.3\%$); however, HAL was more potent ($DE_{50}=0.095\pm 0.004$ mg/kg) than LMH-2 ($DE_{50}=13.8\pm 1.15$ mg/kg) and GBP ($DE_{50}=68.4\pm 6.5$ mg/kg). Regarding the antihyperalgesic effect, LMH-2 ($E_{max}=96.3\pm 1.2\%$) also was the most effective treatment, while HAL ($E_{max}=86.9\pm 7.41\%$) and GBP ($E_{max}=86.9\pm 4.8\%$) showed similar efficacies. These results prove that LMH-2, a σ_1R antagonist, could be a useful pharmacological strategy for treating neuropathic pain in patients with diabetic neuropathy.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.19

Topic: D.02. Somatosensation – Pain

Title: In vivo Efficacy of SUVN-6206012 (P2X7R antagonist) on Chronic Constriction Injury Induced Neuropathic Pain in Two Different Species

Authors: *V. GOURA, P. JAYARAJAN, R. KALLEPALLI, S. GAGGINAPALLI, S. RAVELLA, A. MOHAMMED, M. SRIRANGAVARAM, R. SUBRAMANIAN, R. NIROGI; Suven Life Sci. Ltd., Suven Life Sci. Ltd., Hyderabad, India

Abstract: Purinergic P2X7 receptors (P2X7R) are more divergent among purine receptor family in regard to its function, molecular structure and expression. It is well reported in the literature through *in vitro* studies that the affinity towards P2X7R varies between the species due to significant difference in receptor pharmacology among mouse, rat and human. In the current research we compared the *in vivo* efficacy of compounds from P2X7R antagonist research program using two different species. With our focussed structure activity relationship work, SUVN-6206012 has been identified as novel P2X7R antagonist. SUVN-6206012 efficacy was evaluated in neuropathic pain model such as chronic constriction injury (CCI) induced pain in mouse and rat. Paw withdrawal thresholds (PWTs) were evaluated using Von Frey filaments. SUVN-6206012 showed significant increase in PWTs in both mouse and rat model of CCI, when compared to vehicle treated group. SUVN-6206012 showed analgesic like activity in the mouse and rat. Similarly, in support to the literature evidence, SUVN-6206012 exhibited species difference in *in vitro* functional assay. SUVN-6206012 showed higher functional binding affinity

towards human and mouse than rat. Interestingly, *in vivo* efficacy data is in congruent with *in vitro* affinity, like increase in PWTs are relatively higher in mice when compared with rats. These results further support that SUVN-6206012 may translate similar *in vivo* efficacy in the clinic due to its higher binding affinity towards P2X7 receptors in human.

Disclosures: **V. Goura:** A. Employment/Salary (full or part-time);; Suven Life Sciences Ltd. **P. Jayarajan:** A. Employment/Salary (full or part-time);; Suven Life Sciences Ltd. **R. kallepalli:** A. Employment/Salary (full or part-time);; Suven Life Sciences Ltd. **S. Gagginapalli:** A. Employment/Salary (full or part-time);; Suven Life Sciences Ltd. **S. Ravella:** A. Employment/Salary (full or part-time);; Suven Life Sciences Ltd. **A. Mohammed:** A. Employment/Salary (full or part-time);; Suven Life Sciences Ltd. **M. Srirangavaram:** A. Employment/Salary (full or part-time);; Suven Life Sciences Ltd. **R. Subramanian:** A. Employment/Salary (full or part-time);; Suven Life Sciences Ltd. **R. Nirogi:** A. Employment/Salary (full or part-time);; Suven Life Sciences Ltd..

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.20

Topic: D.02. Somatosensation – Pain

Support: DA052690

Title: Cannabinoid approaches to reduce post-surgical pain

Authors: ***S. G. KINSEY**, C. B. RODRIGUEZ;
Sch. of Nursing, Univ. of Connecticut, Storrs, CT

Abstract: The endocannabinoid system has garnered interest in recent years due to its analgesic therapeutic potential. For example, inhibiting monoacylglycerol lipase (MAGL) exhibits antinociceptive, anti-allodynic, and inflammatory properties in various rodent models. Thus, we hypothesized that MAGL inhibition, via JZL184, attenuates allodynia caused by hindpaw incision (HPI) a post-surgical model of pain in mice. Under isoflurane anesthesia, equal numbers of adult male and female C57Bl/6J mice were subjected to HPI, in which a small incision was made on the plantar surface of one hind paw. The incision was closed with a single suture. Approximately 24 hours post-surgery, JZL184 (1-40 mg/kg, ip), the NSAID diclofenac sodium (50 mg/kg, ip), or vehicle (5% ethanol, 5% kolliphor EL, and 90% saline) was administered. After two hours, paw withdrawal threshold is quantified using an up-down approach with von Frey filaments to measure the magnitude of mechanical allodynia. A separate group of mice was treated repeatedly with JZL184 (8 mg/kg, sc) post HPI. HPI induced mechanical allodynia that persisted for up to 10 days post-surgery. Paw withdrawal threshold did not differ between paws contralateral to HPI or sham-operated mice. At 24 hours post-surgery, acute treatment with either diclofenac or JZL184 (≥ 4 mg/kg, ip) attenuated mechanical allodynia induced by HPI. JZL184-

induced anti-allodynia was blocked by pretreatment with the CB₂ selective antagonist SR144528 (3 mg/kg, ip) indicating a CB₂ mechanism. Analgesia was maintained over repeated JZL184 administration, with complete resolution of HPI-induced allodynia six days earlier than in vehicle treated controls. No sex differences were observed. These data support targeting the endocannabinoid system for post-operative pain treatments.

Disclosures: S.G. Kinsey: None. C.B. Rodriguez: None.

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

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Program #/Poster #: 463.21

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01NS109936
NIH Grant P30GM145497

Title: Antinociceptive actions of mitochondrial uncoupling drugs in rat and mouse

Authors: C. STRAUB¹, I. M. BONET², S. M. DINSDALE³, R. GEGUCHADZE³, D. J. GOODE³, I. D. MENG⁴, J. D. LEVINE², *D. C. MOLLIVER³;

¹Biomed. Sci., Univ. of New England, Col. of Osteo. Med., Biddeford, ME; ²Div. Neurosci/NIH Pain Ctr., Univ. of California San Francisco, San Francisco, CA; ³Biomed. Sci., Univ. of New England Col. of Osteo. Med., Biddeford, ME; ⁴Biomed. Sci., Univ. of New England Col. of Osteo. Med., Biddeford, ME

Abstract: We recently reported that heat hyperalgesia induced by injection of prostaglandin PGE₂ in the mouse hindpaw is mediated in part by the cAMP effector Epac2. Acute hyperalgesia was correlated with an EPAC2- and protein kinase C-dependent increase in mitochondrial respiration. 2,4-dinitrophenol (DNP), a protonophore mitochondrial membrane potential (MMP) uncoupling drug, reduced MMP in vitro (20μM) and PGE₂ hyperalgesia in vivo (5mg/kg, i.p.) but had no effect on baseline heat withdrawal thresholds, suggesting that mitochondrial function is linked to nociceptor sensitization. To further explore this phenomenon, we tested 2 structurally distinct uncoupling drugs, DNP and BAM15, in a battery of pain models. In mouse, systemic DNP or BAM15 (1mg/kg) reduced persistent heat and mechanical hyperalgesia induced by hindpaw injection of 1% carrageenan (CGN), as well as in the sciatic nerve crush model of neuropathic pain. In a rat model of uveitis, systemic DNP reversed sensitization of the capsaicin-evoked eye wipe response by ultraviolet light exposure. We next tested BAM15 effects in rat models of opioid-induced mechanical hyperalgesia. Hindpaw injection of BAM15 (0.2μg/μl) 1 hour prior to a low systemic dose (0.03mg/kg) morphine prevented hyperalgesia observed 30 minutes after morphine. This morphine regimen also causes hyperalgesic priming manifested by prolonged hyperalgesia in response to hindpaw injection of PGE₂ 4 days after morphine. Local injection of BAM15 1 hour after PGE₂ attenuated prolonged PGE₂ hyperalgesia in morphine-

primed rats observed 4 hours after PGE2 injection. However, priming was restored 1 week after BAM15 injection, indicating that BAM15 does not prevent maintenance of morphine-induced priming. These results indicate that mitochondrial uncoupling drugs have robust anti-hyperalgesic effects in diverse rodent pain models. To confirm a direct action of these drugs on nociceptors, we recorded from acutely isolated mouse DRG neurons. Ten minute exposure to DNP (20 μ M) or BAM15 (2 μ M) suppressed excitability through hyperpolarization and increased rheobase and membrane permeability, consistent with an increase in K⁺ conductance. These effects were blocked by co-application of 1 μ M glibenclamide, suggesting activation of ATP-sensitive K⁺ (K_{ATP}) channels. This effect was observed in a subset of capsaicin-sensitive and IB4-binding neurons, but not in neurons negative for both indicators (n=8-13 cells each). Experiments are underway to identify the molecular mechanisms responsible for activation of K_{ATP} channels by DNP and BAM15 and to determine whether additional mechanisms contribute to anti-hyperalgesic effects in vivo.

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Poster

463. Neuropathic and Chronic Pain

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.22

Topic: D.02. Somatosensation – Pain

Support: Helen Buchanan and Stanley Joseph Seeger Endowment at The University of Texas MD Anderson Cancer Center

Title: Combination Drug Therapy for Chemotherapy-Induced Peripheral Neuropathy

Authors: *Y. LU¹, Q. YANG², S. ABDI¹;

¹Dept. of Pain Med., The Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; ²Dept. of Neuroscience, Cell Biol. and Anat., Univ. of Texas Med. Br., Galveston, TX

Abstract: Chemotherapy induced peripheral neuropathy (CIPN) is a common and serious adverse effects experienced by cancer patients treated with chemotherapy. The management of CIPN is a challenge to clinicians as the analgesic effect of currently available drugs such as anticonvulsants and antidepressants is at best moderate. One of the reasons might be the fact that the aforementioned agents are used in general as single agents. Thus, we aimed to study the effect of a combination of Retigabine (RTG, anticonvulsant) and Duloxetine (DLX, antidepressant) in an animal model of Paclitaxel-induced peripheral neuropathy (PINP). We also wanted to study if sex is a determinant for the analgesic response to the drugs. CIPN was induced with intraperitoneal injection of 2mg/kg paclitaxel on four alternate days in male and female Sprague-Dawley rats. After the rats developed mechanical allodynia (MA) on day 14, 12 male (Gr. 1-3, N=4 per group) and 12 female rats (Gr. 4-6, N=4 per group) were administered with

either RTG 10mg/kg (Gr 1 and 4), DLX 10mg/kg (Gr 2 and 5) or a combination of RTG + DLX at the aforementioned doses (Gr 3 and 6). Our results clearly show that both drugs as single or combination agents improved MA in male rats. Further, the combination of the two drugs was more effective in reducing MA compared to single drugs. Interestingly, only the combination therapy was effective in reducing MA in female rats. Most importantly, there was no side effects observed with either of the drugs or their combination during the experimental period. In conclusion, a combination of RTG and DLX alleviates chemotherapy-induced neuropathic pain in rats without side effects. However, each drug might have differential effect on male and female rats. Further studies need to be done to substantiate these preliminary findings and the underlying mechanisms.

Disclosures: Y. Lu: None. Q. Yang: None. S. Abdi: None.

Poster

463. Neuropathic and Chronic Pain

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.23

Topic: D.02. Somatosensation – Pain

Support: NIH NS102722
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DoD W81XWH1810431

Title: Pharmacological Antagonism of the CGRP Receptor Reduces Cancer Nociception

Authors: *N. HUU-TU¹, F. DONG², A. KHAN², J. HWANG², N. KHAN², S. AKULA², B. FINNIE², J. LIM², R. ALEMU², V. TRAN², N. W. BUNNETT³, B. L. SCHMIDT²;

¹Bluestone Ctr. For Clin. Res., ²NYU Col. of Dentistry, Bluestone Ctr. for Cli, New York, NY; ³NYU Col. of Dentistry, Dept. of Mol. Pathobiology, New York, NY

Abstract: Oral squamous cell carcinoma (oral SCC) is notoriously painful. Opioids offer inadequate relief. CGRP is a neuropeptide secreted from free nerve endings to tissues. We hypothesize that pharmacological inhibition of calcitonin receptor-like receptor/receptor activity modifying protein-1, CLR/RAMP1, a CGRP receptor found on oral SCC cell membrane reduces cancer nociception in oral cancer mouse models. To generate a xenograft oral SCC cancer model in the paw, we inoculated 2×10^5 human oral SCC cells (HSC-3, JCRB0623, Japan) into the left hind paw of the NU/J *Foxn1*tm mice (Number 002019, the Jackson Laboratories, Bar Harbor, ME). To generate a syngeneic oral SCC tongue cancer mouse model, we inoculated 5×10^3 mouse oral SCC cells (MOC-2, gifted from Uppaluri lab) into the tongue of C57BL/6J wild-type mice (Number 000664, the Jackson Laboratories, Bar Harbor, ME). After the mouse cancer models developed cancer nociception, we treated the mice with a potent and selective CGRP

receptor inhibitor (BIBN 4096, catalog number 204697-65-4, TOCRIS, 1 mg.kg⁻¹, IP) or control. To measure cancer nociception in the paw and to test the effect of the CGRP receptor inhibitor, we used the paw von Frey assay. Separately, we used the facial von Frey assay to measure cancer nociception in the tongue cancer model and to evaluate the effect of the CGRP receptor inhibitor on orofacial nociception. CGRP is anterograde transported from neuron body to the periphery. To study the effect of CGRP accumulation in the cancer paw, we ligated the sciatic nerve in the HSC-3 paw cancer mouse model and subsequently performed immunohistochemistry using anti-CGRP and anti-PGP9.5 (an axonal marker) antibodies. The presence of CLR/RAMP1 receptor for CGRP on cancer cells was also confirmed by immunohistochemistry. Our results reveal that CGRP accumulated in the cancer paw and that CGRP receptor inhibitor-treated mice (paw and tongue models) exhibited significantly less cancer nociception at 1-, 3-, 6-, and 12-hours post-treatment. CGRP receptor inhibitor reduced cancer nociception (0.36 grams versus 0.20 grams, and 1.8 versus 1.2) in paw cancer mice and tongue cancer mice respectively at 3 hours post-treatment. We infer from these results that pharmacological inhibition of the CGRP receptor reduces cancer nociception. Our findings establish the foundation for the development of treatment approaches for oral SCC pain that target the CGRP receptor.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.24

Topic: D.02. Somatosensation – Pain

Title: The Centre for Advanced Neurosurgical Diagnostics Innovation in Pain (CANDIP) - Development of a multidimensional database for patients with chronic pain

Authors: *M. R. WALKER¹, P. SRISAIKAEW¹, D. JORGENS¹, T. H. LATYPOV^{1,3}, K. D. DAVIS^{1,3,4}, C. HONEY¹², C. IORIO-MORIN¹³, G. R. LORELLO⁵, M. MCANDREWS^{1,6}, D. J. MIKULIS^{1,3,7}, M. MOAYEDI⁸, F. RUDZICZ^{14,9}, T. SANKAR¹⁵, D. A. SEMINOWICZ¹⁶, K. ULUDAG^{1,3,10}, G. ZADEH^{1,3,11,2}, M. HODAIE^{1,3,11,2};

¹Krembil Res. Inst., ²Div. of Neurosurgery, Toronto Western Hosp., Univ. Hlth. Network, Toronto, ON, Canada; ³Inst. of Med. Sci., ⁴Dept. of Surgery, ⁵Dept. of Anesthesiol. and Pain Med., ⁶Dept. of Psychology, ⁷Joint Dept. of Med. Imaging, ⁸Fac. of Dent., ⁹Dept. of Computer Sci., ¹⁰Dept. of Med. Biophysics, ¹¹Div. of Neurosurgery, Fac. of Med., Univ. of Toronto, Toronto, ON, Canada; ¹²Div. of Neurosurgery, Fac. of Med., Univ. of British Columbia, Vancouver, BC, Canada; ¹³Dept. of Surgery, Univ. de Sherbrooke, Sherbrooke, QC, Canada; ¹⁴Vector Inst. for Artificial Intelligence, Toronto, ON, Canada; ¹⁵Neurosurg., Univ. of Alberta, Edmonton, AB, Canada; ¹⁶Dept. of Med. Biophysics, Western Univ., London, ON, Canada

Abstract: Objective: There is often limited information available for neurosurgical decision making for pain conditions. Fields such as neuroimaging, cognition, and genomics can provide diagnostic and prognostic support; however, no platform exists to integrate this information. A common repository of multidimensional data and processing tools is needed to facilitate modern, data-driven analyses for more accurate diagnostics, better outcomes, and improved healthcare access. Therefore, we propose the establishment of the Centre for Advanced Neurosurgical Diagnostics Innovation in Pain (CANDIP), a multidimensional open resource for advanced pain diagnostics.

Methods: We analyzed the requirements of such a centre to provide sufficiently rich data and statistical power for detailed and generalizable AI-based disease profiling. We focus on trigeminal neuralgia (TN), a severe chronic orofacial pain which is amenable to surgical treatment, while designing future scalability for other conditions. Embracing open science and equity, diversity, inclusion, and accessibility (EDIA) principles drive the platform's impact while protection of personal health information is crucial.

Results: We developed a harmonized data collection protocol consisting of multiple modalities and sites across Canada to satisfy the dimensionality, variability, and generalizability requirements. Recruitment consists of 250 TN and healthy participants each. MRI brain scans include T1w, T2w, DWI, and resting-state fMRI. Processing tools are provided for correcting distortions and extracting data derivatives. Clinical metrics (25 variables) include pain duration, intensity, surgical history, and treatment outcomes. Rooted in the principles of EDIA, standardized demographics (45 variables) include age, sex, gender, ethnicity, education, and occupation. Validated cognitive/psychological questionnaires (15 variables) assess domains including attention, memory, learning, and depression. Whole blood samples for genomics are processed through DNA methylation analysis with Illumina-450k arrays. Deidentified data are securely stored using the extensible neuroimaging archive toolkit (XNAT). User-access and data-use forms have been developed, providing secure and open access.

Conclusions: CANDIP is a multidimensional and scalable resource consisting of neuroimaging, cognitive/psychological assessments, demographics, clinical metrics, and biospecimens. The large dataset and informatics tools will facilitate modern AI-based analyses with translational impact in diagnostics, personalized treatment recommendations, and equitable access to care.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.25

Topic: D.02. Somatosensation – Pain

Support: DoD grant W81XWH2110756

Title: Inhibition of diabetic neuropathic pain by intrathecal and intravenous administration of MMP-9 monoclonal antibody in mice

Authors: *Y. MATSUOKA¹, K. FURUTANI¹, K. LEE³, X. GE³, R.-R. JI^{1,2};

¹Anesthesiol., ²Neurobio., Duke Univ. Med. Ctr., Durham, NC; ³Mol. Med., The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Our previous study has shown that matrix metalloproteinase 9 (MMP-9) plays a central role in the development of neuropathic pain after nerve injury by regulating glial activation and neuroinflammation (Kawasaki et al, 2008). Clinical trials using small molecule inhibitors of MMPs have failed due to the lack of specificity and toxicity. By using functional selection, we developed a monoclonal antibody (mAb) that can effectively alleviate chemotherapy-induced neuropathic pain following intravenous injection (Lopez et al., 2019). Diabetes-induced peripheral neuropathy (DPN) may develop in 50% of diabetic patients and there is a lack of effective treatment for painful DPN. In this study, we tested the effects of the MMP-9 mAb in mouse models of DPN. DPN was induced by intraperitoneal injection of 150 mg/kg of streptozotocin (STZ) in CD1 mice, and MMP-9 mAb was administered intrathecally (IT) or intravenously (IV) three times every other day. Mechanical and cold pain was assessed in von Frey filament and acetone tests at early-phase (1 week) and late phase (4 weeks) of diabetes. To assess non-evoked on-going pain, conditioned place preference (CPP) assay was performed. Whole cell patch-clamp recordings were made from the spinal dorsal horn neurons of STZ-treated mice to examine the effects of MMP-9 and mAb on synaptic transmission. The results showed that either IT or IV injection of MMP-9 mAb significantly increased reduced mechanical allodynia, by increasing paw withdrawal mechanical threshold in both early and late phases. Following IV injections, accumulating effect of analgesia was observed after the third injection. We also observed CPP in mice treated with MMP-9 antibody compared with vehicle-paired mice, indicating the effectiveness of the mAb in reducing ongoing pain. Furthermore, MMP-9 mAb effectively reduced diabetic neuropathic pain in a genetic model of DPN in db/db mice. Finally, MMP-9 treatment in spinal cord slices enhanced spontaneous excitatory postsynaptic currents (EPSCs) in dorsal horn neurons, whereas the mAb treatment reduced EPSCs in neurons of diabetic animals. Our findings suggest that MMP-9 monoclonal antibody is highly effective in alleviating diabetic neuropathic pain via both peripheral and central actions.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.26

Topic: D.02. Somatosensation – Pain

Title: High-intensity interval training for symptom management in a patient with transverse myelitis

Authors: J.-F. DANEAULT;

Rehabil. and Movement Sci., Rutgers Univ., Newark, NJ

Abstract: INTRODUCTION: Transverse Myelitis (TM) is a rare disorder caused by inflammation of the spinal cord. The classical symptoms of TM include weakness of the limbs, sensory alterations, pain, as well as bowel and bladder dysfunction. Long-term care of patients with TM includes the management of symptoms with medication and physical therapy but their effectiveness is variable across individuals. High-intensity interval training (HIIT) has been shown to improve functional capacity, psychological function, pain, and quality of life in other populations. OBJECTIVES: The goal of the current study was to identify the impact of HIIT on pain in a patient with TM. CASE DESCRIPTION: The patient was a 42-year-old male diagnosed with idiopathic TM. The patient had no history of any medical conditions and tested negative for all autoimmune disorders typically associated with TM. Magnetic resonance imaging showed a lateralized spinal lesion at C2-C3. After 3 months of standard long-term TM medical care, the patient still reported mild weakness, paresthesia, pain, and allodynia on the right side of his body. The patient then started a 6-month cycling HIIT program consisting of 3 HIIT sessions per week with 2-3 additional recovery sessions of mild to moderate continuous cycling per week. The patient continued taking his prescribed medication. The weekly HIIT sessions were structured and personalized to the patient's state. Physical activity, sleep, and heart rate were monitored throughout the program using an activity tracker. RESULTS: The patient completed, on average, 4 sessions per week (range 2-6) over 6 months. The session duration ranged from 15 minutes to over 60 minutes. The patient experienced days with increased symptom severity or fatigue during which he did not complete the sessions. There were no adverse events. The patient reported marginal increases in pain during some high intensity intervals, but this pain dissipated after the session with the cool down and relaxation. The patient observed qualitative improvements in daily pain severity over the course of the program. He also reported feeling better after starting the program. Additionally, a qualitative evaluation of the spinal lesion showed a slight decrease in size. Quantitatively, we observed significant improvements in mobility (i.e., number of steps and daily active time) and sleep quality based on activity tracker data. CONCLUSION: These results suggest that HIIT may be a potential adjunctive intervention for the management of long-term symptoms of TM. Larger cohort studies and work on identifying the underlying mechanisms of HIIT that lead to the improvement of TM pain are needed.

Disclosures: J. Daneault: A. Employment/Salary (full or part-time);; Rutgers University.

Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.27

Topic: D.02. Somatosensation – Pain

Support: RFMH 903-2940A
NIH Grant 1F31NS127547-01
NIH Grant R01NS086444

Title: Tianeptine Alleviates Sensory and Affective Components of Neuropathic Pain

Authors: *E. A. PEKARSKAYA¹, R. A. SERAFINI², J. G. SOARES¹, J. A. JAVITCH¹, V. ZACHARIOU²;

¹Columbia Univ., New York, NY; ²Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Neuropathic pain (NP) results from chronic injury or disease and is characterized by numerous sensory and affective symptoms. Primary treatments consist of monoamine targeting antidepressants, gabapentanoids, and opioids, but a significant percentage of patients do not tolerate or respond to these medications. As such, identifying alternative treatments is necessary. Our group's prior work determined that the mu-opioid receptor (MOR) is a critical target of the atypical antidepressant tianeptine (TIA). TIA is an effective antidepressant that lacks major side effects typically observed with opioid analgesics, such as tolerance, physical dependence, and addiction. Here, we demonstrate that TIA (30mg/kg i.p. BID for two weeks) has strong anti-allodynic properties in the murine spared nerve injury (SNI) model 10 weeks after injury as measured by the Von Frey assay, while also preventing emotional manifestations of chronic pain as measured by marble burying (OCD/anxiety-like), open field (anxiety-like), and novelty suppressed feeding (depression-like). No broad analgesic effects were observed, reinforcing the efficacy of TIA in alleviating maladaptive NP symptoms instead of physiological nociception. These findings fall in line with its effectiveness in mouse models of depression. However, how and where TIA is acting to relieve these symptoms is unclear.

In this study, we used bulk and single nucleus RNA sequencing to assess the effect of TIA on transcription in key affective and pain-processing brain areas, such as the Nucleus Accumbens (NAc) and the Habenula (Hb), both of which have aberrant activity in clinical chronic pain and depression populations. We found that TIA reverses several SNI-induced gene expression changes ($\log_2FC > |0.32|$; $p\text{-nom} < 0.05$) in the NAc at the whole tissue level, potentially by upregulating neuroplasticity-associated pathways (Qiagen Ingenuity Pathway Analysis). Habenular tissue, which has some of the highest expression levels of MORs in the brain, is currently being processed through single-nucleus RNA sequencing. We hypothesize TIA affects specific Oprm1+ cells of the Hb that contribute to the regulation of downstream monoaminergic signaling pathways. Future work includes identifying cell subtype-specific pathways altered by TIA treatment and using information from our transcriptomic data to create refined analogs that improve treatment efficacy.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.28

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS112263

Title: Peripheral myeloid cells control resolution of painful Chemotherapy-Induced Peripheral Neuropathy (CIPN)

Authors: *G. T. NARATADAM^{1,2}, P. A. BARBA-ESCOBEDO³, J. MECKLENBURG³, S. A. SHEIN⁴, A. V. TUMANOV⁴, A. N. AKOPIAN^{3,5};

¹South Texas Med. Scientist Training Program, ²Physiol. and Pharmacol. Discipline, Grad. Sch. of Biomed. Sci., ³Dept. of Endodontics, The Sch. of Dent., ⁴Dept. of Microbiology, Immunol. & Mol. Genetics, Long Sch. of Med., ⁵Dept. of Pharmacology, Long Sch. of Med., UT Hlth. San Antonio, San Antonio, TX

Abstract: Chemotherapies have provided excellent relief from cancer mortality but are associated with severe side effects. One of these side effects is Chemotherapy-Induced Peripheral Neuropathy (CIPN). CIPN presents as a distal symmetrical polyneuropathy causing numbness, tingling, and sensory loss and in a subset of patients, pain in the hands and feet. Painful CIPN is a main cause for chemotherapy dose reduction and treatment cessation, leading to increased cancer mortality and reduced quality of life in survivors. It is known that a subset of painful CIPN patients will have a resolution of pain while some continue to have persistent pain, however the mechanisms that mediate pain resolution are unknown and constitute a large gap of knowledge. The aim of this study is to identify endogenous mediators controlling CIPN pain resolution. To examine this, we developed pre-clinical CIPN pain mouse models using the chemotherapy paclitaxel, where adult mice treated with specific injection protocols develop resolving or persistent nociceptive phenotype measured via von Frey. Using these models, we performed bulk RNA sequencing on hind paw, whole dorsal root ganglion (DRG), DRG sensory neurons and spinal cord at initiation, pre-resolution, post-resolution, and persistency time points. PANTHER gene ontology analysis to identify biological processes identified that resolving painful CIPN is preceded by upregulation of inflammatory processes and immune system related genes in the hind paw. Myeloid cells are a known immune driver for inflammatory processes in the periphery. Using inducible cell ablation to ablate myeloid cells, we identified that peripheral, but not central, myeloid cells are critical for CIPN pain resolution in male but not female mice. Further RNA sequencing analysis identified multiple pro-inflammatory pathways correlated with CIPN resolution phenotype. Overall, these studies provide mechanistic insights for CIPN pain resolution and identify a unique therapeutic mechanism for permanent treatment of painful CIPN. Future studies will focus to delineate the molecular signaling factors required for CIPN pain resolution.

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Poster

463. Neuropathic and Chronic Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 463.29

Topic: D.02. Somatosensation – Pain

Support: Japan Society for the Promotion of Science 19K21225
Japan Society for the Promotion of Science 20K18476

Title: Effect of intranasal treatment with the anti-HMGB1 neutralizing antibody on mechanical hypersensitivity in a mouse model of hemi-Parkinson's disease

Authors: *Y. NAKAMURA¹, F. SATO¹, K. YAMANO¹, S. MA¹, T. KOCHI^{1,2}, K. HISAOKA-NAKASHIMA¹, D. WANG³, K. LIU³, H. WAKE^{3,4}, M. NISHIBORI³, N. MORIOKA¹; ¹Pharmacol., ²Dent. Anesthesiol., Hiroshima Univ., Hiroshima, Japan; ³Pharmacol., Okayama Univ., Okayama, Japan; ⁴Pharmacol., Kindai Univ., Osaka-Sayama, Japan

Abstract: Parkinson's disease (PD) is a common neurodegenerative disorder characterized by dopaminergic neuronal death in the substantia nigra. Patients with PD exhibit not only motor symptoms such as tremor, bradykinesia, and rigidity but also pain symptoms. However, the pain symptoms are often neglected, and pain treatment for these patients is ineffective because of a lack of understanding of the mechanisms of PD-associated pain. High mobility group box-1 (HMGB1) is an alarmin/damage-associated molecular patterns protein that can be passively or actively released from cells to function as a proinflammatory molecule. Our previous studies have reported that HMGB1 is involved in the development and maintenance of chronic pain in the peripheral nerve injury model and intracerebroventricular injection with recombinant HMGB1 (rHMGB1) evokes mechanical hypersensitivity. Thus, an increase of HMGB1 in the brain is associated with the development or maintenance of PD-associated pain. However, little is known about the relationship between HMGB1 and PD-associated pain. Here, the current study investigated the effect of anti-HMGB1 neutralizing antibody (nAb) on mechanical hypersensitivity in a mouse PD model. For the preparation of the hemi-PD model, mice were unilaterally injected with 6-hydroxydopamine (6OHDA) into two sites in the right striatum. Mechanical hypersensitivity was evaluated by using von Frey filaments. To deliver antibody into the central nerve system (CNS), intranasal (i.n.) treatment was performed. After 6OHDA injections into the mouse striatum, motor dysfunction, dopaminergic neuronal death, and mechanical hypersensitivity were caused. Additionally, the HMGB1 concentration in cerebrospinal fluid was significantly higher in hemi-PD mice compared with the control group. Moreover, the i.n. treatment with anti-HMGB1 nAb ameliorated mechanical hypersensitivity, but not motor dysfunction and dopaminergic neuronal death, in hemi-PD mice. In conclusion, i.n. treatment with anti-HMGB1 nAb ameliorated mechanical hypersensitivity by inhibiting HMGB1 function in the CNS. Therefore, blockade of central HMGB1 function might be a new therapeutic strategy for pain associated with PD.

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Poster

464. Visceral Pain

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Program #/Poster #: 464.01

Topic: D.02. Somatosensation – Pain

Support: NIH HEAL/SPARC U01 NS113867-01
NIH R15HL137143-01A1

Title: Trpv1-ir axon innervation of gastric muscular and submucosal layers of rats

Authors: *J. MADAS¹, J. MA¹, A. MISTAREEHI¹, A. KWIAT¹, K. T. BENDOWSKI¹, D. NGUYEN¹, J. CHEN¹, T. L. POWLEY², Z. CHENG¹;

¹Univ. of Central Florida, Orlando, FL; ²Perdue Univ., W Lafayette, IN

Abstract: Visceral pain is initiated by the activation of nociceptive nerves at their peripheral terminals (receptors). Nociceptive afferent axons can be detected with a variety of different markers (e.g., CGRP, SP, TRPV1). Previously, the topographical distribution and morphology of SP-IR and CGRP-IR axons and terminals were determined in the flat-mounts of the muscular layers of the whole stomach. In this study, we used TRPV1 as marker to label nociceptive afferent axons in flat-mounts of the muscular and submucosal layers of the whole ventral stomach in SD rats. Using a confocal microscope and a Zeiss M2 Imager microscope, we scanned the entire flat-mounts and determined the distribution and morphology of TRPV1-IR axons and terminals. We found that 1) TRPV1-IR axons formed extensive terminal networks in both the gastric muscle and submucosal layers. 2) In longitudinal and circular muscles, TRPV1-IR varicose axons ran in parallel with the direction of the muscles. 3) In the myenteric ganglia, TRPV1-IR axons formed varicose terminal contacts with individual myenteric neurons. 4) There were a number of TRPV1-IR myenteric neurons in all stomach regions. 5) In the submucosal plexus, submucosal ganglia were very sparse. TRPV1-IR axons formed varicose terminal contacts with individual neurons in the submucosal ganglia. 6) In the submucosa, we also found that TRPV1-IR varicose axons traveled within the connective tissue. 7) TRPV1-IR axons innervated submucosal blood vessels. Our data provide for the first time a comprehensive innervation map of TRPV1-IR axons and terminals in the whole gastric muscle and submucosa layers with single cell/axon/synapse resolution. This work provides an anatomical foundation for functional studies of TRPV1-IR axons in various regions of the stomach and their remodeling in diseases. This study was supported by NIH HEAL/SPARC U01 NS113867-01 and NIH R15HL137143-01A1.

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Poster

464. Visceral Pain

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Program #/Poster #: 464.02

Topic: D.02. Somatosensation – Pain

Support: NIH Grant U01NS113873

Title: Sex differences in zymosan-induced behavioral visceral hypersensitivity and colorectal afferent sensitization

Authors: T. GUO¹, J. LIU², L. CHEN³, Z. BIAN¹, G. ZHENG¹, *B. FENG¹;

¹Univ. of Connecticut, Storrs, CT; ²Biomed. Engin., Univ. of Connecticut, Vernon, CT;

³Biomed. Engin., Univ. of Connecticut, Storrs, CT

Abstract: Female patients with irritable bowel syndrome (IBS) report more intense visceral pain sensation than male patients. Female rats show more sensitive and cycle-dependent visceromotor responses (VMR) to graded colorectal distension (CRD). Both clinical psychophysical evidence and preclinical behavioral data support an apparent sex difference in visceral nociception. However, very little is known regarding the potential differences in sensory neural encoding of distal colon and rectum (colorectum) between males and females. In this study, we systematically assessed the sex difference in colorectal neural encoding by conducting high-throughput optical recordings in intact dorsal root ganglia (DRG) from control and visceral hypersensitive mice. We crossbred GCaMP6f mice with VGLUT2-Cre mice to express GCaMP6f gene in most colorectal sensory neurons. Mice receiving intracolonic treatment of zymosan (30mg/mL, 0.2mL) for three consecutive days developed behavioral visceral hypersensitivity as validated by enhanced VMR to CRD. We then harvested the colorectum with pelvic nerve, lumbar splanchnic nerve and T12 to S1 DRG in continuity in an ex vivo preparation for GCaMP6f recordings. A total of 2275 colorectal afferents were characterized from both sexes in control and zymosan-treated groups. DRG neurons responding to either colorectal distension (15, 30, 45 and 60 mmHg) and/or mucosal shearing (20-30 mL/min) were functionally categorized into four classes: mucosal, muscular-mucosal and high- and low-threshold muscular afferents. In control group, the number of colorectal afferents recorded per mouse were slightly lower in male mice than in female ones (38.7 vs. 45.2) with no significant difference in their distributions across thoracolumbar (TL) and lumbosacral (LS) DRG. In zymosan group, more afferents were recorded from each male mice than female ones (53.8 vs. 44.1) showing significantly higher TL innervation (T12, L1, and L2 DRG) in male group. Within the male groups, zymosan treatment resulted in a significant increase in the proportion of TL innervation as compared with saline treatment. In contrast, female mice showed no difference in the proportion of colorectal neurons between saline- and zymosan-treated groups. Our results

have revealed a significant sex difference in colorectal afferent innervation and sensitization in the context of behavioral visceral hypersensitivity. The current outcomes draw further focused research on the neurophysiological differences between sexes that could drive the differential gastrointestinal symptoms in male and female IBS patients.

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Poster

464. Visceral Pain

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 464.03

Topic: D.02. Somatosensation – Pain

Title: Murine model of endometriosis correlates cystic load with pain response along with observable signs of anxiety and depression in C57/Bl6 mice

Authors: *P. J. SWEENEY¹, K. H. PARK¹, J. E. FRIEDMAN², H. Y. LEE³, Y. KIM³, S. LEE¹, A. LEE¹, A. LEE³, L. C. H. PARK³;

¹Naason Sci. Inc., Osong, Korea, Republic of; ²Naason Sci., Rehovot, Israel; ³Naason Sci., Osong, Korea, Republic of

Abstract: Endometriosis is a disease that affects 10 to 15% of women. It can be asymptomatic but can manifest as pain, heavy menses, infertility, and increased risk of ovarian cancer. It is hormonally dependent, with ectopic deposition of endometrial-like tissue outside the womb, in the ovaries, fallopian tubes and in the peritoneum. Endometriosis induces a prolonged inflammatory response and can affect quality of life through pain and discomfort with anxiety and depression experienced in some women. In the preclinical modelling of the disease a preferred method has been to transfer endometrial tissue from hormonally primed mice to create endpoints to quantify treatment efficacy. These involve the examination of the pelvic region for the number, size and volume of implanted endometriomas. Less frequently studies have observed sequelae of the disease such as abdominal pain, anxiety and depression. We adapted a syngeneic model of endometriosis with 2 groups of (N=20) female C57/Bl6 mice consisting of age-matched donors, hormonally primed with implanted progesterone, and estrogen administration to induce menses and generate endometrial tissue. Tissue was then taken postmortem from donors and processed via an adaptation of previously published protocols and introduced to the uterus of the naïve recipients. Results showed an over 80% presence of cystic like tissue distribution in abdominal area of the recipient. Mortality in the recipient group was 0 and all inoculated subjects survived until the 8-week endpoint. All animals were observed in the home cage with a monitoring system that allows for temporal observation of locomotion and behavior in group housed conditions. Animals were subjected to peripheral and abdominal von Frey filament testing for nociceptive response 3 X weekly.

During the observation and testing period inoculated animals exhibit a decreased pain threshold and quantifiable signs of anxiety and depression. Retrospective analysis shows that animals with

a higher cystic load, either larger cysts or a higher number of cysts, show a correlation with abdominal von Frey pain response as well as the degree to which they manifest signs of anxiety and depression. Simultaneously, naïve controls and a sham group, created by employing a suture to the colonic mesentery, do not show a similar pattern of tactile allodynia and anxiety and depression under similar conditions. These findings present a case for correlating cystic load sensitivity to pain and to allow observation the success of inoculation during the in-life phase. The model also allows for observation of psychiatric symptoms like anxiety and depression that are associated with endometriosis in women.

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Poster

464. Visceral Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 464.04

Topic: D.01. Somatosensation

Support: NIH Grant R01DK125543

Title: Urinary bladder distension evokes increases in urothelial Ca²⁺ signaling

Authors: *G. M. HERRERA, G. W. HENNIG, M. T. NELSON, T. J. HEPPNER;
Univ. of Vermont, Burlington, VT

Abstract: The urothelial cells that line the lumen of the urinary bladder are believed to be responsible for sensing distension as the bladder fills with urine. However, very little is known about the cellular signaling mechanisms that underlie mechanosensation in the urothelium. Here we make use of transgenic mice that conditionally express the genetically encoded Ca²⁺ indicator GCaMP6f under the control of the mouse uroplakin II promoter (mUPII/rtTA-M2). These mice exhibit robust Ca²⁺-dependent fluorescence in all cell layers of the urothelium, enabling live cell imaging of Ca²⁺ signaling in native urothelium for the first time. To explore urothelial Ca²⁺ signaling, we used an ex vivo model in which the isolated urinary bladder was cannulated and filled with physiological saline solution at a rate of 1.8 ml/hr. Bladder capacity was defined as the volume at which intravesical pressure reached 25 mmHg. Fluorescence was measured using a wide field fluorescence microscope and a novel pentaplanar reflectance imaging macroscopy chamber. At low bladder volumes (<20% capacity), periodic Ca²⁺ signals were observed with a prevalence of 6,100 ± 1,790 ZUMS per minute (see Longden et al, 2021 Sci Adv 7(30): eabh0101 for explanation of Ca²⁺ prevalence quantitation). The occurrence of oscillatory Ca²⁺ signals increased greatly at higher volumes. At volumes ~50% of capacity, Ca²⁺ signal prevalence increased >3-fold to 21,790 ± 6,480 ZUMS per minute. Volumes near 100% capacity were associated with a decrease in Ca²⁺ prevalence to 2,750 ± 1,970 ZUMS per minute. In summary, the mUPII/rtTA-M2-GCaMP6f mice represent a useful tool for studying urothelial cell

physiology. These mice enable monitoring of Ca²⁺ signals in native intact urothelium under physiological conditions. Changes in Ca²⁺ signaling frequency may underlie part of the response of the urothelium to act as a sensory structure to transduce the filling state of the bladder to the central nervous system. This work was supported by a grant from the National Institute of Diabetes and Digestive and Kidney Diseases (R01DK125543).

Disclosures: **G.M. Herrera:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Med Associates, Inc.. **G.W. Hennig:** None. **M.T. Nelson:** None. **T.J. Heppner:** None.

Poster

464. Visceral Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 464.05

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01 DK119183
NIH Grant P30 DK079307
NIH Grant R01 DK129473

Title: Chronic pelvic pain develops in sensory neuron conditional *Asic3* null mice subjected to chemical injury

Authors: N. MONTALBETTI¹, M. DALGHI³, G. APODACA², *M. D. CARATTINO¹;
¹Univ. of Pittsburgh, ²Dept. of Med., Univ. of Pittsburgh, Pittsburgh, PA; ³Dept. of Med., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Sensitization of primary afferents is an important underlying mechanism for visceral hypersensitivity and pain. We investigated the contribution of acid-sensing ion channels (ASICs) in sensory neurons to the development of pain in a model of chemical-induced cystitis. ASICs are assembled as heterotrimers in bladder sensory neurons; however, the deletion of the ASIC3 subunit results in a loss-of-function phenotype with sensory neurons unable to discharge action potentials in response to acidification. In this study, conditional sensory neuron *Asic3* knockout (KO) mice (*Asic3^{fl/fl};Avil-Cre^{+/-}*) and control littermates (*Asic3^{fl/fl};Avil-Cre^{-/-}*) received cyclophosphamide (CYP) (IP 80 mg/kg), or saline, every other day for five days. Experimental observations were made after one day (acute) or 14 days (chronic) after the last dose of CYP. In the acute setting, both control and conditional *Asic3* KO mice treated with CYP exhibited an irritated bladder phenotype with a high number of voiding events of small volume. However, voiding activity normalized in both groups within two weeks of receiving CYP. In contrast, conditional *Asic3* KO mice treated with CYP developed pelvic allodynia that persisted for at least two weeks, whereas control mice had no pain phenotype. In the chronic setting, no apparent edema or inflammatory cells were observed in bladders of control or conditional *Asic3* KO mice, indicating that the pelvic allodynia seen in conditional *Asic3* KO mice treated with CYP is likely

driven by abnormal functioning of the nervous system and not by inflammation. To assess whether, in the chronic setting, the referred pelvic allodynia seen in conditional *Asic3* KO mice treated with CYP is caused by sensitized primary afferents, we examined the firing evoked by sustained suprathreshold electrical stimulation of acutely isolated bladder sensory neurons. Sensory neurons were classified based on the sensitivity of the action potential to tetrodotoxin (TTX), as TTX-resistant (TTX-R) or TTX-sensitive (TTX-S). Strikingly, ~ 40% (11/17) of the bladder sensory neurons with TTX-R action potentials from conditional *Asic3* KO mice treated with CYP exhibited aberrant firing (i.e., sensitization) in response to suprathreshold stimulation, compared to 3% (1/33) in control mice. These findings indicate that the pelvic allodynia seen in conditional *Asic3* KO mice is driven in part by sensitized bladder afferents. Taken together, our studies support the notion that ASICs operate at the nerve terminals to modulate nociceptor excitability and sensitization.

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Poster

464. Visceral Pain

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Program #/Poster #: 464.06

Topic: D.02. Somatosensation – Pain

Support: NIH HEAL/SPARC U01 NS113867-01
NIH R15HL137143-01A1

Title: Topographical Distribution and Morphology of SP-IR Axons in the Antrum, Pylorus, and Duodenum of Rodents

Authors: A. MISTAREEHI¹, K. T. BENDOWSKI¹, J. MADAS¹, A. BIZANTI¹, N. KOGUT¹, D. NGUYEN¹, J. MA¹, J. CHEN¹, T. L. POWLEY², *Z. CHENG¹;

¹Univ. of Central Florida, Orlando, FL; ²Perdue Univ., W Lafayette, IN

Abstract: Substance P (SP) is a commonly used marker of nociceptive axons. Previously, we studied the topographical innervation of SP-IR axons in the flat-mounts of the muscular layers of the whole stomach and found that SP-IR axons innervated the antrum-pyloric region very densely. However, the distribution and morphology of SP-IR axons in the submucosa and mucosa is not well documented. In this study, the mouse and rat antrum-pylorus-duodenum (APD) were sectioned (100 μ m; mouse: 30 sections/each, n=9; rats: 70 sections/each, n=9) and immunohistochemically labeled for SP. To determine the distribution and morphology of SP-IR axons and calculate their density in different layers of APD, a Zeiss M2 Imager was used to scan each serial section. Each section was presented as a montage of approximately 50 (mouse) and 200 (rats) all-in-focus maximal projection images. To determine the detailed structures of SP-IR axons and terminals, we used the confocal microscope to scan regions of interest. In all APD

regions, we found that in mice and rats: 1) SP-IR fibers innervated all layers including the longitudinal/circular muscles, myenteric ganglia, submucosa, submucosal ganglia, muscularis mucosa, and mucosal epithelium. Many SP-IR axons were also vesicular acetylcholine transporter-IR (VACHT, parasympathetic marker). 2) In muscular layers, SP-IR varicose axons densely innervated the smooth muscles and formed heavy terminals which encircled numerous individual myenteric neurons. 3) In the submucosa, SP-IR axons innervated blood vessels and submucosal ganglia and formed a network in duodenal Brunner's glands. 4) In the mucosa, SP-IR axon bundles were found in the muscularis mucosa at the base of mucosa. Some SP-IR axons entered the gastric subepithelium and duodenal villi. 5) SP-IR axon density varied across the layers of the APD regions: density in the muscles was much higher than in the submucosa and mucosa. 6) The muscular wall of the antrum and duodenum showed a higher density than the pyloric sphincter. 7) Mice and rats had a similar innervation pattern. However, SP-IR axon innervation in rats was much denser than in mice particularly in the mucosa. This work provided a comprehensive view of the distribution and morphology of SP-IR axons in all layers of APD at single-cell/axon/synapse resolution. These data will establish a foundation for functional mapping of the nociceptive innervation of the stomach and its pathological remodeling in gastrointestinal diseases and will be used to create a 3-D atlas of the SP-IR innervation of the APD region. Supported by NIH HEAL/SPARC U01 NS113867-01 and NIH R15HL137143-01A1.

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Poster

464. Visceral Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 464.07

Topic: D.02. Somatosensation – Pain

Support: NIH HEAL/SPARC U01 NS113867-01
NIH R15HL137143-01A1

Title: Topographical Distribution and Morphology of Calcitonin Gene-Related Peptide (CGRP) Axons in the Flat-Mounts of the Whole Stomach in Female Mice

Authors: *J. MA¹, A. MISTAREEHI¹, D. NGUYEN¹, J. MADAS¹, A. KWIAT¹, J. CHEN¹, K. T. BENDOWSKI¹, M. HEAL², M. LIN³, R. CHRISTIE³, P. HUNTER³, T. L. POWLEY⁴, Z. CHENG¹;

¹Univ. of Central Florida, Orlando, FL; ²MBF Biosci., Williston, VT; ³Univ. of Auckland, Auckland, New Zealand; ⁴Perdue Univ., W Lafayette, IN

Abstract: Millions of Americans suffer from chronic pain in the gastrointestinal tract. However, peripheral nociception has not been well studied which has slowed the development of innovative therapies for chronic pain. In this study, we performed a topographical mapping of the nociceptive afferent axons in the flat-mounts of whole ventral and dorsal stomach muscular layers of mice (C57BL/6J, female, 3-5 months, n=6) using calcitonin gene-related peptide (CGRP) as a marker. Then, we used a confocal microscope and a Zeiss M2 Imager microscope to scan the whole stomachs. Over 300 maximum projection images from the confocal/Zeiss microscopes were assembled to create a complete montage of each stomach. Furthermore, a NeuroLucida 3D Digitization and Tracing System was used to trace CGRP-IR axons and terminals in the whole stomach. We found that 1) CGRP-IR axon bundles entered the stomach along the blood vessels near the esophagus and along the lesser curvature. 2) CGRP-IR axons formed extensive terminal networks in both ventral and dorsal stomachs. 3) CGRP-IR axons densely innervated the blood vessels (arteries and veins). 4) In longitudinal and circular muscles, CGRP-IR varicose axons ran in parallel with the direction of the muscles. 5) CGRP-IR axons formed a complex network between the longitudinal and circular muscle layers. 6) In the myenteric ganglia, CGRP-IR axons formed varicose terminal contacts with individual myenteric neurons. 7) We did not observe any clearly identifiable CGRP-IR myenteric neurons. 8) CGRP-IR axon innervation of the stomach showed a similar pattern to SP-IR axons and terminals between the ventral and dorsal stomachs. 9) SP-IR axon innervation was much denser in the antrum and cardia regions than in the fundus and corpus. 10) CGRP-IR axons had a similar innervation pattern in male (another study, n=6) and female mice. 11) The CGRP-IR axon innervation maps will be integrated into 3D stomach scaffolds (male and female). Control: In control mice (n=8), we injected either tracer DiI or Fluorogold (i.p.) into the ventral and dorsal stomach muscular layers and found that the main extrinsic source of CGRP-IR afferent axons in the stomach was from the T7-T11 DRG and to a lesser extent the vagal nodose ganglia, but not from the celiac ganglia or the dorsal motor nucleus of the vagus. Our data provides a topographical map of the CGRP-IR innervation of the whole stomach at single cell/axon resolution in female mice. This work provides an anatomical foundation for functional studies of CGRP-IR axons in various regions of the stomach and their remodeling in diseases in female mice. This study was supported by NIH HEAL/SPARC U01 NS113867-01 and NIH R15HL137143-01A1.

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Poster

464. Visceral Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 464.08

Topic: D.02. Somatosensation – Pain

Support: NIH R01 152219

Title: Activation of select airway afferent subpopulations evokes cardiopulmonary reflexes

Authors: *T. DARCEY, T. TAYLOR-CLARK;
Univ. of South Florida, Tampa, FL

Abstract: Activation of airway sensory nerves causes respiratory and autonomic reflexes. The majority of the sensory nerves are only sensitive to noxious stimuli, such as inflammation, infection, irritants and pollutants. Activation of these nociceptive sensory nerves evokes protective mechanisms such as apnea, cough and bradycardia. However, these reflexes may also contribute to disease morbidity when excessively or inappropriately activated. Airway nociceptive sensory nerves, which are largely projected from the vagal ganglia (nodose and jugular ganglion), are heterogeneous with respect to gene expression and neuroanatomy, and our objective is to characterize the reflexes evoked by activation of specific afferent subsets. To selectively activate vagal afferent subpopulations *in vivo*, mice were exposed to nebulized selective stimuli such as capsaicin (transient receptor potential (TRP) vanilloid 1 (V1) agonist), allyl isothiocyanate (AITC, TRP ankyrin 1 (A1) agonist), adenosine (nodose-selective agonist) and clozapine-N-oxide (CNO, selective agonist for the designer receptors exclusively activated by designer drugs (DREADD) stimulatory receptor hM3Dq). hM3Dq expression was selectively expressed in sensory subpopulations under the control of Cre recombinase in TRPV1-cre (all nociceptors), Tac1-cre (peptidergic/jugular-originating nerves) and P2X2-cre (nodose-originating nerves). ECGs were recorded via radiotelemetry following implantation of biopotential sensing modules, and respiration was measured via whole body plethysmography. Dose response experiments have been performed for capsaicin, AITC and CNO to determine the minimum dose required to evoke the cardiopulmonary reflex, all of which produce significant bradypnea and bradycardia. Capsaicin and AITC produced comparable levels of bradypnea, but AITC evoked a stronger bradycardia than capsaicin. Bradypnea also appeared at lower doses for both capsaicin and AITC compared to the higher doses required to evoke bradycardia. Adenosine administration produced bradypnea but minimal bradycardia. Our data indicate that stimulation of nociceptive afferents (TRPV1+ and/or TRPA1+) selectively evokes bradypnea and bradycardia in freely moving mice. However, our data also suggests that functionally-distinct nociceptive subpopulations may differentially regulate cardiopulmonary reflexes.

Disclosures: T. Darcey: None. T. Taylor-Clark: None.

Poster

464. Visceral Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 464.09

Topic: D.02. Somatosensation – Pain

Support: St. Louis VA Health Care System Pilot

Title: Viscerosomatic pain in a rat model of Gulf War Illness

Authors: C. O. LIGON¹, E. NOOR-MOHAMMADI¹, B. DIECKGRAEFE², J. ELWING², G. S. SAYUK², *A. C. JOHNSON^{3,1};

¹Dept. of Physiol., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; ²VA Hlth. Care Syst., St. Louis, MO; ³VA Hlth. Care Syst., Oklahoma City, OK

Abstract: Introduction: Gulf War Illness (GWI) is a chronic multi-symptom illness that causes fatigue, mood disorders, pain, and digestive complaints. Despite the approximately 250,000 Veterans affected by this condition, the mechanisms responsible for the pathogenesis of GWI are not well understood, and consequently, there are few therapies. One potential etiology for GWI involves the prophylactic exposure of the Veterans to insecticides and anti-cholinesterase compounds to counteract chemical warfare agents along with combat stressors. While models have been developed that expose rodents to similar compounds, the effect on visceral sensation has not been evaluated. The goal of the current study was to evaluate chronic viscerosomatic sensitivity in a rat model of GWI. **Methods:** Twenty-four adult male Sprague Dawley rats were randomized to GWI model or control group. Rats in the GWI model were exposed to oral pyridostigmine bromide (PB, 1.3 mg/kg), topical N,N-diethyl-m-toluamide (DEET, 40 mg/kg) and topical permethrin (PM, 0.13 mg/kg) daily from days 0-28. Immediately after compound exposure, rats in the GWI group were exposed to an acute stressor by being placed in a restraint device for 5 min. Control rats received oral sterile PBS (vehicle for PB) and topical 70% ethanol (vehicle for DEET and PM) without stress exposure. On day 83, somatic sensitivity was assessed via application of von Frey filaments to the hind paw. Colonic sensitivity then assessed via isobaric balloon distension (20, 40, and 60 mmHg) on day 84, and referred bladder sensitivity was measured at day 90 via stimulation of the suprapubic region with von Frey filaments. Results were expressed as mean \pm SD and analyzed with repeated measure-multifactor ANOVA or Student's t-tests. **Results:** Rats in the GWI group displayed increased somatic sensitivity compared to control (72.7 ± 18.4 g vs. 90.4 ± 9.6 g withdrawal threshold, $p = 0.0095$). Additionally, GWI rats displayed hypersensitivity to colonic distension (60 mmHg: 23.8 ± 5.7 vs. 18.0 ± 4.7 contractions, $p = 0.0010$). Finally, analysis of the abdominal withdrawal reflex to suprapubic stimulation revealed main effect of group with no post-hoc differences, indicating a mild increase in referred bladder sensitivity in the GWI model group. **Conclusions:** Using a rat model of GWI, we demonstrated that exposure to GWI-related neurotoxicants and stress induced long-term viscerosomatic hypersensitivity. Based on the chronic nature of the hypersensitivity, future studies will evaluate epigenetic mechanisms in limbic brain regions that modulate descending pain inhibition to investigate mechanisms to develop novel therapies for Veterans with GWI.

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Poster

464. Visceral Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 464.10

Topic: D.02. Somatosensation – Pain

Support: NIDDK K01DK114395-06
Start-up funds

Title: Pelvic tactile allodynia is dependent on mast cell activation in a non-invasive mouse model of endometriosis

Authors: *K. ROMAN, P. PRASOON, B. K. TAYLOR;
Dept. of Anesthesiol. and Perioperative Med., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Endometriosis (EM) impacts approximately 10% of women in the world (Zondervan et al., 2020) and over 4 million in the United States (Agarwal et al., 2019). The mechanism(s) that drive chronic pelvic pain (CPP) in EM (EM-CPP) are unclear. Human EM lesions are infiltrated with mast cells (MCs) (Matsuzaki et al., 1998). In other forms of CPP, such as chronic prostatitis, MC activation is increased (Roman et al., 2014). To test the hypothesis that MC activation is a casual factor in EM-CPP, we characterized pelvic tactile allodynia in a new non-invasive mouse model of EM (Fattori et al., 2020). Briefly, female C57BL/6J (B6) donor mice (6 weeks old) received a subcutaneous injection of estradiol benzoate (EB; 10 μ g in 100 μ l of sesame seed oil) and 4 days later they were euthanized and each uterine horn was excised (~1cm) and placed in Hank's Balanced Salt Solution (HBSS; 600 μ l) and minced (>1mm). Recipient B6 female mice at 6 weeks of age were habituated for at least 5 days before they received an intraperitoneal (i.p.) injection of either HBSS (500 μ l; "Sham mice") or HBSS+minced uterine horn tissue from donor mice (500 μ l; "EM mice"). To assess the development of pelvic tactile allodynia, recipient/EM mice were placed on a wire mesh suspended on top of a metal rig and calibrated von Frey (vF) filaments (0.008, 0.02, 0.07, 0.16, 0.4, 1, 2, and 6 grams) were applied to the pelvic region using the up-down method. Testing was conducted before (baseline) and 7, 14, 21, and 28 days after i.p. injection. On day 28, the MC stabilizer ketotifen fumarate (Keto; 4.5mg/kg) or 0.9% saline was injected and vF thresholds were assessed at 3, 9, 18, and 36 hrs. In our first experiment, we evaluated the relationship between the dose of EB given to donor mice and the degree of tactile hypersensitivity in recipient mice (n=4/dose; n=4/vehicle at each dose). Neither 1 μ g nor 3 μ g changed mechanical threshold, while both 10 μ g or 30 μ g decreased mechanical thresholds from day 7 to 28. Thus, we used 10 μ g of EB in subsequent experiments (p<0.05 at each timepoint, Tukey). We next evaluated the effect of Keto or saline on mechanical hypersensitivity in Sham control or EM mice. In Sham, neither saline (n=6) nor Keto (n=7) changed mechanical thresholds at any timepoint. In EM mice, Keto (n=8) but not saline (n=7) reversed hypersensitivity (F(12,96)=2.168, p=0.0192, Drug X Time, 2-way ANOVA) at the 9, 18, and 36 hr. timepoints (p<0.05 at each timepoint, Tukey). In summary: 1) donor tissue from mice that received 10 μ g of EB induces pelvic tactile allodynia in recipient mice, 2) inhibition of MC activity alleviates pelvic tactile allodynia in EM mice, and 3) the EM mouse model developed by Fattori et al., works in our laboratory.

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Poster

464. Visceral Pain

Location: SDCC Halls B-H

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Program #/Poster #: 464.11

Topic: D.02. Somatosensation – Pain

Support: NIH DK 51413

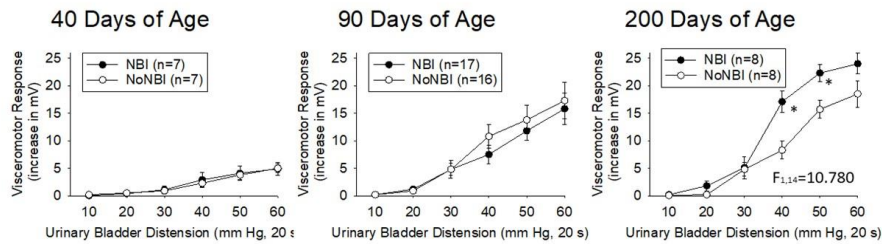
Title: Aging female rats which experienced neonatal cystitis develop bladder hyperalgesia without a secondary insult

Authors: *T. J. NESS¹, C. DEWITTE²;

¹Anesthesiol. and Perioperative Med., Univ. of Alabama At Birmingham, Birmingham, AL;

²Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: **BACKGROUND:** The incidence of the painful bladder syndrome, interstitial cystitis, increases in prevalence with aging with a sharp increase beginning in the sixth decade of life. The mechanistic basis of this increase is unknown. Many of the phenotypic features of interstitial cystitis are present in a rat model in which rat pups experience neonatal bladder inflammation (NBI) induced by the intravesical administration of the inflamogen zymosan on days P14-16 of life and then experience a second insult (e.g., bladder re-inflammation, acute stress, peri-segmental hindpaw inflammation) as an adult [Ness et al, 2021]. These rats demonstrate bladder hypersensitivity to distension manifest as increased robustness of cystometric, neuronal, cardiovascular and reflex responses to urinary bladder distension. The present study sought to determine whether the effects of aging alone might serve as a secondary insult producing similar hypersensitivity measures in NBI-treated female rats. **METHODS:** The vigor of visceromotor responses to UBD was determined in rats which had experienced NBI, with their controls, at ages 40, 90 and 200 days of age. **RESULTS:** There was an effect of aging on responses to UBD with more robust visceromotor responses to UBD noted in rats which had experienced NBI and which were 200 days old whereas a similar phenomenon was not noted in 40 and 90 day old rats. There was also a greater potential for bladder tissue damage following hydrodistension in older rats which had experienced NBI **CONCLUSIONS:** Bladder hypersensitivity increased as a function of age in rats which had experienced NBI suggesting that the degenerative changes that occur with aging may be sufficient to act as a second insult in subjects susceptible to hypersensitivity due to a previous bout of neonatal cystitis.



Disclosures: T.J. Ness: None. C. DeWitte: None.

Poster

464. Visceral Pain

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 464.12

Topic: D.02. Somatosensation – Pain

Support: NIDCR R01 DE029074

Title: Sex Differences in Referred Pain of Visceral Origin Following Stress and Orofacial Inflammation

Authors: *L. G. HERNANDEZ-ROJAS¹, J. T. DA SILVA¹, S. HANSON¹, A. J. SCOTT², R. K. ERNST², O. K. MELEMEDJIAN¹, D. A. SEMINOWICZ¹, R. J. TRAUB¹;

¹Dept. of Neural and Pain Sci. and the Ctr. to Advance Chronic Pain Res., ²Dept. of Microbial Pathogenesis, Univ. of Maryland Sch. of Dent., Baltimore, MD

Abstract: Temporomandibular disorder (TMD) and irritable bowel syndrome (IBS) are two highly prevalent chronic overlapping pain conditions that coexist in >50% of patients with either disorder, are more predominant in women and are exacerbated by stress. Mechanisms underlying the comorbidity of chronic pain originating in distally unrelated tissue types are still unknown and the effects of stress recurrence are also poorly understood. We hypothesize that changes in brain function initiated by pre-existing pain combined with stress contributes to development of de novo referred pain and the recurrence of stress evokes greater and longer referred hypersensitivity related to de novo pain. Using an animal model (masseter muscle inflammation followed by stress) that induces de novo Comorbid visceral Pain Hypersensitivity (CPH) in rats, we reported more robust and longer duration CPH in female rats compared to males (Da Silva et

al. IASP 2021, Hernandez et al. SFN2021). In this study, referred pain from the colon; hypersensitivity to mechanical stimulation at the base of the tail; was examined in rats subjected to stress alone (Stress-Induced Hypersensitivity, SIH) or orofacial pain plus stress (CPH). Preliminary data from stress alone in females showed referred pain two weeks post stress, resolving by three weeks ($p=.001$, $n=4$). In the case of CPH females, referred pain was present at least seven weeks post stress ($p<.001$, $n=12$) mirroring the visceromotor response. For CPH males, referred pain was resolved by seven weeks post stress ($p=.007$, $n=4$). A second round of stress was induced in SIH and CPH rats after resolution of the hypersensitivity of the first stress stage. In this case, SIH rats showed increased referred pain at least four weeks following the second stress session ($p=.010$, $n=4$). For CPH female rats, the referred pain was present at least 4 weeks following the second stressor ($p=.001$, $n=4$). In order to confirm the mechanohypersensitivity was indeed referred pain from the colon, intracolonic injection of lidocaine attenuated the referred pain following the first and/or second stress sessions in SIH and CPH rats. For CPH females, lidocaine attenuated the referred hypersensitivity at week 7 post first stressor ($p=.017$, $n=4$) and at week 4 following the second stressor ($p=.005$, $n=4$). Data collection is ongoing in males. These preliminary results suggest that referred visceral mechanosensitivity is a suitable method for assessing de novo Comorbid visceral Pain Hypersensitivity and Stress-Induced Hypersensitivity in rats. Also, this study supports our previous work comparing SIH and CPH groups, suggesting that hypersensitivity in males may resolve faster than in females

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Poster

464. Visceral Pain

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Topic: D.02. Somatosensation – Pain

Support: National Institute of Diabetes and Digestive and Kidney Diseases Grant 5R01DK120108
National Institute of Diabetes and Digestive and Kidney Diseases Grant 1R01DK124580

Title: Nerve growth factor signaling in urinary bladder dysfunction with cyclophosphamide-induced cystitis

Authors: *H. W. HSIANG¹, B. GIRARD³, S. CAMPBELL², M. A. VIZZARD³;
¹Univ. of Vermont, ²Univ. of Vermont, Burlington, VT; ³Larner Col. of Med. at UVM, Larner Col. of Med. at UVM, Burlington, VT

Abstract: Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic inflammatory pain condition characterized by urinary bladder inflammation and afferent hypersensitization leading to bladder dysfunction. While the etiology of IC/BPS remains mysterious, converging evidence suggests a role for changes in neurotrophin signaling, particularly that of nerve growth factor (NGF). NGF signals through two distinct receptors, TrkA and the pan-neurotrophin p75NTR, that produce opposing or facilitative effects depending on the expression of coreceptors, ligand availability, and cellular context. Given its complexity and tissue-specificity, thorough characterization of NGF signaling in the bladder is imperative to the development of effective therapies for IC/BPS. Here, we identified changes in urinary bladder expression of various NGF signaling-related proteins in mice with cyclophosphamide (CYP)-induced cystitis using immunohistochemical and enzyme-linked immunosorbent assays. Additionally, using conscious, open-outlet cystometry, we demonstrated that disruption of NGF signaling via pharmacological inhibition of TrkA or p75NTR improves bladder function in CYP-treated mice at a timescale of half an hour. Specifically, intravesical administration of a TrkA inhibitor was associated with a 1.5-fold increase in intermicturition interval and bladder capacity in acute (4-hour) CYP-treated mice and a 1.6-fold increase in chronic (8-day) CYP-treated mice. p75NTR inhibition was associated with a 1.8-fold increase in acute CYP-treated mice and, interestingly, a 0.6-fold decrease in chronic CYP-treated mice. These findings demonstrate the therapeutic potential of targeting NGF signaling and identify potential targets in developing effective therapies for IC/BPS and other inflammatory disorders of the bladder.

Disclosures: **H.W. Hsiang:** A. Employment/Salary (full or part-time);; University of Vermont. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Pfizer. **B. Girard:** A. Employment/Salary (full or part-time);; University of Vermont. **S. Campbell:** A. Employment/Salary (full or part-time);; University of Vermont. **M.A. Vizzard:** A. Employment/Salary (full or part-time);; University of Vermont. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Pfizer.

Poster

465. Touch Reception and Mechanosensitive Channels

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 465.01

Topic: D.03. Somatosensation – Touch

Support: NIH/NIGMS P20GM103436
Alfred P. Sloan Foundation Research Fellowship in Neuroscience

Title: Transcript level expression of COCH in sensory corpuscles of Pekin ducks (*Anas platyrhynchos*)

Authors: ***T. R. HART**, E. R. SCHNEIDER;
Biol. Dept., Univ. of Kentucky, Lexington, KY

Abstract: Light-touch transduction in vertebrate skin is mediated by mechanoreceptive neurons ending in specialized organs called sensory corpuscles. Studies suggest the non-neuronal cells composing these corpuscles are themselves touch sensitive and interact with neuronal afferents to transduce touch sensation. However, the mechanisms by which corpuscles contribute to touch transduction remain uncertain, particularly for Pacinian corpuscles. Understanding interaction between cells at this neuronal-non-neuronal interface could provide important insights for touch transduction. The bill skin of Pekin ducks (*Anas platyrhynchos*) is rich in Herbst and Grandry corpuscles, which are analogous to human Pacinian and Meissner corpuscles. To screen for molecular markers of corpuscle cells, we performed RNA sequencing and differential gene expression analysis using skin samples from adult ducks and late-stage duck embryos, both of which contain functioning touch receptors. Samples were collected in quadruplicate via circular biopsy punches from three skin sources: adult bill, embryo bill, and embryo foot. Adult and embryo bill skin served as corpuscle-enriched tissue and were compared with corpuscle-scarce embryo foot skin. Differential gene expression analysis using DESEQ2 was used to screen for genes upregulated in corpuscle-rich tissue. RNA fluorescent *in situ* hybridization and immunohistochemistry were then used to localize gene expression in embryonic and adult bill skin. Among the genes tested, we report for the first time the expression of the COCH gene in the inner core lamellar cells of Herbst corpuscles. COCH encodes a secreted extracellular matrix protein, cochlin, which is associated with glaucoma and DNFA9, diseases associated with irregularities in physiological fluid shear in the eye and inner ear, respectively. Though the function of cochlin is poorly understood, *in vitro* studies have shown cochlin forms multimers in response to fluid shear and its expression induces co-expression of the mechanosensitive ion channel TREK-1 (Goel et al., PLOS One, 2011), suggesting cochlin may play a role in touch transduction. Our differential expression analysis also showed upregulation of KCNK2 (TREK-1) in the bill tissue (embryo bill vs. foot, logFC = 2.16, $q < 5e-13$). This combined with COCH expression in inner core cells of Herbst corpuscles suggests COCH may play a role in mechanosensation.

Disclosures: T.R. Hart: None. E.R. Schneider: None.

Poster

465. Touch Reception and Mechanosensitive Channels

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 465.02

Topic: D.03. Somatosensation – Touch

Title: Mechanoreceptor and innervation density in marmoset hands

Authors: *V. SUKUMAR¹, M. FEYERABEND², W. INOUE³, A. PRUSZYNSKI⁴;
¹UNIVERSITY OF WESTERN ONTARIO, UNIVERSITY OF WESTERN ONTARIO, LONDON, ON, Canada; ²Schulich Sch. of Med. and Dent., ³Univ. of Western Ontario, London, ON, Canada; ⁴Physiol. and Pharmacol., Western Univ., London, ON, Canada

Abstract: Mechanoreceptors are specialized structures that respond to the stresses and strains in the skin that arise when it is deformed. In the glabrous skin of the hand, A β afferent fibers branch extensively before innervating multiple low threshold mechanoreceptors (Meissner corpuscles, Merkel complexes), leading to complex receptive fields. Recent studies have shown that these first order A β afferent fibers signal fine details of touched objects, suggesting the complex innervation constitutes a peripheral neural mechanism for extracting the spatial features of touched objects and surfaces. In the present work, we have established an approach for determining the density of low threshold mechanoreceptors and the morphology of their innervation in marmoset hands using immunohistochemistry and confocal imaging. We present the difference in mechanoreceptor and innervation densities across different regions of the marmoset hand. This work provides a baseline of the peripheral morphology in marmosets and will facilitate the study of the changes occurring in the periphery after nerve injury.

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Poster

465. Touch Reception and Mechanosensitive Channels

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Program #/Poster #: 465.03

Topic: D.03. Somatosensation – Touch

Support: Undergraduate Research Opportunity Program Grant, Mills College
Jill Barrett Undergraduate Research Program in Biology Grant, Barrett Foundation
Mills College Faculty Research Grant

Title: The *C. elegans* precipice response is influenced by force vectors

Authors: *R. M. MITCHELL, S. MCCOY, S. ZHANG, W. PULICE, D. PATTILLOS, K. HODNETT, J. J. YOUNG;
Biol., Mills Col., Oakland, CA

Abstract: The precipice response in *C. elegans* is a behavioral phenomenon wherein a worm will strongly reverse upon the first encounter of its nose with the edge of an agar chunk. Although mentioned in a WormBook section on mechanosensation (Chalfie, WormBook 2014), until recently this behavior remained uncharacterized. Our previous work defined the behavior as a reversal within two seconds of the nose tip moving past the edge of an agar chunk that must complete at least one full sine wave and showed that mechanosensation—and in particular, anterior harsh touch sensation—is required for normal rate of precipice response. We designed precipice assays to compare N2 wild-type worms to three mutant strains with varying levels of touch deficiency: *mec-3(e1338)*, *mec-10(e1515)*, and *trp-4(sy695)* and found that only *mec-3* worms with total loss of gentle and harsh touch sensation had a significantly decreased rate of

precipice response (Mitchell *et al.*, microPublication Biology 2021).

We will report on additional experiments that further elucidate the mechanisms of the precipice response in *C. elegans*. One question that remained after our initial experiments was how worms would behave when they experienced different force vectors as their heads moved over the edge of a chunk. Wild-type *C. elegans* have a strong tendency to exhibit the precipice response when they are situated atop an agar chunk and their head moves over the edge (Mitchell *et al.*, microPublication Biology 2021). We wondered whether this was primarily due to a mechanical stimulus akin to anterior harsh touch when their nose tip lost connection with solid substrate, or if the precipice response is mediated more by proprioceptive neurons that can detect stretch when the worm's head is pulled down by gravity. If the latter is a more accurate description of what triggers the precipice response, then we might expect worms to behave differently when they are situated on the side of an agar chunk than when they are on the top and are therefore experiencing different force vectors. We have indeed found that the precipice response is elicited more frequently when N2 worms encounter an edge while on the top of an agar chunk (65%, n = 159) as opposed to when they are assayed on the side of a chunk (50%, n = 115; $\chi^2 = 6.0237$, $p = 0.01$). These results indicate that the precipice response is influenced by the particular force vectors experienced by the head, and not solely by the experience of the head losing contact with the agar surface. We are currently working to identify the specific neurons involved in the precipice response, as well as investigating a potential role for habituation in this behavior.

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Poster

465. Touch Reception and Mechanosensitive Channels

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Program #/Poster #: 465.04

Topic: D.03. Somatosensation – Touch

Support: Pennsylvania Department of Health Grant (SLS)
Penn State College of Medicine (SLS)

Title: Functional characterization of endogenous Piezo1 mechanosensitive channels in Neuro2a cells

Authors: *S. STELLA, A. ISELY;

Neural and Behavioral Sci., Penn State Univ. Hershey-College of Med., Hershey, PA

Abstract: Mechanosensitive cation channels have the ability to sense and respond to mechanical stimuli in the surrounding environment. In a variety of sensory neurons, the Piezo family of mechanosensitive ion channels have been shown to be essential for mechanical responses mediated by the gating of cations, like Ca^{2+} following the activation of these channels. Piezo1 channels have been shown to be endogenously expressed by the neuroblastoma cell line,

Neuro2a. The principal aim of this study is to characterize the pharmacology and functional expression of Piezo1 channels in Neuro2a cells using the chemical agonist, Yoda-1 as the activator of endogenous channels. We measured $[Ca^{2+}]_i$ changes in Neuro2a cells using the Ca^{2+} -sensitive dye, Calbryte 520, and monitored channel activity with the Piezo1 selective chemical agonist Yoda-1. Additionally, immunocytochemistry was performed on cultured Neuro 2a cells with specific antibodies to the Piezo1 protein to determine the localization and expression levels. A Neuro2a cell line (Piezo1 $-/-$) where the Piezo1 gene had been deleted using CRISPR served as a negative control. Cell response properties were confirmed by evoking $[Ca^{2+}]_i$ changes from application of 100 μ M ATP and depolarization-evoked $[Ca^{2+}]_i$ increases (with elevated $[K^+]_o$) to confirm functional cell viability. Piezo1 expression was robust in Neuro2a cells with multiple antibodies with different epitopes showing immunoreactivity throughout the cell that was absent in cells where the Piezo1 gene was deleted. Yoda1 consistently evoked dose-dependent $[Ca^{2+}]_i$ increases in Neuro2a cells with an EC_{50} of $\sim 50 \mu$ M that was inhibited by several mechanosensitive channel antagonists, including GsMTx-4 (5 μ M). Likewise, Yoda1 did not evoke any calcium increases in Neuro2a cells where the Piezo1 gene was absent. Taken together, the evidence provided in this study demonstrate that the Neuro2a cell line combined with Yoda-1 serves as a powerful pharmacological tool to screen and investigate the properties of Piezo1 mechanosensitive channels using calcium imaging.

Disclosures: S. Stella: None. A. Isely: None.

Poster

465. Touch Reception and Mechanosensitive Channels

Location: SDCC Halls B-H

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Program #/Poster #: 465.05

Topic: D.03. Somatosensation – Touch

Support: EU Horizon 2020 grant agreement 813713 (NeuTouch)

Title: Sub-surface skin strain patterns during natural object interactions

Authors: *G. CORNIANI¹, Z. S. LEE¹, M. J. CARRÉ¹, R. LEWIS¹, B. P. DELHAYE², H. P. SAAL¹;

¹Univ. of Sheffield, Sheffield, United Kingdom; ²Inst. of Neurosci., Univ. catholique de Louvain, Woluwe-saint-Lambert, Belgium

Abstract: Physical interactions between human skin and object surfaces result in stereotypical spatiotemporal patterns of mechanical strain within and between different skin layers that depend on the mechanical, elastic, and geometrical features of both the object and the skin. Understanding the biomechanics of the skin and subcutaneous tissues is fundamental for our understanding of the human tactile sensory system, because mechanoreceptors, responsible for the encoding of tactile stimuli, are responsive to various aspects of skin deformation. Due to technical limitations, little is known about the behaviour of the skin below its immediate surface

in vivo and for dynamic stimuli, even though sub-surface skin strains are ultimately transduced by mechanoreceptors to form the first stage of sensory encoding in the tactile system. In this study, we employed Optical Coherence Tomography (OCT), a non-invasive imaging technique to capture the skin's internal morphology during object interactions. Specifically, we tracked landmarks, such as the skin surface, the epidermal ridges, the stratum corneum, and the dermis-epidermis ridges, where type-1 mechanoreceptors are situated. We recorded OCT images at a rate of 20 frames per second during loading and sliding interactions of the fingerpad with different contact surfaces. The recorded images present a sliced side view of the fingerpad with high spatial resolution.

To study the biomechanics of the captured tissues, we segmented the different visible layers and tracked features of the movement of the skin during the contact with the surfaces. We characterised the compression of the skin tissues under different loading conditions by measuring the variation in thickness and the geometrical deformation of the epidermal ridges by computing the waviness of the profile. Next, we reconstructed time-varying strain patterns to derive the local skin strain changes at the depth of the mechanoreceptors. In particular, strain rates were computed using the Green-Lagrange strain equations, resulting in two axial components aligned with the skin surface and orthogonal to it, as well as a shear component. We found repeatable, stereotypical spatiotemporal patterns in all three strain components, which depend on the geometrical features of the surface and vary in magnitude in different skin subsurface layers. These findings will help understand how surface deformations propagate into deeper layers towards mechanoreceptor locations and shed light on the function of skin morphology in encoding tactile stimuli.

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Poster

465. Touch Reception and Mechanosensitive Channels

Location: SDCC Halls B-H

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Program #/Poster #: 465.06

Topic: D.03. Somatosensation – Touch

Title: Investigating somatosensation in the naked mole-rat using high-speed videography

Authors: *R. SCHWARK, P. CHANG, I. ABDUS-SABOOR;
Zuckerman Inst., Columbia Univ., New York, NY

Abstract: The naked mole rat (*Heterocephalus glaber*) is a eusocial species of rodent which lives in large subterranean colonies in arid regions of east Africa. Colonies consist of a single breeding female, 1-3 breeding males, and dozens of non-reproductive drones organized in a dominance hierarchy. These animals have evolved an endless variety of adaptations, including decreased pain sensitivity, extreme lifespan length, individual vocal identification, and an exquisitely sensitive somatosensory system. Despite decades of work, an in-depth exploration of

these somatosensory abilities, and their relation to the naked mole-rat's social behavior, remains relatively unexplored. Here we use high-speed videography to shed light on the precise nature of somatosensation in these animals, including in the nociceptive and social dimensions. In contrast to mice, applying innocuous mechanical stimuli to the hindpaw was never sufficient to garner a response in mole-rats, although prolonged application of a noxious pinprick stimulus was able to elicit a pain-like withdrawal response. However, naked mole-rats did respond to innocuous mechanical stimulation when the dorsal back skin was brushed. Interestingly, an extremely aversive response was elicited when the same brush stimulus was applied to the snout. These results suggest that the naked mole-rat has extremely sensitive mechanosensory abilities, but that the accompanying behavioral response is profoundly different depending on the location of the stimulated tissue on the body. Furthermore, this data illustrates that naked mole-rat pain is more nuanced than previously described, and is divergent from that of other rodents.

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Poster

465. Touch Reception and Mechanosensitive Channels

Location: SDCC Halls B-H

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Program #/Poster #: 465.07

Topic: D.03. Somatosensation – Touch

Title: Involvement of Mas-related G protein-coupled receptor (Mrgpr) pathway in chronic itch

Authors: N. IZUMIMOTO, M. KONNO, M. MORIYAMA, N. YUZAWA, T. IWAMURA, ***K. HASEBE**, H. NARUMI;

Toray Industries, Inc., Kanagawa, Japan

Abstract: Chronic pruritus is one of the debilitating symptoms which occurs with various causes like systemic and dermatological diseases or side effect of medications and frequently is refractory to treatment. Atopic dermatitis (AD) is a common dermatologic disease that is accompanied by severe chronic pruritus, which is one of the most important symptoms in the disease. Furthermore, in patients with AD, the severe pruritus not only influences the patient's quality of life, but also elicits intense and persistent scratching, which aggravates the lesions. Thus we have to develop an efficient strategy for controlling pruritus and scratching in treating AD. Recently, it has been reported that mas-related G protein-coupled receptor (Mrgpr) involves in the control of histamine-independent itch and the modulation of Mrgpr might be a promising target for the treatment of chronic itch. It is known that there is the ortholog of Mrgpr to regulate the function of the sensory nerve (X1~X4 subtypes in humans, and A3/C11 and D subtypes in rodents). In the present study, we used the transgenic mice expressed MrgprX1 in the sensory nerve and Mrgpr cluster KO (Mrgpr-KO) mice and evaluated the scratching behaviors in the acute itch model evoked by Mrgpr agonist, BAM-8-22. In addition, the modulation of Mrgpr and the effect of steroid drug in the MC-903-induced chronic itch model in both transgenic mice were examined. As results, BAM-8-22 (50 nmol/site, intra-dermal injection) did not induced the

scratching behaviors in the Mrgpr-KO mice over vehicle-treatment, on the other hand, it evoked scratching behaviors in the Mrgpr-X1 transgenic mice compared to the vehicle-treated animals. In the chronic itch model evoked by the repetitive application of MC903 (4 nmol/site, once daily for 6 days) produced the scratching bouts in both Mrgpr-KO and MrgprX1-transgenic mice over vehicle-treatment, but the ones in the MrgprX1 mice evidently outweighed them in the Mrgpr-KO mice. In addition, dexamethasone, a steroid drug, significantly attenuated the scratching behaviors evoked by MC903 in both Mrgpr-KO and MrgprX1-transgenic mice to the same extent. From these observations, it is suggested that the itch in the AD patients might be able to be inhibited completely by the combination of steroid and MrgprX1 antagonist.

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Poster

465. Touch Reception and Mechanosensitive Channels

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Program #/Poster #: 465.08

Topic: B.03. Ion Channels

Title: Mechanical activation of Piezo1 by shear stress using high throughput automated patch clamp

Authors: A. BRUGGEMANN¹, N. MURCIANO¹, M. G. ROTORDAM¹, M. RAPEDIUS¹, N. BECKER¹, M. J. LUDLOW³, G. PARSONAGE³, K. CUTHBERTSON³, R. FOSTER³, R. HAEDO⁴, G. OKEYO⁴, *A. R. OBERGRUSSBERGER², D. J. BEECH³;
²Nanion Technologies GmbH, ¹Nanion Technologies GmbH, Munich, Germany; ³Univ. of Leeds, Leeds, United Kingdom; ⁴Nanion Technologies Inc., Livingston, NJ

Abstract: Piezo channels are mechanosensitive cation channels that play important roles in biological functions including touch, proprioception, shear stress, stretch sensation and blood pressure regulation. Piezo1 channels have been shown to sense mechanical stimuli in central nervous system vasculature and are expressed in retina and cortex capillaries. Piezo1 is also important for promoting vascular pathfinding in a variety of organs including brain, and loss of function mutations of Piezo1 impair endothelial pathfinding. Interestingly, overactivation of Piezo1 by Yoda-1 impairs neuronal myelination causing neuronal damage, whereas inhibition of Piezo1 has a neuroprotective effect.

A significant challenge in Piezo1 channel drug development using automated patch clamp assay recordings is evoking highly reproducible Piezo 1 current amplitudes using pseudo-physiological mechanical stimulation. Here we show that optimization of pipetting parameters coupled with modification of the NPC-384 chip of the SyncroPatch 384 lead to Piezo1-mediated currents activated by mechanical stimulation. Data from mouse and human Piezo1 channels expressed in HEK293 cells activated by either mechanical or chemical stimuli will be shown, as well as the combination of both methods. Using this approach we were able to show that very specific

modification of the Yoda-1 molecule permits the development of new Piezo1 agonists with improved physico-chemical properties. These new tool compounds should improve Piezo1 channel modulation in experimental physiological models.

In this way, mechanical stimulation of Piezo1 channels using a high throughput planer patch clamp system could be demonstrated. The possibility of comparing and combining mechanical and chemical stimulation in a high throughput patch clamp assay facilitates the biophysical and pharmacological characterization of Piezo channels and thereby provides an important experimental tool for studying Piezo channel biology in CNS function.

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Poster

465. Touch Reception and Mechanosensitive Channels

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Topic: D.03. Somatosensation – Touch

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Title: Novel properties of Tentonin 3, a Slowly-inactivating mechanosensitive ion channel: mechanosensitivity of orthologs, cytoskeleton dependency, a novel binding protein and its specific inhibitor

Authors: P. LEE¹, *G. HONG¹, U. OH²;

¹Korea Inst. of Sci. & Technol., ²Korea Inst. of Sci. and Technol. (KIST), Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of

Abstract: Tentonin 3/TMEM150C (TTN3) is a slowly-inactivating mechanosensitive ion channel with physiological linkages regarding muscle coordination, baroreceptor function, and insulin secretion. Due to the disappearance of TTN3 mechanosensitive currents in Piezo1 ablated HEK cells, TTN3 is rather considered as a regulatory protein modulating Piezo 1. Here, we

present new evidences supporting TTN3 as an ion channel rather than a regulator. The mechanosensitivity of TTN3 was conserved among orthologs. The channel activity showed high dependency on cytoskeletal integrity and focal adhesion rather than Piezo1 protein. We also identified a TTN3-specific inhibitor and a binding protein. Our new data suggests TTN3 acts as a mechanically-activated channel.

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Poster

466. Plasticity in the Somatosensory System

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Program #/Poster #: 466.10

Topic: D.03. Somatosensation – Touch

Support: NINDS F31NS103439
NIAMS R01 R01AR051219

Title: The spatiotemporal dynamics of sensory neuron and Merkel-cell remodeling are decoupled during epidermal homeostasis

Authors: *R. CLARY^{1,2}, B. A. JENKINS², E. A. LUMPKIN¹;
¹Univ. of California, Berkeley, Berkeley, CA; ²Neurobio. & Behavior Training Program, Columbia Univ., New York, NY

Abstract: Spinal afferents innervate the skin to form a sensory interface that is responsible for encoding tactile, thermal, noxious and itch stimuli. Additionally, the skin serves as a protective barrier; epithelial cells renew monthly to maintain the barrier, which in turn raises the question of whether sensory afferents homeostatically remodel to preserve innervation patterns. We addressed this question *in vivo* using longitudinal two-photon imaging of TrkC-tdTomato-labeled somatosensory axons (n=19 axons), which innervated Atoh1-GFP-expressing Merkel cells (n=309) in adult mouse skin. Both axon terminals and Merkel cells were highly plastic at three day intervals over a one-month period. Remodeling was observed in 63% of Merkel cells (addition, removal, relocation) and 89% of terminal branches (sprouting, growth, regression, removal). We tested whether remodeling was synchronous across arbors; interestingly, plasticity in Merkel cells was synchronized during a period of rapid epithelial turnover. When Merkel cells remodeled, the degree of plasticity between Merkel-cell clusters and their axons was correlated (slope=0.64; $P_{\text{slope}} < 0.0001$; $R^2 = 0.37$). Moreover, individual axonal branches were stabilized by contacts with Merkel cells. Together, these results confirm a role for epithelial-neural crosstalk in axonal plasticity. Conversely, axons were highly dynamic even when Merkel cells were stable, indicating that intrinsic neural mechanisms also drive axon plasticity. Using the TrkC-tdTomato reporter allowed us to visualize the entire extent of afferent terminals, where previous studies using Neurofilament Heavy antibodies only labeled the intermediate filament cytoskeleton. With TrkC-tdTomato, we visualized two terminal morphologies that innervated Merkel cells: transient

swellings called boutons (11% of contacts) and stable cups termed kylikes (89% of contacts). In *Atoh1* knockout mice that lack Merkel cells, axons showed higher complexity than control mice, with exuberant branching and no kylikes (median complexity index: WT=13,607, KO=69,753; P=0.0006; Mann-Whitney rank test). Thus, Merkel cells limit axonal branching and promote branch maturation. Together, these results reveal a previously unsuspected high degree of plasticity in somatosensory axons that is biased, but not solely dictated, by plasticity of target epithelial cells. This system provides a framework to identify intrinsic and extrinsic mechanisms that govern axonal patterning during epithelial homeostasis.

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Poster

466. Plasticity in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: NIH R21 MH123906
NIH RF1 MH114103

Title: Anatomical plasticity in SST interneurons during sensory association learning

Authors: *M. MOSSO¹, A. RAY², R. SWINDELL², F. BOLIO¹, E. PARK¹, D. A. KULJIS², A. L. BARTH³;

²Biol. Sci., ¹Carnegie Mellon Univ., Pittsburgh, PA; ³Biol. Sci., Carnegie Mellon U., Pittsburgh, PA

Abstract: Somatostatin (SST)-expressing inhibitory interneurons can reduce their firing activity during learning. It has been hypothesized that this reduction may facilitate plasticity at excitatory synapses. Here we deployed high-throughput, fluorescence-based methods for a quantitative analysis of SST inputs and outputs in mouse somatosensory cortex during training in a sensory association task. We used molecular genetic techniques to selectively label either excitatory and inhibitory synapses onto SST neurons, using virally-encoded FingR-intrabodies for the synaptic scaffolding proteins PSD95 and gephyrin. Separately, axonal boutons from SST neurons were virally labeled in S1 with synaptophysin-GFP. Animals of both sexes were used. We used volumetric confocal imaging of different cortical layers in fixed tissue for fluorescence-based reconstruction of SST synapse and bouton area and/or volume using the image processing software, Imaris. Our analysis suggested that the SST output is rapidly reduced at the onset of training in superficial but not deep layers. Changes in PSD95 puncta size was also reduced during training, but required longer training periods to emerge. Electrophysiological recordings examining SST output were consistent with this rapid and layer-specific reduction at the onset of training. High-throughput anatomical approaches can thus stimulate new and testable hypotheses about altered circuit function during learning.

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Poster

466. Plasticity in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: NI-IISc Postdoctoral Fellowship
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NIH RF1 MH114103

Title: Dendrite specific thalamocortical plasticity in L5 pyramidal neurons during sensory learning

Authors: *A. RAY¹, J. A. CHRISTIAN¹, M. MOSSO¹, E. PARK¹, L. AWASTHI², K. I. WILLIG³, A. L. BARTH¹;

¹Biol. Sci., ²Carnegie Mellon Univ., Pittsburgh, PA; ³Dept. of NanoBiophotonics, Max-Planck-Institute for Biophysical Chem., Göttingen, Germany

Abstract: Layer 5 (L5) pyramidal (Pyr) neurons in the neocortex are targeted for synaptic changes during learning, but the way these synaptic alterations are distributed across the somatodendritic arbor has not been well-described. This is an important question, as the proximal and apical dendrites have different computational properties and synaptic plasticity within each region may have markedly different effects on firing activity. Here we use fluorescence-based reagents for pre- and postsynaptic labeling to monitor changes in thalamocortical synapses onto the proximal and apical dendrites of L5 Pyr neurons. Experiments were carried out in somatosensory (barrel) cortex from male and female mice after training in a sensory association task. Using axonal fills and molecular-genetic tags for post-synapse identification in Rbp4-Cre transgenic mice, we found that thalamocortical synapses from the higher-order P_{OM} thalamus onto L5 Pyr showed rapid morphological changes in both pre- and postsynaptic structures at the earliest stages of sensory association training. Increases in thalamocortical synaptic size were compartment-specific, occurring selectively in the proximal dendrites of L5 Pyr and not at inputs onto their apical tufts in layer 1 (L1). Both axonal and dendritic changes were transient, normalizing back to baseline as animals became expert in the task. Anatomical measurements were corroborated by electrophysiological recordings at different stages of training. Thus, fluorescence-based analysis of cell-type specific synapses can reveal compartment-specific plasticity that will constrain models for the role of dendritic function during learning.

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Poster

466. Plasticity in the Somatosensory System

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 466.13

Topic: D.03. Somatosensation – Touch

Support: BBSRC Grant 517267

Title: Interactions between primary and secondary somatosensory cortex

Authors: *H. WYSZYNSKA, S. KANG;
Cardiff Univ., Cardiff, United Kingdom

Abstract: The rodent primary (S1) and secondary somatosensory cortices (S2) are known to play a role in processing whisker sensory information. S1 and S2 are reciprocally connected, suggesting that information is likely processed through an interaction between these structures. However, this interaction is not well understood. In order to characterize how S1 and S2 interact, we inhibited the whisker receptive area of either S1 (barrel cortex) or S2 while recording from both structures simultaneously using a Neuropixel 1.0 probe. Inhibition was achieved by expressing DREADDs (hM3Dq) in inhibitory interneurons and administering CNO (3.5mg/kg) i.p. during recordings in anaesthetised mice. As expected, DREADD activation dramatically reduced both whisker responses and spontaneous firing rates within the region expressing the construct. Network activity was reorganised such that some cells showed higher firing rates (putative inhibitory interneurons) while others exhibited lower firing rates or were silenced. In addition, we found that inhibition of S1 led to a reduction in network activity in S2, while inhibition of S2 had no effect on the average network firing rate in S1. This finding implies that S1 drives network activity in S2 but not vice versa. We also looked at whisker evoked activity in the two areas by choosing a whisker that evoked responses in both cortices. We found that S2 inhibition had little influence on the peak firing response of S1 cells following whisker stimulation. However, the timing of S1 whisker responses were disrupted within the network. Latency to whisker stimulation tended to increase in a subset of the S1 population following inhibition of S2. Similarly, latency to whisker stimulation tended to increase in S2 following inhibition of S1. While whisker responses were reduced in S2 following S1 inhibition, they were not abolished, indicating elements of parallel processing in S2, presumably from independent thalamic POM input. In conclusion, the data indicates that S1 firing modulates activity in S2 and vice versa. Inhibiting S1 led to a decrease in S2 firing rates on average, consistent with S2 receiving a strong excitatory input from S1. In contrast, S2 blockade did not affect overall excitability in S1 but rather led to a reorganization of firing rates and temporal firing associations within that structure. HW was supported by BBSRC studentship.

Disclosures: H. Wyszynska: None. S. Kang: None.

Poster

466. Plasticity in the Somatosensory System

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 466.14

Topic: D.03. Somatosensation – Touch

Support: BBSRC Grant BB/T007028/1
MRC Grant MR/W004844/1

Title: Role of primary and secondary somatosensory cortices in texture discrimination learning

Authors: *A. PANDEY, Z. MASSERI, R. HONEY, K. FOX;
Sch. of Biosci., Cardiff university, Cardiff, United Kingdom

Abstract: Rodents learn about their environment using their whiskers and can learn to perform simple whisker dependent texture discriminations (Pacchiarini et al, 2019). Whiskers are represented in the barrel cortex of the primary somatosensory cortex (S1), which is reciprocally connected to the secondary somatosensory cortex (S2). S1 and S2 neurons respond to whisker stimulation whilst sensing textures as demonstrated in head fixed mice. However, the role of S1 and S2 in learning a texture discrimination has not been studied in freely moving mice. We asked whether S1 and S2 are required for texture discrimination during a natural foraging behavior. We trained mice to dig for a food reward buried in a sawdust filled bowl that could be identified from the texture on the surface of the bowl. A second bowl had a different texture and no reward. Floxed excitatory DREADDs (hM3D(Gq)) were expressed bilaterally in S1 or S2 of PV-Cre mice. In 3 cases we found an overlap of DREADD expression in S1 and S2 and these were excluded from further analysis. S1 or S2 were silenced by increasing the activity of PV positive interneurons by IP injection of CNO (Clozapine-N-oxide, 3.5mg/kg) 30 minutes before the task began. Mice were tested on 3 consecutive days. Inactivation of S1 rendered mice unable to discriminate between grooved and smooth textures. An ANOVA showed that the control and experimental groups were highly significantly different ($F_{(3,19)} = 6.904$, $p < 0.005$). Inactivation of S2 also compromised the animal's ability to discriminate between two textures. An ANOVA for saline and CNO injected groups showed an effect of S2 inactivation ($F_{(5,53)} = 5.07$, $P < 0.001$). Inhibition of S2 did not affect learning a similar task with an odour as the discrimination factor, suggesting that the effect in S2 is modality specific. Therefore, we conclude that both, S1 and S2 are required for learning to discriminate between textures and impairment to either one prevents texture learning. Supported by BBSRC BB/T007028/1 and MRC MR/W004844/1. Pacchiarini, N., Berkeley, R., Fox, K., & Honey, R. C. (2020). Whisker-mediated texture discrimination learning in freely moving mice. *Journal of Experimental Psychology: Animal Learning and Cognition*, 46(1), 40-46. <https://doi.org/10.1037/xan0000212>

Disclosures: A. Pandey: None. Z. Masseri: None. R. Honey: None. K. Fox: None.

Poster

466. Plasticity in the Somatosensory System

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 466.15

Topic: D.03. Somatosensation – Touch

Support: BBSRC BB/T007028/1
MRC MR/W004844/1

Title: Layer 2/3 spine dynamics in barrel cortex when learning a texture discrimination

Authors: A. PANDEY, N. PACCHIARINI, A. GREWAL, A. GRIFFITHS, E. HARDCASTLE, I. HEALY-MILLET, R. HONEY, ***K. D. FOX**;
Cardiff Univ., Cardiff Univ., Cardiff, United Kingdom

Abstract: Mice can learn to discriminate between two textured surfaces using their whiskers (Pacchiarini et al. 2020), but cannot learn this discrimination if either S1 or S2 are inhibited bilaterally (Pandey et al. this poster session). But does learning cause plasticity in S1 cortex or does the plasticity take place elsewhere? To answer this question, we trained freely moving mice on a texture discrimination task having previously implanted them with cranial windows positioned over the barrel cortex. Mice chose between two textures before digging for a food reward. We viewed the behavior of dendritic spines located on layer 2/3 pyramidal cells expressing GFP at time intervals before, during and after learning. Previous studies had shown that sensory deprivation affects basal but not apical dendrites on layer 2/3 pyramidal cells (Seaton et al. 2020) and so we imaged both dendritic locations. We found that spines showed plasticity on basal but not apical dendrites during the learning phase of the texture discrimination task (Basal: $F(2,41)=22.72$, $p=0.0001$; Apical: $F(2,23)=1.38$, $p=0.27$). Mice undergoing the same behavioral procedure, but without the need to discriminate to obtain the reward, did not show spine plasticity ($F(2,23)=0.15$, $p=0.86$). Mice undergoing an odour-based discrimination rather than texture, similarly did not show spine plasticity in barrel cortex ($F(2,17)=1.46$, $p=0.26$). We conclude that spine plasticity in primary somatosensory cortex is produced when a rewarded discrimination is learned and that this plasticity is modality specific and restricted to the basal dendrites. Supported by BBSRC BB/T007028/1 and MRC MR/W004844/1

Pacchiarini, N., Berkeley, R., Fox, K., & Honey, R. C. (2020). Whisker-mediated texture discrimination learning in freely moving mice. *Journal of Experimental Psychology: Animal Learning and Cognition*, 46(1), 40-46. <https://doi.org/10.1037/xan0000212>

Seaton, G., Hodges, G., de Haan, A., Grewal, A., Pandey, A., Kasai, H. & Fox, K. (2020) Dual-Component Structural Plasticity Mediated by α CaMKII Autophosphorylation on Basal Dendrites of Cortical Layer 2/3 Neurons. *Journal of Neuroscience* 40 (11) 2228-2245; <https://doi.org/10.1523/JNEUROSCI.2297-19.2020>

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Poster

466. Plasticity in the Somatosensory System

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 466.16

Topic: D.03. Somatosensation – Touch

Support: Eunice Kennedy Schriver National Institute of Child Health & Human Development, NIH Grant R01HD094588

Title: Rapid and delayed forelimb to lower jaw remapping in rat forepaw barrel subfield (FBS) in primary somatosensory cortex (SI) following forelimb deafferentation

Authors: *V. PELLICER MORATA¹, L. WANG², A. DE JONGH CURRY³, J. W. TSAO⁴, R. S. WATERS¹;

¹Anat. and Neurobio., Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN; ²Neurol., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; ³Biomed. Engin., Univ. of Memphis, Memphis, TN; ⁴Neurol., NYU Langone Med. Ctr., NYC, NY

Abstract: Introduction: Stroking the lower jaw skin surface of individuals with upper limb amputation often elicits phantom limb sensation (PLS) and/or phantom limb pain (PLP) in the missing limb. One explanation is the deafferented limb somatosensory cortex (SI) becomes responsive to previously unexpressed input from the adjacent lower jaw representation; a phenomenon called cortical reorganization. Here we report rapid cortical reorganization in the forepaw barrel subfield (FBS) in primary somatosensory cortex (SI) following forelimb amputation (AMPr), brachial plexus nerve cut (BPnc), and brachial plexus anesthesia (BPA), [deafferentations often observed in the clinic], and delayed reorganization following forelimb amputation (AMPd). In parallel studies, we present evidence that the newly expressed lower jaw input for rapid cortical reorganization derives from the neighboring lower jaw barrel subfield (LJBSF) in SI, and that the pathway is masked by GABAergic inhibition.

Methods: Anesthetized Sprague-Dawley rats, underwent forelimb amputation, BPnc or BPA, and craniotomy was performed to reveal the underlying brain surface. A carbon fiber electrode was inserted in SI (layer IV) to record receptive fields of forelimb/lower jaw neurons following mechanical/electrical stimulation; latency measures were obtained using electrical stimulation applied to the lower jaw and forelimb skin surfaces. In AMPr, BPnc, BPA rats, mapping occurred immediately following deafferentation; in AMPd rats, mapping occurred 6-27 wks after amputation. Lesions were placed at selected sites in SI to identify electrode recording sites.

Results: 1) Neurons in FBS respond exclusively to input from forepaw in forelimb intact rats, 2) Following each type of deafferentation, newly expressed lower jaw input appeared immediately in the anterior FBS, previously occupied by input from digits one (D1), D2, and thenar pad (TH), 3) evoked response latencies for the new lower jaw input were significantly delayed compared to evoked response latencies observed in intact rats following forepaw stimulation, and 4) in delayed forelimb amputees (AMPd), the resulting lower jaw reorganization in SI occupied the entire FBS.

Conclusions: Differential mechanisms underlie rapid and delayed lower jaw reorganization in rat FBS following forelimb deafferentation that may involve both sources (cortical/subcortical) of the newly expressed input and mode of reorganization. While this study does not directly address

PLP and PLS, the findings are relevant for elucidating mechanisms underlying cortical reorganization which is one etiological-theory of phantom limb phenomenon.

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Poster

466. Plasticity in the Somatosensory System

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 466.17

Topic: D.03. Somatosensation – Touch

Support: Eunice Kennedy Schriver National Institute of Child Health & Human Development, NIH Grant R01HD094588

Title: Lower jaw barrel subfield (LJBSF) in rat somatosensory cortex provides a source of lower jaw input in the anterior forepaw barrel subfield (FBS) immediately following forelimb deafferentation

Authors: L. WANG¹, V. PELLICER MORATA², A. DE JONGH CURRY³, J. W. TSAO⁴, *R. S. WATERS⁵;

¹Neurol., ²Anat. and Neurobio., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; ³Biomed. Engin., Univ. of Memphis, Memphis, TN; ⁴Neurol., NYU Langone Med. Ctr., NYC, NY; ⁵Anat. and Neurobio., Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Introduction: The forepaw barrel subfield (FBS) in layer IV of rat primary somatosensory cortex (SI) receives exclusive input from the contralateral forepaw and wrist. Following forelimb deafferentation (forelimb amputation, brachial plexus nerve cut, or brachial plexus anesthesia), FBS neurons respond immediately to “new input” from the lower jaw. The newly expressed input is restricted to the anterior part of the FBS previously occupied by the representation of digit one (D1), D2, and thenar pad (TH). However, in forelimb amputees examined 6-to-27 weeks after amputation, the newly expressed lower jaw input is distributed throughout the entire FBS. In this study, we provide anatomical evidence, in part, that the source of the newly expressed lower jaw is the neighboring lower jaw barrel subfield (LJBSF).

Methods: In anesthetized Sprague-Dawley rats, neuroanatomical anterograde (biodextran amine [BDA]) or retrograde (cholera toxin B subunit [CT-B]) tracers were injected into a physiologically identified site in the LJBSF or FBS, respectively. Tracers were injected in lower jaw or forelimb representations in intact rats (n=28) and forelimb amputees 6 or more weeks after amputation (n=14).

Results: Tracer injections in forelimb intact and forelimb amputated rats confirmed a direct projection from LJBSF to anterior FBS, as well as projections to secondary somatosensory cortex (SII), and motor cortex (MI). No evidence was found supporting a LJBSF projection to posterior FBS. When CT-B was injected in posterior FBS, in both forelimb intact and amputees,

labeled cell bodies were found subcortically only in the ventral posterior lateral (VPL) nucleus serving the forelimb, but not in the ventral posterior medial (VPM) nucleus serving the lower jaw. Interestingly, posterior injections also labeled cell bodies in SII.

Conclusions: a) No differences in pattern of labeling were found for injections made in forelimb intact and forelimb amputees, b) LJBSF provides a source of the immediately expressed “new” lower jaw input in the anterior FBS, c) LJBSF or thalamic nuclei, VPM, provide an unlikely source of new lower jaw input to posterior FBS, and 4) we cannot rule out that an indirect projection from LJBSF to SII may provide lower jaw input to the posterior FBS.

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Poster

466. Plasticity in the Somatosensory System

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Program #/Poster #: 466.18

Topic: D.03. Somatosensation – Touch

Support: Eunice Kennedy Schriver National Institute of Child Health & Human Development, NIH Grant R01HD094588

Title: Removal of GABAergic inhibition unmasks input from lower jaw barrel subfield (LJBSF) to forepaw barrel subfield (FBS) in rat primary somatosensory cortex (SI)

Authors: ***A. DE JONGH CURRY**¹, **L. WANG**², **J. W. TSAO**³, **R. S. WATERS**⁴;
¹Biomed. Engin., Univ. of Memphis, Memphis, TN; ²Neurol., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; ³Neurol., NYU Langone Med. Ctr., NYC, NY; ⁴Anat. & Neurobio., Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: **Introduction:** The rat forepaw barrel subfield (FBS) and lower jaw barrel subfield (LJBSF) in layer IV of primary somatosensory cortex (SI) are somatotopically organized and receive peripheral input from contralateral forepaw and lower jaw, respectively. The LJBSF projects to the anterior FBS. In forelimb intact rats, this corticocortical connection is not expressed. However, in forelimb deafferented rats, input from the lower jaw becomes immediately expressed in the FBS. In this study we provide functional evidence that the masking of lower jaw responses in forelimb intact rats is under endogenous GABAergic regulation.

Methods: In anesthetized Sprague-Dawley rats, a carbon-fiber electrode was inserted in the FBS to record electrical responses to mechanical stimulation applied to forepaw to produce a map of the FBS. Studies were carried out in forelimb intact rats. Upon completion of mapping, selected sites in the FBS were chosen to study the role of GABAergic inhibition. The single carbon fiber electrode was replaced by a micropipette attached to a carbon fiber electrode where the tip distances were less than 60 microns apart. The pipette was filled with the GABA_A blocker bicuculline methiodide (BMI). Spontaneous activity was recorded before, during, and after

iontophoresis of blocker. Mechanical and electrical stimulation of the lower jaw were used to test for the presence of lower jaw input in the FBS. Signal processing was accomplished with IGOR-Pro. Cytochrome oxidase staining was used to show the barrel subfields of SI to reconstruct the recording sites based on the electrical lesions placed at selected sites in FBS after recording.

Results: Iontophoresis of the GABA_A blocker, BMI: a) unmasked previously unexpressed lower jaw input in the anterior FBS, while maintaining input from forepaw digits and pads, b) reversibly altered spontaneous background firing from irregularly firing to burst firing and, c) enlarged forepaw receptive fields of all digits and pads.

Conclusion: GABA_A inhibition is responsible, in part, for masking lower jaw input in the FBS of forelimb intact rats and is a likely mechanism underlying cortical reorganization of the anterior FBS that follows forelimb deafferentation.

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Poster

466. Plasticity in the Somatosensory System

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Program #/Poster #: 466.19

Topic: D.03. Somatosensation – Touch

Support: K25 R23380
R01 R23400

Title: Neural-thread enabled longitudinal tracking of neurovascular coupling and brain plasticity after ischemic stroke

Authors: *H. RATHORE^{1,2}, F. HE⁴, J. ZHANG³, B. NOBLE³, R. YIN³, H. ZHU³, C. XIE^{3,2}, L. LUAN^{3,2};

¹Rice Neuroengineering Initiative, Houston, TX; ²Applied Physics, ³Rice Univ., Houston, TX;

⁴Shanghai Inst. of Optics and Fine Mechanics, Shanghai, China

Abstract: Neurovascular coupling(NVC), the close spatial and temporal relationship between neural activity and hemodynamics, plays a central role of regulating the cerebral blood flow and maintaining normal brain function. However, the quantitative relationship between changes in hemodynamics and neural activity, particularly the spatiotemporal variations within the evoked brain region, remains murky. More importantly, although a broad spectrum of neurological and cerebrovascular diseases are known to affect neurovascular coupling, the quantitative change of the coupling function and its dependence on the severity or the time course of the disorders remain largely unknown. Here, we integrate large-scale ultraflexible NeuralThreads (NET) with functional optical imaging for simultaneous mapping of laminar neural activity at multiple locations, cerebral blood flow, and oxygenation. This novel multimodal neural platform enables quantification and tracking of neurovascular coupling in a spatiotemporally resolved manner and

over chronic periods. Using mouse stroke models and single whisker stimulation paradigms, we explore the origin of spontaneous neuroplasticity in the brain following an ischemic stroke, targeted at a single barrel inside the Barrel cortex. Our current understanding of neuroplasticity involves the functional remapping of the peri-infarct regions to those brain regions that remain unaffected by the stroke, however the exact mechanism of the reorganization of neural tissue remains unknown. We attempt to answer this question by performing a longitudinal stroke study and extracting data from the diseased brain as it progresses through the acute, sub-acute as well as the recovery stages.

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Poster

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Topic: D.03. Somatosensation – Touch

Support: MSCA-IF-2018 ID-845685
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Paris Émergence(s) 2017

Title: Brain-wide functional and structural remodeling in the adult brain mapped at cellular resolution

Authors: *A. VIEITES PRADO¹, C. KIRST^{2,3}, C. ROUSSEAU⁴, C. NGUYEN⁴, S. SKRIABINE⁴, P. GASPAR¹, N. RENIER¹;

¹INSERM, Paris, France; ²Dept. of Anat., Kavli Inst. for Fundamental Neurosci., San Francisco, CA; ³Lawrence Berkeley Natl. Lab., Berkeley, CA; ⁴Paris Brain Inst., Paris, France

Abstract: Critical periods of plasticity of brain networks are time windows during which the development of networks strongly can adapt to environmental changes. Understanding the nature and regulation of brain plasticity beyond these periods is a daunting task. Indeed, structural and functional remodeling are expected to be more limited, and also there is no clear consensus on where in the brain those adaptations can occur. Using a mouse model of permanent sensory deprivation in the adulthood we studied functional and structural plasticity in the mature brain. Using whole-brain mapping of Fos and calcium imaging with fiber photometry, we measured dynamic adaptive changes during the first weeks following deprivation, leading to permanent changes in brain activity at whole-brain scale. We then reconstructed structural axonal markers at whole-brain scale to generate unbiased maps of changes in fiber densities at the micron scale following adult deprivation. This allowed us to identify axonal remodeling processes in the primary and secondary somatosensory areas of the cortex among others. Finally, to further characterize the plasticity of these areas, the connectivity

of the identified plastic regions was studied using viral tracers. **Our study highlights the spatiotemporal dynamics of adult networks beyond the critical periods of development. We found that levels of activity are chronically different after permanent sensory deprivation and that these changes can drive long-range axonal remodeling processes.**

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Poster

466. Plasticity in the Somatosensory System

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Program #/Poster #: 466.21

Topic: D.03. Somatosensation – Touch

Support: Wellcome Trust Seed Award in Science 215186/Z/19/Z

Title: Surgical repair of the major nerves of the hand in humans leads to significant changes in the spatial arrangement of digit responses in primary somatosensory cortex.

Authors: M. WEBER¹, F. MCGLONE², A. MARSHALL³, O. ONYEKWELU⁴, L. BOOTH⁵, R. TIMIRCAN¹, A. FINCH¹, S. WATT¹, E. JESUDASON⁵, V. LEES^{6,4}, *K. F. VALYEAR^{1,7}; ¹Bangor Univ., Bangor Univ., Bangor, United Kingdom; ²Liverpool John Moores Univ., Liverpool, United Kingdom; ³Univ. of Liverpool, Liverpool, United Kingdom; ⁴Manchester Univ. Fndn. Hosp. Trust, Manchester, United Kingdom; ⁵Betsi Cadwaladr Univ. Hlth. Board, Bangor, United Kingdom; ⁶Univ. of Manchester, Manchester, United Kingdom; ⁷Bangor Imaging Unit, Bangor, United Kingdom

Abstract: The primary somatosensory cortex is organised such that individual digits of the hand are represented separately, in a spatially ordered fashion. This topographical organisation is found to change in non-human primates after median nerve transection and repair (Wall et al. 1986). These changes in cortical topography may reflect changes in the periphery. As the regeneration of a cut nerve that has been surgically repaired is not topographically guided, sprouting fibres establish new connections, innervating end receptors at different locations relative to the pre-injury architecture. These peripheral changes may drive cortical reorganisation. Peripheral rewiring occurs after hand-nerve repair in humans, commonly observed as aberrant touch localisation within the projection territory of the repaired nerve, yet whether the spatial arrangement of digit-specific responses in cortex also changes remains unclear. Here, we use fMRI to characterise digit specific responses in the primary somatosensory cortex of 15 patients with median/ulnar nerve repairs and 18 controls with healthy hands. Vibrotactile stimulation was delivered separately to each digit, and digit-specific responses were identified using conventional contrast methods. Patients and controls also underwent detailed testing of touch localisation, and patients completed the Rosen test, a standardised measure of hand function after nerve injury. Our fMRI results confirm the predicted spatial arrangement of

digit responses within the primary somatosensory cortex of healthy controls. The thumb is represented laterally within the posterior bank of the central sulcus, followed by digits two through five represented progressively more medially. This canonical spatial order breaks down, however, in patients. The pattern of response overlap between pairs of digits departs significantly from that of healthy controls for the injured but not for the uninjured hand, and patients also show an overall increase in response overlap across all digits. Errors of touch localisation are increased for the patient's injured hand, and correlate with sensory Rosen scores. Touch localisation error, however, does not reliably correlate with changes in sensory cortex. Altogether, our results reveal changes in the somatotopic organisation of sensory cortex after hand-nerve injury and repair, consistent with predictions from animal models, yet whether these changes reflect reorganisation of the periphery remains unclear.

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Poster

466. Plasticity in the Somatosensory System

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Program #/Poster #: 466.22

Topic: D.03. Somatosensation – Touch

Support: CHRONOS Project
Swiss National Science Foundation through the National Centre of Competence in Research (NCCR) Robotics
The Bertarelli Foundation

Title: Wearable display to restore thermal sensation in upper-limb amputee patients

Authors: ***F. IBERITE**¹, **S. SHOKUR**^{2,1}, **J. MUHEIM**², **O. AKOUISSI**², **A. CLERC**², **A. FELOUZIS**², **M. KALFF**², **L. TESTA-MOULLET**², **M. PLOUMITSAKOU**⁴, **F. MOROSATO**⁵, **E. GRUPPIONI**⁵, **S. MICERA**^{1,3};

¹The Biorobotics Inst., Scuola Superiore Sant'Anna, Pisa, Italy; ²STI, ³Sti, EPFL, Genève, Switzerland; ⁴Lab. for Behavioral Neurol. & Imaging of Cognition, Dept. of Fundamental Neurosci., Univ. of Geneva, Geneva, Switzerland; ⁵Ctr. Protesi INAIL, Vigorso di Budrio (BO), Italy

Abstract: We aim at conveying temperature sensation into neuroprosthetics for upper limb amputee patients. Adding thermal sensation can improve users' experience with a prosthetic arm. Indeed, beyond the obvious advantage of informing if an object is cold, warm, or dangerously hot, enhancing existing prosthetics with temperature feedback has the potential to increase the embodiment of the prosthetic arm and create the basis for an affective touch. Moreover, temperature feedback is essential to distinguish contact with different types of material; for

example, the touch of a copper slab or piece of wood is associated with specific profiles of temperature changes at the fingertip. Here we present a Wearable Thermal Display (WTD), allowing us to convey real-time feedback on specific spots of the residual arm. The device comprises three components: an active thermal sensor (ATS), a control unit, and a thermal stimulator. The ATS consists of two platinum thin-film conductive tracks encapsulated into polyimide and placed on a PDMS support. One trace is used to measure the temperature through its change of resistivity when the other heats the sensor to the baseline temperature of the human skin. The Peltier module is mounted on the subject's arm, presenting the desired temperature. A central unit provides telemetry, using the temperature of the ATS as a setpoint for the thermal display. We characterized the ATS to ensure linearity in the temperature range of interest. Additionally, the design was optimized for accurate sensing, uniform temperature distribution, and low power consumption. We validated our WTD with healthy and amputee participants in three functional tasks. For these tasks, the ATS was placed on the fingertip of a prosthetic hand (not worn by the subject), and the Peltier module was in contact with the subjects' upper arm; participants were blindfolded throughout the experiments. (1) The subjects could distinguish between different temperatures (40 C°, 25 C°, 15C°) with an accuracy of close to 100%, with a delay of 1-2 [s]. (2) The WTD could be used to provide the sensation produced by the contact with three different materials (copper, glass, plexiglass), and (3) the sensation produced by the contact with wet/dry objects. Our thermal display proved to be a viable, non-invasive feedback device for delivering a wide spectrum of thermal and thermally related sensations, such as wetness and material discrimination. Additionally, the ATS opens to an improved experience for prosthesis users, as the warmth of the active element is matched with the baseline temperature of the skin.

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Poster

466. Plasticity in the Somatosensory System

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 466.23

Topic: D.03. Somatosensation – Touch

Support: JST ERATO Grant Number JPMJER1701 (Inami JIZAI Body Project)
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JSPS KAKENHI JP22H03946

Title: Neural correlates of independent sixth finger embodiment

Authors: *K. ARAI¹, Y. TAKAHARA², D. UEDA², M. FUKUNAGA³, G. GANESH⁴, Y. MIYAWAKI^{2,5};

¹Dept. of Advanced Interdisciplinary Studies, Univ. of Tokyo, Tokyo, Japan; ²The Univ. of Electro-Communications, Tokyo, Japan; ³Div. of Cerebral Integration, Natl. Inst. for Physiological Sci., Aichi, Japan; ⁴de Robotique et de Microélectronique de Montpellier (LIRMM), CNRS, Montpellier, France; ⁵Ctr. for Neurosci. and Biomed. Engineering, The Univ. of Electro-Communications, Tokyo, Japan

Abstract: The representation of one's self in the brain is plastic, and several studies over the past decades have shown that, given the right conditions, humans can readily embody artificial limbs into their body (Aymerich et al., 2016), leading to changes in neural activations in the brain (e.g., Ehrsson, 2012). However, embodiment of limbs has been invariably studied for substitute artificial limbs, controlled by the movements of an innate limb and limbs from which non-visual sensory feedback is provided on an existing limb. Our recent behavioral study examined for the first time whether humans can also embody an "independent" supernumerary limb, a sixth finger in our case, and showed that this was indeed possible (Umezawa et al., 2022). But how is this independent supernumerary limb represented in the brain? To answer this question, we used a 7T fMRI scanner and compared the brain activities of 16 healthy volunteers during a finger-tapping task (with their innate finger) before and after habituation to our sixth (robot) finger. In habituation tasks, participants worked in two conditions: (1) In the embodiment condition, participants had to repeatedly tap each of their six fingers (five innate and one robotic) according to visual cues. The sixth finger was operated on using the null space forearm muscle activation (similar to Umezawa et al. 2022), while feedback of the sixth finger movement was provided to the side of the palm using a sliding pin. (2) In the non-embodiment condition, the participants performed the same habituation, but the sixth finger moved randomly, and no haptic feedback of its movement was provided. Subjective evaluations using a standard embodiment questionnaire showed that the participants perceived a significantly higher sense of agency, body image, and sense of ownership towards our robotic sixth finger after the embodiment condition compared to the non-embodiment condition. Correspondingly we observed that brain activity changes were significantly higher for the embodiment condition compared to the non-embodiment condition in the primary somatosensory and motor cortex. The correlations between these results suggest that the embodiment may change the primitive representation of body parts in the sensorimotor cortex.

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Poster

466. Plasticity in the Somatosensory System

Location: SDCC Halls B-H

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Topic: D.03. Somatosensation – Touch

Support: ERC (715022, EmbodiedTech)

Title: Can the somatosensory system integrate a tactile model for an extra robotic body part?

Authors: *L. DOWDALL¹, G. DOMINIJANNI², M. MOLINA¹, D. CLODE³, T. R. MAKIN⁴;

¹Univ. Col. London, London, United Kingdom; ²Ctr. for Neuroprosthetics and Inst. of Bioengineering, École Polytechnique Fédérale De Lausanne (EPFL), Lausanne, Switzerland;

³Univ. of Cambridge, Cambridge, United Kingdom; ⁴Univ. of Cambridge, London, United Kingdom

Abstract: Augmentation technology is a rapidly expanding field, and with it there is growing interest in how such devices interface with the body. When learning to control augmentation devices, one important sensory input is the tactile feedback received from where the device is worn on the body, described as intrinsic touch. We asked whether the brain gathers information from intrinsic tactile inputs to construct an internal representation of the device. We particularly wanted to determine whether the brain integrates intrinsic touch inputs with the somatosensory inputs from the biological fingers.

To investigate changes in somatosensory functioning following training with a supernumerary robotic finger (the Third Thumb, Dani Clode Design) we utilised a spatiotemporal localisation task. Participants judged the temporal order of two consecutive trains of vibrations on their biological finger and the Third Thumb. We first determined how a single session of motor training with the Third Thumb impacts temporal order judgements. Participants (n=20) trained to collaborate with the Third Thumb using one pre-specified finger. Improvements in localisation ability were seen for the trained finger-pair, but not for an untrained finger-pair. This suggests integration of the intrinsic tactile feedback from the Third Thumb and somatosensory inputs from the trained finger had taken place. Changes in a measure of bias for the trained finger-pair also implies a recalibration of the tactile input, indicating training shifts the perceptual model of the Third Thumb. Next, to thoroughly explore the possibilities of altered sensory representation, we examined tactile temporal order judgements in two groups of participants (n=50 in total) before and after a week of altered finger-synchronisation motor training: either due to extended Third Thumb training, or training to play the piano. To monitor co-usage of the Third Thumb with the biological fingers, markerless tracking is being used. To further assess changes to inter-finger sensory representation, we are using fMRI to study the representational similarity patterns across the biological fingers and Third Thumb (via intrinsic touch) before and after training using a soft pneumatic actuator stimulation system. We predict there will be improved localisation ability (and representational similarity) in the Third Thumb training group between the Third Thumb and the biological fingers it collaborates with most frequently, as the brain has gained familiarity integrating these somatosensory inputs. This work will allow us to demonstrate the brain's ability to integrate an artificial limb into the biological body's sensory model.

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Poster

466. Plasticity in the Somatosensory System

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Program #/Poster #: 466.25

Topic: D.03. Somatosensation – Touch

Title: Explaining the exception by describing the rule: revisiting a historical study on the statistics of the deep winding within the central sulcus

Authors: *R. SCHWEIZER^{1,3}, A. M. MUELLEN^{2,1};

¹Functional Imaging Lab., ²Cognitive Neurosci. Lab., German Primate Ctr., Göttingen, Germany; ³Dept. of Cognitive Neurosci., Maastricht Univ., Maastricht, Netherlands

Abstract: Heschl (1877), the Austrian anatomist, described for 1087 brains not only the prevalence of a rare anatomical variation, the ‘bridged’ central sulcus (CS), but also obtained the heights of a ‘deep winding’ within the CS. Based on this distribution being skewed towards ‘deep windings’ with larger heights, he concluded that the rare ‘bridged’ CS is an extreme form of the profound ‘deep winding’. We replicated this study on 1112 T1-weighted magnetic resonance images (MRI) of the Human Connectome Project Young Adult (HCP-YA) dataset. Through visual inspection of surface reconstructions, 9 brains (0.8%) with a ‘bridged’ CS were identified, corresponding to Heschl’s report of 6 brains (0.6%). To determine the height of the “deep winding” within BrainVISA (Morphologist, 4.5.0) after cortical surface and sulci reconstruction, the CS was parametrized resulting in a CS mesh from which the CS depth $y(i)$ [mm] was determined for positions $x(i) \in [1:101]$ (medial $i=1$; lateral $i=101$), resulting in CS depth profiles from 2165 hemispheres. All depth profiles were smoothed to enable extraction of turning points. Stability was analyzed across datasets applying a two-dimensional kernel density estimation of depth and position for all maxima, minima, and the global minimum. The global minimum at $i \in [47:101]$ exhibiting a high intrinsic stability was designated as PPfpm-II, reflecting the lateral end of the ‘deep winding’, with the adjacent medial maximum extracted as PPfpm-I reflecting its peak height (extraction validity: 93.3%). Height was defined as the absolute depth difference of PPfpm-II minus PPfpm-I and normalized as percentage by total depth at PPfpm-II. The heights of 1983 hemispheres present as a normal-like distribution with a median of 18.4% ($\pm 0.3\%$ SE) relative height which - based on the average CS depth of -23 mm (± 1.8 mm SE) - results in an absolute height of the ‘deep winding’ at the median of ca. 3.8 mm. The height distribution is truncated at 0 mm height and exhibits a significant positive skew towards larger heights with a maximal value at 76%, i.e. a ‘deep winding’ with a height of ca. 17.5 mm. The comparison with Heschl’s dataset shows that both studies find height distributions with a skew towards extended ‘deep windings’. The broader height distribution of the HCP-YA dataset is supposedly due to the MRI based analyses. Despite the different datasets and analyses, a high concordance was found with regard to the prevalence of the ‘bridged’ CS, and the height distribution of the ‘deep winding’. These results refine and confirm Heschl’s conclusions with the ‘bridged’ CS being an extreme form of the ‘deep winding’ within the CS.

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Poster

467. Olfaction: Behavior and Perception II

Location: SDCC Halls B-H

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Topic: D.04. The Chemical Senses

Support: Francis Crick Institute: core funding from Cancer Research UK (FC001153), the UK Medical Research Council (FC001153), and the Wellcome Trust (FC001153)
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Title: Mice can extract information about odour source distance from temporally complex odour plumes

Authors: *A.-C. MARIN^{1,2}, T. ACKELS^{1,2}, J. J. HARRIS¹, D. DASGUPTA^{1,2}, A. ERSKINE^{1,2}, T. P. A. WARNER³, A. T. SCHAEFER^{1,2};

¹Francis Crick Inst., London, United Kingdom; ²Dept. of Neuroscience, Physiol. & Pharmacol., Univ. Col. London, London, United Kingdom; ³The Francis Crick Inst., London, United Kingdom

Abstract: Natural odour plumes are shaped by airflow turbulence, resulting in high frequency odour intensity fluctuations that contain information about odour source location. We aimed to investigate whether mice can extract and use this information in a distance discrimination task. We built a wind tunnel and odour delivery devices to reliably generate and record odour plumes. In addition, we created an “olfactory virtual reality” by replicating the recorded odour plumes with a high-temporal-bandwidth multichannel odour delivery device. In a high-throughput behavioural conditioning system, we trained mice to perform distance discrimination tasks, either in “olfactory virtual reality”, or directly using odour plumes generated in the wind tunnel. Using odour sources placed at different distances in the wind tunnel, we found that mice (n=24) learnt to respond differently to odour plumes based on source distance. Due to the difficulty of the task, only some mice (6/24) successfully learnt to perform the Go/No-Go task, as defined by consistent performance above 60%. However, all mice showed differential lick response patterns between rewarded and unrewarded trials, suggesting they can indeed discriminate between near and far plumes. In a separate cohort of mice (n=3 anaesthetised mice), we performed calcium imaging of neurons in the olfactory bulb while presenting distant odour plumes in olfactory virtual reality, and found that a subset of Mitral and Tufted cells (46/531 cells) responded differentially to different distances. As total odour concentration was kept constant, we suggest these neurons respond to temporal features of odour plumes, which are informative of source distance. We conclude that mice are able to perform distance discrimination using olfactory information available in temporally complex odour plumes, and that the differential processing of this information can already be observed at the level of the olfactory bulb.

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Poster

467. Olfaction: Behavior and Perception II

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Program #/Poster #: 467.02

Topic: D.04. The Chemical Senses

Support: NSF/CIHR/DFG/FRQ/UKRI-MRC Next Generation Networks for Neuroscience Program

Title: Information-theoretic analysis of active bi-antennal sensing for olfactory navigation

Authors: *J. D. VICTOR¹, A. C. TRUE², J. P. CRIMALDI²;

¹Weill Cornell Med. Col., New York, NY; ²Univ. of Colorado, Boulder, CO

Abstract: Olfactory navigation is made challenging by the turbulent nature of natural plumes. To meet this challenge, organisms typically use pairs of sensors as well as active sensation, which shapes the spatiotemporal characteristics of the olfactory environment even before it is sensed. In this context, active sensation includes not only exploration of the environment by a locomoting organism, but also, behaviors such as antennal motion or sniffing. These behaviors can influence the region of space that is sampled even without locomotion and they can result in a local mixing of odor concentrations within this region. To explore the utility of these strategies— independent of the specific algorithm used for odor navigation—we combined an information-theoretic approach with measurements of the spatiotemporal characteristics of real plumes. The information-theoretic analysis determined the mutual information between odor concentrations at a pair of sensors, and relative location of the sensor pair to the plume source. Spatiotemporal odor distributions were obtained via planar laser-induced fluorescence measurements of real plumes. We analyzed four plumes with realistic advection speeds (5 to 20 cm/s), with and without a nearby boundary, and computed mutual information between location and samples of odor concentration at 49 sites within each plume. To analyze the benefit of using a pair of sensors, we compared the information about location obtained from the odor concentration at a pair of sensors to that obtained from a single sensor, and how this information depended on the resolution for concentration. Consistent with previous results, little information is gained by increasing the resolution for odor concentration beyond 3 to 4 bits (8 to 16 levels). At each level of resolution, two sensors yielded more information than one, with progressively more information gain as sensor separation increased. Information gain was maximized by coding strategies that encoded odor concentrations jointly, but with different strategies for different plume types: for more turbulent plumes, the optimal coding strategy signaled the presence of a high concentration at either sensor without regard to sensor identity; for more diffusive plumes, the optimal coding strategy signaled the signed difference between the odor concentration at the two sensors. Mixing the local environment prior to sampling generally

increased the amount of information, even for the more turbulent plumes. In sum, bi-antennal (or binaral) sampling has considerable advantages for odor navigation, and these advantages are maximized by coding strategies that depend on plume characteristics.

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Poster

467. Olfaction: Behavior and Perception II

Location: SDCC Halls B-H

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Program #/Poster #: 467.03

Topic: D.04. The Chemical Senses

Support: R01HD087509

Title: Developmental predator odor experience and its epigenetic effect on innate fear

Authors: ***N. J. COLLINS**¹, **S. E. ROSS**¹, **M. C. O'SHEA**², **O. K. BIGHAM**², **L. W. AYERS**³, **J. B. ROSEN**², **T. L. ROTH**²;

¹Univ. of Delaware Grad. Program In Behavioral Neurosci., ²Univ. of Delaware, Newark, DE;

³Widener Univ., Chester, PA

Abstract: While early life stress (ELS) in both humans and rodents may lead to later incidences of psychopathology, not everyone exposed to ELS goes on to develop adverse symptomology. Maternal buffering, or the presence and availability of a caregiver during an adverse event, has the propensity to alter both neurocircuit development and increase stress resiliency. Previous work in our laboratory demonstrated the capacity of predator odor exposure (TMT, a synthetic component of fox feces) to induce a robust freezing response in rodents compared to a noxious control odor BTA (butyric acid) or water. Moreover, if TMT is administered to pups during the first three weeks of life (PN1-21) in the presence of their dam, dams engage in longer bouts of nursing in the compared to BTA exposure. Further, when these pups were tested for freezing to TMT during adolescence (PN30), the pups developmentally exposed to TMT reduced their freezing response compared to developmentally exposed BTA animals (Ayers et al., 2015). The present study first seeks to replicate and expand on these findings, including examining sex differences in offspring outcomes and determining underlying epigenetic changes. Preliminary data suggests a sex-specific effects, such that females, but not males, exposed to TMT display the reduction in freezing previously reported. Finally, given that maternal programming is known to alter epigenetic markers in the developing brain, several candidate genes in the olfactory bulb, amygdala, medial prefrontal cortex, and periaqueductal grey were examined. Preliminary data suggests differences in gene expression with developmental TMT exposure in several candidate genes, suggesting a link between the early life environment and later behavioral phenotypes of the progeny.

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Poster

467. Olfaction: Behavior and Perception II

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Topic: D.04. The Chemical Senses

Support: NIH Grant DC015137
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Title: Structural Plasticity in the Olfactory Bulb: The Interplay between Synaptic Plasticity and Adult Neurogenesis in Learning and Memory

Authors: *B. SAKELARIS, H. RIECKE;
Northwestern Univ., Evanston, IL

Abstract: The olfactory bulb (OB) is one of the few areas in the mammalian brain that undergo substantial structural changes in their neural circuitry well into adulthood. These changes, which include the formation and removal of synapses as well as the neurogenesis and apoptosis of interneurons, are presumed to play a key role in the perceptual learning and forgetting of animals upon repeated odor exposure. In the OB, excitatory mitral cells (MCs) form an odor representation that is shaped in part by a large population of inhibitory granule cells (GCs). Learning occurs when this odor representation is altered through network changes, which are driven by the formation and removal of reciprocal MC-GC synapses and the birth and death of adult-born GCs (abGCs). The loss of memories on the other hand can be attributed to a combination of synaptic rewiring and the death of abGCs that helped encode learned odors. Here, we present a biophysically inspired computational model of this structural plasticity in the rodent OB to investigate synaptic integration of abGCs and their impact on learning and discrimination. In doing so, we illuminate factors that aid the integration of adult-born neurons into the OB and how the death of abGCs can facilitate learning. This model extends previous models (Adams et al. 2019, Meng & Riecke 2020) in several ways. First, reflecting the local and global dynamics of calcium in GCs, we model spinogenesis as a function of the local activity at the spine as well as the large-scale activity of the GC on which the spine is located. The local contribution allows different spines to undergo different processes even though they may be on the same dendrite. Furthermore, neurogenesis is modeled by continuously adding GCs to the network which are endowed with age-dependent parameters that reflect increased excitability and plasticity as reported for abGCs during their critical period. Finally, GC death is modeled as an activity-dependent process predominantly affecting immature cells. We show that our model explains key experimental results not captured by previous models, including how different waves of abGCs are responsible for encoding odors presented at different times and how the retention and loss of memories due to interfering stimuli depends on the interval between the presentations of the stimuli (Forest et al. 2019). We go on to show that enhanced plasticity and excitability are key factors which not only aid memory acquisition, but also retention. Lastly the model shows that the activity-dependent death of adult-born neurons reduces spurious signals

which may obscure a future stimulus, enabling the animal to learn new odors more easily while preserving old memories.

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Poster

467. Olfaction: Behavior and Perception II

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Topic: D.04. The Chemical Senses

Support: Hope College
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Sherman Fairchild Summer Research Stipend Award

Title: Olfactory functional loss and recovery following degeneration and regeneration of the lesioned olfactory bulb of zebrafish

Authors: ***S. L. DEWITT**¹, E. A. THOMAS¹, A. B. GRAY², E. CALVO-OCHOA¹;
¹Biol. and Neurosci. Program, Hope Col., Holland, MI; ²Biol., Kalamazoo Col., Kalamazoo, MI

Abstract: The olfactory system of zebrafish serves the sense of smell by perceiving chemical cues and mediating olfactory behavior and is renowned for its ability to adjust to morphological stress through various neuroplasticity mechanisms. Odor-driven behaviors such as feeding, kinship, and alarm are crucial for survival. Zebrafish are able to detect odorants through the stimulation of odor-specific olfactory sensory neurons (OSNs) in the olfactory epithelium (OE), which in turn activates the olfactory bulb (OB) for odor discrimination. Complete morphological recovery following direct damage to the olfactory epithelium of zebrafish has been well established. However, the effects of a brain lesion on olfactory function in zebrafish have yet to be studied. In this study, we investigated the olfactory response to odorants following an excitotoxic lesion in the olfactory bulb. We used adult zebrafish of both sexes and produced a unilateral focal excitotoxic lesion in the olfactory bulb by injecting quinolinic acid. Next, we assessed olfactory behavior following 1- and 21- days post-lesion (dpl). For this, we studied olfactory function by means of olfactory-mediated behavioral tasks in response to three groups of odorants with physiological relevance: alanine (food cues), taurocholic acid with urea (kinship cues), and cadaverine (alarm cues). These assays were recorded and analyzed using an animal tracking software. We collected and assessed the following behavioral parameters: speed, distance traveled, preference index, erratic swimming, and freezing time. To obtain a morphological correlate of olfactory system lesion and recovery, we assessed OE and OSN integrity by histological stains and immunohistochemistry. We found that control (non-lesioned) fish displayed expected behavioral responses to the odorants tested. On the other hand, lesioned fish (1 dpl) displayed a loss of olfactory function in response to all odorants concomitant with

extensive neurodegeneration of OSNs in the OE. In addition, we found that recovered fish (21 dpl) displayed a restored olfactory response to all odorants as well as complete morphological recovery of the OE. Our results are the first to characterize olfactory function loss associated with degeneration of the olfactory system caused by a lesion in the olfactory bulb. Furthermore, we show that both olfactory function and morphology are restored to control levels by 21 days. This research may give insight into recovery mechanisms in the mammalian brain and provide knowledge for future therapeutic solutions to olfactory loss due to traumatic brain injuries or neurodegenerative diseases in humans.

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Poster

467. Olfaction: Behavior and Perception II

Location: SDCC Halls B-H

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Program #/Poster #: 467.06

Topic: D.04. The Chemical Senses

Title: An optogenetic stimulation approach to quantify the contribution of individual glomeruli to olfactory percepts.

Authors: *W. G. BAST, C. AGHAMOHAMMADI, P. GUPTA, T. A. ENGEL, D. F. ALBEANU;

Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Can we predict how similar the smell of two odors will be if we know which odorant receptors (OR) are activated by each of them? This seemingly simple question remains unsolved as little is known about how the brain maps differences in odorant receptor activation patterns to distinct olfactory percepts. The difficulty in controlling odor stimuli at the level of individual receptor types makes it challenging to disentangle the contribution of specific ORs in shaping olfactory perception.

To overcome this limitation, we exploited the anatomical clustering of ORs to individual glomeruli that naturally occurs in the early olfactory system. We identified several dozens of glomeruli on the olfactory bulb surface of OMP-Cre x ReAChR mice and determined their responses to 123 chemically diverse monomolecular odorants. We created synthetic olfactory stimuli by optogenetically activating combinations of identified glomeruli using digital micro-mirror device (DMD)-based patterned photo-stimulation. To obtain a metric of perceptual distance between stimulus pairs, we asked expert mice engaged in a Go/No-Go task to report differences in stimulus identity between different photo-activated glomerular sets. In this manner, we aimed to quantify the contribution of OR identity and their functional properties to the perceptual similarity between the synthetic olfactory stimuli sampled.

Our preliminary results are consistent with previous proposals that the number of overlapping glomeruli between stimuli pairs explains in part their perceived similarity. However,

incorporating into our model the odor response tuning of the glomeruli that compose each synthetic olfactory stimulus reduced the discrepancy between the model predictions and the measured perceived similarities by more than 30% on average. Thus, in addition to the degree of glomerular overlap, odor tuning contributes to the perceptual similarity between stimulus pairs. A further extension of the model quantified the contributions of each glomerulus in a reference pattern. Our results suggest the existence of a perceptual glomerular hierarchy within each glomerular activation pattern: on average, some glomeruli were six times more potent than others in creating the specific odor percept elicited by the optogenetically-induced stimulus. In summary, our approach provides a framework to relate the perceptual similarity of olfactory stimuli to individual OR identity and their response features and allows to elucidate the logic of the downstream processing neural circuits in olfactory perception.

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Poster

467. Olfaction: Behavior and Perception II

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Program #/Poster #: 467.07

Topic: D.04. The Chemical Senses

Support: OIST corporation
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Title: Cell-type and context-specific perisomatic inhibition of olfactory bulb output

Authors: *S. LINDEMAN, I. FUKUNAGA;
Sensory and Behavioural Neurosci. Unit, Okinawa Inst. of Sci. and Technol., Onna, Japan

Abstract: Sensory representations in the olfactory bulb are dynamic and dependent on brain state and behavioural context. Previous studies report that responses of mitral cells (MCs) and tufted cells (TCs), the main output neurons of the olfactory bulb, show differences in the way they are modulated, but exactly how this is achieved is not resolved. Local inhibitory interneurons receive cortical top-down feedback and neuromodulatory signals. They are therefore candidate mediators for the cell-type specific modulation. First, we aimed to localise the source of modulation in MCs and TCs. We used two-photon calcium imaging to image from the apical dendrites and somata of MCs and TCs in mice performing a fine odour discrimination task. This revealed that odours evoked considerably divergent responses based on reward contingency, predominantly at the level of MC somata, and not in TC somata, nor in their apical dendrites. In particular, the MC somatic responses were dominated by inhibition for the rewarded odour (evoked amplitude = -0.045 ± 0.061 and -0.021 ± 0.054 , rewarded versus unrewarded odour resp., mean \pm s.d., $p < 0.001$, 1-way ANOVA, $n = 450$ MCs, 8 mice). Due to the perisomatic specificity of this phenomenon, we focused on adult-

born granule cells (abGCs), which are known to be important for fine odour discrimination, distinguishing superficial vs. deep gemmules based on imaging depth. We found that responses to rewarded odours were particularly enhanced in deep gemmules, indicating their potential role in the inhibitory modulation observed in MC somata.

Further, in investigating the source of the differential perisomatic inhibition on MCs, we found that the dominance of inhibitory responses on the rewarded odours in MC somata is absent in disengaged mice (evoked amplitude = -0.014 ± 0.050 and -0.009 ± 0.057 , rewarded versus unrewarded odour resp., mean \pm s.d., $p = 0.4$, 1-way ANOVA, $n = 147$ MCs, 3 mice). As cortical top-down feedback is hypothesized to provide behaviourally relevant contextual information to the olfactory bulb, we studied its involvement by pharmacologically silencing the anterior piriform cortex in engaged mice. This resulted in a reduction of perisomatic inhibition of MC activity (evoked amplitude \pm s.d. = 0.001 ± 0.058 and -0.008 ± 0.073 , rewarded versus unrewarded odour resp., mean \pm s.d., $p = 0.217$, 1-way ANOVA, $n = 178$ MCs, 4 mice), as well as dampening of evoked responses in abGC gemmules.

Together, our data suggest that the cell-type specific modulation of olfactory bulb output neurons in a behaviourally relevant context may involve depth-specific abGC dendrites, as well as contextual input onto them.

Disclosures: S. Lindeman: None. I. Fukunaga: None.

Poster

467. Olfaction: Behavior and Perception II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 467.08

Topic: D.04. The Chemical Senses

Support: NIH R01 MH101293

Title: Changes in olfactory bulb GABA(B)receptor signaling induced by fear learning

Authors: *A. BAKIR, J. P. MCGANN;
Rutgers Univ. Dept. of Psychology, Piscataway, NJ

Abstract: Olfactory-cued fear conditioning induces plasticity throughout the brain's olfactory system. Learning-induced, stimulus-specific plasticity has been observed as early as the olfactory sensory neurons (OSNs), which exhibit greatly enhanced odor-evoked synaptic output following olfactory fear conditioning. One candidate mechanism for this enhancement is the GABA(B) receptor, which presynaptically modulates glutamate release from the OSNs and whose expression is downregulated in OSNs following conditioning (Bhattarai et al. 2020). To evaluate the role of GABA(B) receptors in olfactory fear learning on olfactory bulb circuits, we used in vivo calcium imaging to observe the effect of local GABA(B) receptor blockade on population-level calcium dynamics in OSNs, periglomerular cells, and mitral cells from anesthetized mice that had undergone odor-cued conditioning or control exposures. In mice, in the conditioning

control groups, blockade of GABA(B) receptors increased the odor-evoked response amplitudes in all three cell types and comparably for all odors tested, reflecting a global increase in odor-evoked neurotransmitter release from the OSNs and activity in downstream neurons. However, in the animals that received odor-shock fear training, GABA(B) receptor blockade caused a significantly smaller increase in the neural responses evoked by the shock-predictive odor than for control odors in all three populations of neurons. GABA(B) receptor blockade also induced changes in the kinetics of the bulbar response over the course of a six second odor presentation. In mitral cell populations, the effect of the GABA(B) blockade became larger across consecutive inhalations, but in mice who had received odor-cured fear conditioning the effect was even more skewed toward the end of the odor presentation. These data support the idea that changes in GABA(B) receptor-mediated inhibitory signaling play an important role in associative learning throughout the olfactory glomerular circuit.

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Poster

467. Olfaction: Behavior and Perception II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 467.09

Topic: D.04. The Chemical Senses

Title: Understanding the neuronal substrates of sensorimotor predictions via a novel closed-loop olfactory task (*Smellocator*) for mice

Authors: *M. DUSSAUZE, P. GUPTA, U. LIVNEH, D. F. ALBEANU;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: *We all know the startling feeling when sitting on a seat that is just an inch lower than usual!* This brief mismatch reveals a fundamental process that occupies our brain: *predicting the consequences of our actions and verifying whether reality matches our expectations.* Growing evidence suggests that sensory perception is better described as this continuous tally of actual inputs and predictions, than solely as a process of stimulus feature representation. To date, however, little is known about the mechanisms that facilitate the evaluation of sensory inputs against internally generated predictions. To study sensorimotor predictions both at behavioral and circuit-level, we developed a novel closed-loop behavioral task (*Smellocator*), wherein head-fixed mice learn to steer a lever to control the lateral location of an odor source. To obtain rewards, mice move the lever so as to bring the odor source, initialized in different trials at different starting locations, in front of their nose. Mice thus learn to link their motor action to well-defined sensory expectations (odor location). *To read-out the learnt sensorimotor predictions in expert mice,* we violate the learnt expectations by decoupling the stimulus from the current action and creating brief sensorimotor errors. Additionally, across longer timescales, we invert the sensorimotor mapping (direction of odor movement), so as to engage sensorimotor adaptation. Strikingly, we find that *expert mice readily counter these sensorimotor errors and*

display precise corrective movements which provide a behavioral read-out of their individual specific, learnt sensorimotor expectations. In other sensory modalities (vision, audition), sensorimotor error-signals have been observed in the primary sensory cortex. We tested whether similar circuit motifs underlie the error computation and concomitant corrective behaviors during closed-loop olfaction. Using chronically implanted tetrode drives, we monitored neural activity in the olfactory cortex (Anterior Olfactory Nucleus, Piriform Cortex) during behavior. We find that olfactory cortex neurons show *odor-driven responses that are strongly re-shaped by movement related expectations* about the stimulus arrival. Further, transient perturbations often drive olfactory cortex neurons stronger than any other variable in our task including odor onset, target entry and rewards. Using a cross-area comparative approach and neuronal ensemble analysis, we are assessing whether olfactory cortical activity better represents the degree of mismatch between sensory inputs and sensorimotor expectation, or sensory inputs *per se*.

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Poster

467. Olfaction: Behavior and Perception II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 467.10

Topic: D.04. The Chemical Senses

Support: Simons Society of Fellows

Title: Intergenerational inheritance of olfactory experience: fear-conditioning influences immature cell receptor choice

Authors: *Y. AYMAN¹, C. LIFF², E. JAEGER¹, H. LEE⁴, A. KIM³, R. AXEL¹, B. J. MARLIN⁵;

²Zuckerman Inst., ¹Columbia Univ., New York, NY; ³Columbia Univ., New York, NY; ⁴Weill Cornell, New York, NY; ⁵Columbia Univ. Zuckerman Inst., New York, NY

Abstract: In mammals, there is mounting evidence that stress-induced epigenetic changes can alter neural structures across generations. This process, known as intergenerational epigenetic inheritance, affords offspring the ability to adapt to an ever-changing environment. The precise mechanism behind epigenetic regulation in the olfactory epithelium is unknown although morphological changes in response to fear conditioning have been observed. Using iDISCO brain clearing and lightsheet microscopy, we have shown that after a behaviorally neutral odor is paired with a shock, a ~35% increase in the number of specific olfactory sensory neurons (OSNs) is observed in the parental generation (F0) and their offspring (F1) (paired vs. unpaired p<0.0001). Here we provide evidence for the mechanism underlying these epigenetic changes by answering two key questions. Firstly, does fear conditioning extend the lifespan or increase proliferation of the specific OSNs that respond to the conditioned odor. Secondly, is the activation of the mature OSNs necessary for the epigenetic phenotype in F0 and F1. We use 5-

ethynyl-20-deoxyuridine (EdU) to birth date OSNs at single cell resolution in the olfactory epithelium and find that odor shock pairing increases the number of newly born OSNs that respond to the conditioned odor (paired vs unpaired $p < 0.0001$). Moreover, we use DREADDS (hM4Di) to silence the OSNs in vivo and find that the activation of the mature OSNs is necessary for the subsequent increase in newly born cells, as the CNO injected group expressed fewer EdU positive hM4Di receptor expressing cells (CNO vs. saline control $p < 0.05$). Our data indicate a potential mechanism for how environmental signals are conveyed to somatic cells in sensory systems, before being transmitted to germ cells and subsequently to future generations.

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Poster

467. Olfaction: Behavior and Perception II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 467.11

Topic: D.04. The Chemical Senses

Support: NIDCD R01DC019135
GAANN Fellowship
UCR Start-Up Fund

Title: Imminence of Predator Threat Detected by the Accessory Olfactory System

Authors: *Q. T. NGUYEN, A. ROCHA, Y. YAMASHITA, C. STADLER, S. HAGA-YAMANAKA;
Molecular, Cell and Systems Biol., Univ. of California Riverside, Riverside, CA

Abstract: Defensive behaviors in the presence of predator cues are typical innate behaviors in animals. Although predator signals are best detected with the summation of various sensory modalities in nature, olfactory-specific exposure to a predator specimen is sufficient to yield defensive responses in nocturnal prey animals such as mice. Animals display fixed patterns of defensive behaviors such as freezing, flight, and risk assessment in response to olfactory predator cues. Interestingly, olfactory cues from a single predator species can evoke a range of defensive behaviors with different intensities, and conversely, different predator cues can elicit one specific behavioral response. The underlying molecular and neural mechanisms for such behavioral decisions are still not well understood. In this study, we investigated a shift of defensive behavioral responses in mice toward olfactory predator cues collected from the same cat individuals. In our behavioral tests, we observed robust freezing responses to cat odors that were freshly collected, while the freezing response was reduced toward older samples. These behavioral outputs were observed only when mice had direct contact with the cat odor and abolished in mice lacking functional Trpc2. Taken together, these results suggest that fresh cat odors contain temporal cues that signal imminence of predator threat to mice, and the signal is

detected through the accessory olfactory system. Our study may shed light on the molecular and neural mechanisms underlying the defensive behavioral decision making in prey animals.

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Poster

467. Olfaction: Behavior and Perception II

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Program #/Poster #: 467.12

Topic: D.04. The Chemical Senses

Support: TJ Park Science Fellowship

Title: Exoribonuclease ERI-1 regulates *ascr#3*-mediated avoidance behavior in *C. elegans*

Authors: *H. HWANG, Y. CHEON, S. JO, S. OH, K. KIM;
Dept. of Brain Sci., Daegu Gyeongbuk Inst. of Sci. and Technol., Daegu, Korea, Republic of

Abstract: Small RNAs are short, non-coding RNAs that regulate gene expression. Recent studies suggested that the small RNAs modulate animal behavior, but the molecular and neuronal mechanisms by which small RNAs regulate animal behavior are still unclear. *C. elegans* secretes a pheromone mixture called ascarosides. A pheromone component, *ascr#3*(*asc-ΔC9*, C9), has been shown to elicit avoidance behavior in wild-type hermaphrodites. Here, we investigate the roles of small RNAs in *ascr#3* avoidance. We screened candidate mutants of which gene products are known to regulate small RNA pathways and found that four alleles of *eri-1* mutants exhibit defects in *ascr#3* avoidance. *eri-1* encodes 3'-5' exoribonuclease and is shown to mediate exogenous and endogenous RNAi pathways. We found that *eri-1* is expressed in a few neurons including the AVH interneurons and the PVP interneurons. The expression of ERI-1 in AVH rescued defects in *eri-1* mutants and overexpression of ERI-1 in AVH of wild-type animals increased *ascr#3* avoidance. Moreover, genetic ablation of AVH decreased *ascr#3* avoidance behavior, suggesting that *eri-1* in AVH mediates *ascr#3* avoidance. Next, we performed *in vivo* calcium imaging and found that *ascr#3*-evoked calcium transient in ADL-pheromone sensing neurons was abolished in the *eri-1* mutants. We searched putative targets of *eri-1* and found that an FMRFamide-like gene *flp-26* is highly expressed in AVH, and *flp-26* deletion mutants exhibit defects in *ascr#3* avoidance. We are currently investigating the precise mechanisms of how AVH regulates ADL in *ascr#3* avoidance. Interestingly, we found a correlation between *eri-1* expression level in AVH and *ascr#3* avoidance; animals showing high *eri-1* expression exhibit increased *ascr#3* avoidance and vice-versa. Moreover, both *eri-1* expression levels in AVH and *ascr#3* avoidance were increased with maternal age. These results demonstrate that exoribonuclease *eri-1* regulates *ascr#3* avoidance at the circuit level, which provides an opportunity to study the roles of small RNAs in the plasticity of animal behavior.

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Poster

467. Olfaction: Behavior and Perception II

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Topic: D.04. The Chemical Senses

Support: NIH Grant R01DC015784 [JPM]
NIH Grant R01DC017985 [JPM]

Title: A layered, hybrid machine learning analytic workflow for mouse risk assessment behavior

Authors: *J. WANG¹, P. KARBASI², L. WANG², J. MEEKS¹;

¹Dept. of Neurosci., Univ. of Rochester Sch. of Med. and Dent., Rochester, NY; ²BioHPC, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Accurate and efficient quantification of animal behavior has multiple benefits in the quest to understanding the workings of the brain. An emerging approach within the rapidly developing machine learning (ML) field is to combine multiple ML-based algorithms to support automated quantification of animal behavior from video recordings. These so-called hybrid models have emerged because of limitations associated with supervised (e.g., random forest, RF) and unsupervised (e.g., hidden Markov model, HMM) ML classifiers. For example, RF models lack an explicit accounting for temporal relationships across video frames, and HMM latent states are often difficult to interpret. We set out to develop a hybrid model that integrates aspects of RF and HMM models, and did so in the context of a study of threat assessment to predatory odors (e.g., reptile feces or fecal extracts, trimethylthiazoline). We utilized DeepLabCut to estimate the positions of mouse body parts. Positional features were calculated using DeepLabCut outputs and were used to train RF and HMM models with equal number of states, separately. The per-frame predictions from RF and HMM models were then passed to a second HMM model layer (“reHMM”). The outputs of the reHMM layer showed improved interpretability over the initial HMM output, and improved the capacity to analyze temporal aspects of behavior. Finally, we combined predictions from RF and HMM models with selected positional features to train a third HMM model (“reHMM+”). This reHMM+ layered hybrid model unveiled distinctive temporal and high human-interoperable behavioral patterns that mice displayed in the presence of predator odors. For workflow application, we uncovered detailed and dynamic mouse behavioral patterns in the presence of fearful odor trimethylthiazoline and enhanced mouse risk assessment behavior induced by snake feces odor. We conclude that this layered, hybrid machine learning workflow represents a balanced approach for improving the depth and reliability of ML classifiers in chemosensory and other behavioral contexts.

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Poster

467. Olfaction: Behavior and Perception II

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Program #/Poster #: 467.14

Topic: D.04. The Chemical Senses

Support: NSF DBI2014217

Title: Role of Sex Hormone Signaling in the Development of an Innate Courtship Behavior in Mice

Authors: *S. MUKHOPADHYAY¹, K. PATEL¹, P. WU¹, J. LAM¹, L. T. STOWERS²;
¹The Scripps Res. Inst., San Diego, CA; ²Cell Biol., Scripps Res. Inst., La Jolla, CA

Abstract: Sex hormones modulate behaviors of all mammals towards a broad range of sensory stimuli in very stereotypic manners. Innate social behaviors such as mating, courtship, territorial aggression, and parenting are modulated by circulating levels of estrogens, androgens and progesterones. However, our understanding of how this modulation occurs at the level of underlying neural circuits is limited. To further our understanding of this critical control mechanism for social behaviors, we are utilizing a robust mouse courtship behavior, and manipulating global and focal sex hormone signaling. Adult male mice show a stereotypic courtship behavior when presented with female odor (FMU) cues. As part of this, they deposit urine marks (scent mark) throughout the environment, and produce ultrasonic vocalizations (USVs). To gain a detailed understanding of this behavioral development, we are profiling how scent marking develops over puberty, when adult-like sex hormone signaling is established. We find that the response of these animals towards the same olfactory stimulus (FMU) changes drastically between 5 to 7 weeks of age. Although different animals initiate scent marking at different ages, once an animal starts to display scent marking, they continue to do so through adulthood. To zero in on the specific role of sex hormone signaling in regulating this behavioral transition, we are investigating how sex hormone signaling in specific regions of the brain that express cognate receptors affect an animal's interaction with, and response to, a female odor cue. Sex hormone depletion (via castration) abolishes courtship behaviors. Prior literature shows that focal estrogen delivery into the Medial Preoptic Area and the Lateral Septum rescues this behavior in castrated mice. Our results show that delivering estrogen into the dorsal hippocampus of castrated male animals also effects a similar rescue. We are currently utilizing transgenic strategies to manipulate estrogen signaling in these regions to further investigate the region-specific roles of Estrogen signaling. We are also using posture analysis pipelines and detailed behavioral analyses to identify how the interaction with FMU changes through puberty and across different hormone manipulated states. Eventually, we aim to understand how hormonal signaling across a number of receptor expressing regions acts in a coordinated fashion to give rise to new, highly robust behavioral patterns towards very specific environmental stimuli.

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Poster

467. Olfaction: Behavior and Perception II

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Topic: D.04. The Chemical Senses

Support: NIH Grant R01 AA025024

Title: Analysis of volatile organic compounds in soiled bedding that contributes to olfactory modulation of nociception in CFA-treated C57BL/6 mice

Authors: *Y. ZHANG, A. E. RYABININ;
Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Rodents can develop nociceptive hypersensitivity after interacting with familiar conspecifics in painful conditions. Research from several laboratories showed that such social modulation of nociception does not require physical contact between rodents. Particularly, our laboratory has previously reported that mice exposed to the olfactory cues of soiled bedding from conspecifics in complete Freund's adjuvant (CFA)-induced inflammatory pain also developed nociceptive hypersensitivity, implicating the role of olfactory system in the process. This role was confirmed by our recent finding that temporary lesion in the main olfactory epithelium blocked the development of nociceptive hypersensitivity in mice exposed to the soiled bedding. To reveal the olfactory signal from CFA-treated mice that modulates the nociception in conspecifics, we analyzed and compared the volatile organic compounds (VOC) in bedding of CFA-treated mice (CFA-bedding) versus saline-treated control mice (SAL-bedding) in the present study. Male C57BL/6J mice were housed at three per cage in a ventilated room and were used as stimulus mice, and their bedding as olfactory stimulus. After 3 days of habituation to newly changed bedding, bedding from stimulus mouse cages was collected in triplicates as pre-injection soiled samples, and clean bedding was also collected in triplicates as pre-injection background control samples. Then, every stimulus mouse received an intra-plantar injection of CFA or saline in one of their hind paws and was returned to their home cage. Forty-eight hours later, soiled and background control bedding samples were again collected in triplicates as post-injection samples. These bedding samples from multiple cohorts of experiments were analyzed with gas chromatography-mass spectrometry (GC-MS). Our analysis yielded approximately 200-300 VOCs from the bedding samples. With applied selection criteria, we identified 34 compounds only appearing in soiled bedding and another 51 compounds appearing in all soiled and clean samples. Further analyses and comparisons of chemical abundance between pre-injection and post-injection samples, as well as CFA-bedding and SAL-bedding revealed the difference in the volatile chemical profile of bedding samples that may collectively contribute to olfactory modulation of nociception. Built upon the findings from this study, future experiments will be designed to examine the nociceptive effect of these identified compounds individually and/or in combination, thus to further understand social modulation of pain in rodents via olfactory communication.

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Poster

467. Olfaction: Behavior and Perception II

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Topic: D.04. The Chemical Senses

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Title: How honey bees determine the meaning of an odor stimulus

Authors: N. KULKARNI, H. H. LEI, J. VALENCIA, B. SMITH, *H. LEI;
Arizona State Univ., Tempe, AZ

Abstract: Bees encounter numerous olfactory stimuli in nature during foraging trips. However, some stimuli prompt stronger responses than others as not all stimuli are of equal valence value. It is not well understood how the bees evaluate environmental stimuli. In insects, odor stimuli are first detected by olfactory receptor neurons on the antennae and then transmitted to and processed in the antennal lobe (AL). From AL, the information is sent to other higher olfactory centers for further processing. This research aims to compare the neural responses at the periphery (antennae) and in the AL to odors that are differentially associated with sugar reward or salt punishment. Electroantennogram and multichannel extracellular recordings were conducted to monitor the responses at antennae and in AL while the animal was undertaking associative learning tasks. Preliminary results reflecting the behavior of a bee with fixed antennae, showed an increase in the difference in neural response to two odors after having been associated with positive or negative stimuli. Electroantennogram results using fixed antennae, however, showed no difference in response to the two odors. These results suggest that the stimulus evaluation process begins at the AL. Antennae may play significant roles in receiving stimuli regardless of their valence values. Future research aims to extend this procedure to include free antennae to demonstrate differences, if any, in neural response between fixed and free antennae during stimulus evaluation. With further experimentation, this research can provide insight into the role of active sensing in animal behavior as a whole.

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Poster

467. Olfaction: Behavior and Perception II

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Topic: D.04. The Chemical Senses

Support: NIH Grant 1F31MH126622-01A1

Title: Rescue of Social Odor Discrimination in a Mouse Model of the 22q11.2 Deletion Syndrome

Authors: *S. K. BIGLER¹, S. HASSAN², S. A. SIEGELBAUM³;

¹Columbia Univ., Columbia Univ., NEW YORK, NY; ²Columbia Univ., Zuckerman Institute, Columbia Univ., New York, NY; ³Columbia Univ. Coll P & S, Columbia Univ. Postdoctoral Dept. of Neurosci., New York, NY

Abstract: Patients with Schizophrenia Disease (SZD) have difficulty recognizing familiar faces compared to their neurotypical counterparts, making SZD one of the many hallmark diseases in which social memory is impaired. A closer look at the CA2 region of the hippocampus in these patients reveals a marked decrease in the density of parvalbumin-expressing (PV) interneurons compared to controls. These two phenotypes—social memory deficits and lower PV density in CA2—are reflected in a mouse model of the human 22q11.2 Deletion Syndrome (22q11.2DS), a disorder strongly linked to SZD. This mouse model (hereon referred to as *Df(16)A*^{+/-} or “heterozygous” mice) also exhibits reduced feedforward inhibition and changes in intrinsic firing properties of CA2 pyramidal neurons, due in part to upregulation of TREK-1 potassium ion (K⁺) current, suggesting a link between altered CA2 activity and social memory deficits in 22q11.2DS. Interestingly, silencing CA2 in wild-type mice disrupts social memory formation while restoring function to pathologically hyperpolarized CA2 neurons of *Df(16)A*^{+/-} mice by administration of the TREK-1 antagonist spadin rescues their social memory deficit. Furthermore, we have confirmed that the population-level activity of CA2 pyramidal neurons contains sufficient information to distinguish social odors (urines) from distinct, novel conspecifics. We measured CA2’s ability to encode unique social odor stimuli in a head-fixed Go/No-Go Odor Discrimination Task with 2-photon calcium imaging. Water-restricted mice were trained to lick a water port in response to one social odor (GO odor) to receive a water reward and to withhold licking to another social odor (NOGO odor) with no punishment. Here we report a profound behavioral deficit in social odor discrimination and/or social odor-reward association in this task comparing *Df(16)A*^{+/-} mice to their neurotypical counterparts. The next step is to use 2-photon calcium imaging to track CA2 neurons’ abilities to encode and distinguish unique social odors in these heterozygous mice, and to see if CA2 population activity is impacted by TREK-1 blockade with spadin. Our experiments attempt to gain insights into the contribution of abnormal CA2 social coding to the social cognition impairments in this mouse model of the 22q11.2DS.

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Poster

467. Olfaction: Behavior and Perception II

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Topic: D.04. The Chemical Senses

Support: NIH Grant 5R01DC014487

Title: Cortical-bulbar feedback supports behavioral flexibility during rule-reversal

Authors: *D. HERNÁNDEZ TREJO¹, A. CIUPARU³, P. GARCIA DA SILVA⁴, M. C. VELÁSQUEZ⁵, B. REBOUILLAT⁶, M. B. DAVIS¹, M. GROSS², R. C. MUREȘAN³, D. F. ALBEANU²;

¹Neurosci., ²Cold Spring Harbor Lab., Cold Spring Harbor, NY; ³Transylvanian Inst. of Neurosci., Cluj-Napoca, Romania; ⁴Champalimaud Fndn., Lisbon, Portugal; ⁵Nuffield Dept. of Clin. Neurosciences, Oxford Univ., Oxford, United Kingdom; ⁶Dept. D'études Cognitives, École Normale Supérieure, Paris, France

Abstract: Animals must flexibly adjust their behavior to adapt to relevant changes in their environment. Mice excel at recognizing odorants in complex sensory conditions; however, little is known on: (1) how sudden changes in stimulus contingency modify odorant representations and (2) how changes in odorant representations causally relate to behavioral adjustments. The olfactory bulb (OB) relays odor triggered information to higher olfactory areas including the piriform cortex (PCx). The PCx receives input from association areas (e.g., orbitofrontal cortex) and sends abundant feedback that selectively regulate one of the OB output channels (mitral cells). Therefore, the PCx is ideally located to integrate sensorial input and behavioral contingencies and modulate the OB output in tune with the animal behavioral goals. To study the role of cortical feedback on supporting flexible behavior, we trained mice in a Go/No-Go task with rule-reversal guided by odor and tone cues. We monitored cortical-feedback bouton activity across OB layers using multiphoton microscopy (n = 9) in mice that express GCaMP5/7b in anterior PCx (aPCx) neurons. Within the same session, the reward contingency was switched across blocks (~5) of contiguous trials (~45), rewarding either the odorant or tone cues depending on the block rule and animal decision (report lick). Cortical feedback boutons exhibited dense and diverse responses to both odor and tone cues (55% responsive boutons) and their responses are aligned with different trial events. The feedback bouton activity mirrored the block-structure of the task, was reshaped within seconds of rule-reversal, and displayed a high correlation for the same trial outcome. The response changes observed (amplitude, slope) after each rule-reversal slightly lagged in updating in accordance with the switch in behavior. Classifiers (multi-layer perceptrons) trained to decode stimulus identity, trial contingency and behavioral decision rapidly increased their performance during cue-delivery and before the animal's lick report. Preliminary optogenetic experiments using JAWs to suppress the cortical feedback suggest that mice rely on the cortical feedback to flexibly adapt their behavior after each rule-switch. In addition, in mice engaged in a two-tone Go/No-Go task, cortical feedback responses to auditory stimuli evolved throughout training and represented the tone cue reward contingency rather than its identity. Our results suggest that cortical-bulbar feedback multiplexes information about stimulus identity, contingency, and behavioral outcome, and is rapidly re-formatted according to changes in behavioral contingencies.

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Poster

467. Olfaction: Behavior and Perception II

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Topic: D.04. The Chemical Senses

Support: DST-Cognitive Science Research Initiative (DST/CSRI/2017/271)

Title: Characterizing response reversals in olfactory decision-making

Authors: *S. PANDEY, A. K. NAYAK, N. M. ABRAHAM;
Indian Inst. of Sci. Educ. and Res., PUNE, Maharashtra, India

Abstract: A behavioral response from an organism is preceded by sensation and perception of a specific or multiple sensory stimuli followed by decisions based on perceived sensory evidences^{1,2}. In real life, sensory inputs are often noisy and change incessantly, raising the question of when and how accurate decisions are made. The cognitive flexibility adds another layer of complexity to the process of decision-making. Decision reversal allows switching between available choices and rapid stopping of already initiated misconstrued response^{1,4,5}. Here, we aimed to quantify the inappropriate behavioral actions in response to unrewarded stimuli and the underlying neural mechanisms. Mice were trained on an olfactory decision-making task wherein they have to distinguish between a rewarded and unrewarded stimuli by responding with lick or no-lick responses in stimulus-dependent manner. Initially, animals lick for both rewarded and unrewarded odor stimuli. However, as the training progresses, they start to associate reward with one of the odor stimuli and stop licking for unrewarded stimulus. Even after animals reach accurate performance levels (>80%), they tend to initiate lick responses for unrewarded odor in few trials and stop within few milliseconds. We refer this phenomenon as decision reversal (DR). We observed reversals in 5-25% of trials among high performance trial blocks. These reversals/stopping of inappropriate responses were mostly observed during 350-500 ms after the onset of odor stimulus. We observed significantly higher number of reversal trials for binary odor mixture discriminations compared to monomolecular ones. Further, on enhancing the inhibitory synaptic signalling in the olfactory bulb by photoactivating the ChR2 expressing GABAergic granule cells, we observed faster odor discrimination and fewer reversal trials. These findings report decision-reversals that are stimulus complexity-dependent and the pre-cortical control over such a complex cognitive activity.

1.Hanks, T. D. & Summerfield, C. Perceptual Decision Making in Rodents, Monkeys, and Humans. *Neuron* vol. 93 15–31 (2017).2.Brunton, B. W., Botvinick, M. M. & Brody, C. D. Rats and humans can optimally accumulate evidence for decision-making. *Science* (80-.). **340**, 95–98 (2013).3. Sweis, B. M., Thomas, M. J. & Redish, A. D. Mice learn to avoid regret. *PLoS Biol.*

16, (2018).4.Resulaj, A., Kiani, R., Wolpert, D. M. & Shadlen, M. N. Changes of mind in decision-making. *Nature* **461**, 263–266 (2009).5.Van Den Berg, R. *et al.* A common mechanism underlies changes of mind about decisions and confidence. *Elife* **5**, (2016).

Disclosures: S. Pandey: None. A.K. Nayak: None. N.M. Abraham: None.

Poster

467. Olfaction: Behavior and Perception II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 467.20

Topic: D.04. The Chemical Senses

Title: Basal Forebrain GABAergic Subtypes Display Differential Activity During Odor-guided Decision-making

Authors: *E. K. TANTRY¹, E. HANSON MOSS¹, K. BRANDEL-ANKRAPP², B. R. ARENKIEL³;

¹Mol. and Human Genet., ²Neurosci., ³Mol. and Human Genetics, Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Sensory perception relies on the flexible detection and interpretation of sensory stimuli across variable contexts, conditions, and states. Behavioral states like attention and arousal have a profound effect on sensory processing. A known hub of behavioral state regulation is the basal forebrain, which includes dense populations of projection neurons controlling downstream circuit activity related to attention and arousal. However, the process by which the encoding of sensory stimuli is influenced by basal forebrain input is poorly understood. To this end, we utilize the mouse olfactory system as a model to elucidate how basal forebrain circuits modulate olfactory processing and odor-guided decision-making. The basal forebrain has a dense population of both cholinergic and GABAergic neurons projecting to the olfactory bulb. Of these GABAergic neurons, parvalbumin (PV) and somatostatin (SST) have been shown to serve opposing roles in regulating arousal. With a focus on local GABAergic signaling, we coupled fiber photometry with an olfactory-guided go-no/go discrimination task to correlate temporal patterns of PV and SST neuronal activity with features of olfactory task performance such as odor detection, decision-making, reward retrieval, and decision bias. We show that PV-expressing GABAergic neurons are activated during odor presentation and suppressed during reward delivery. Conversely, SST-expressing GABAergic neurons are activated during both odor and reward delivery. The magnitude of the odor-evoked response in PV neurons is inversely correlated to odor discrimination ability, whereas SST neuronal activation is positively correlated with odor discrimination and inversely correlated with decision bias. Finally, we show that chemogenetic inhibition of SST neurons results in an increase in decision bias. These results suggest that subtypes of GABAergic basal forebrain neurons are differentially involved in decision-making and that their specific bidirectional activity influences olfactory discrimination.

Disclosures: E.K. Tantry: None. E. Hanson Moss: None. K. Brandel-Ankrapp: None. B.R. Arenkiel: None.

Poster

467. Olfaction: Behavior and Perception II

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 467.21

Topic: D.04. The Chemical Senses

Support: Stowers Institute for Medical Research
R01 DC014701

Title: A common disinhibition circuit modulates odor processing during attention and associative learning

Authors: *R. GARG¹, Q. QIU¹, R. YU^{1,2};

¹Stowers Inst. For Med. Res., Kansas City, MO; ²Dept. of Anat. and Cell Biol., Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Preparatory attention for upcoming stimulus enhances perception and aids performance. It is also thought to accelerate learned motor responses based on stimulus-value association. Allocation of attention, however, is influenced by task demands, habitual status, and motivation. How a neural circuit underlying an attentive state modulates perception across these conditions is not explored. Using an olfactory discrimination task, we find that both attention and associative learning enhance sensory responses in the main olfactory bulb through a common centrifugal circuit originating from the basal forebrain. In general, dopaminergic short axon cells (SACs) suppress glomerular response to non-rewarded odors. Inhibitory cholinergic input during high task demands selectively releases the inhibition to enhance reward associated odor. Chemogenetic inhibition of SACs and cholinergic input to olfactory bulb confirmed role of this pathway in attention-like behavior enhancements. Thus, the cholinergic input from the basal forebrain to the SACs constitutes a disinhibitory circuit that can be used for attentional modulation of sensory input. Engagement of this centrifugal circuit allows preparatory attention to improve signal saliency, which also depends on learning proficiency. At lower levels of proficiency, fast preparatory attention dispatched this disinhibition to enhance sensory detection and accelerate decision making. Although it is not engaged in proficient animals, pre-stimulus optogenetic activation nonetheless recapitulated attention-like behavior effects. These results identify a circuit that optimizes enhancement of relevant stimuli over a range of task demands and time scales.

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Poster

467. Olfaction: Behavior and Perception II

Location: SDCC Halls B-H

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Program #/Poster #: 467.22

Topic: D.04. The Chemical Senses

Support: NIH Grant DC006640

Title: Olfactory circuit and behavioral deficits in the *Fmr1* KO mouse model of Fragile X syndrome

Authors: *P. KURUPPATH¹, F. R. POUILLE², N. E. SCHOPPA²;

¹Univ. of Colorado, ²Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: Fragile X syndrome (FXS) is the most common monogenetic cause of autism spectrum disorders in humans and is characterized by deficits in cognitive abilities, social interactions and communication, as well sensory processing. At the level of sensory function, studies that have employed mouse models of FXS have provided some circuit-level understanding of the causes of some sensory deficits (e.g., in audition), but olfactory deficits have not been well-studied. Here, we performed experiments in *Fmr1* KO mice in which the gene encoding the Fragile X mental retardation protein is deleted to examine olfactory dysfunction in FXS. As a first step, we performed whole-cell patch-clamp recordings from olfactory bulb slices to identify potential circuit deficits. *Fmr1* KO caused two striking changes in the properties of bulbar output mitral cells (MCs): (1) a large increase in the probability of spontaneous long-lasting depolarizations (LLDs; current variance = $48 \pm 10 \text{ pA}^2$ in wild-type, $n = 10$, versus $330 \pm 95 \text{ pA}^2$ in *Fmr1* KO, $n = 8$; $p < 0.01$, Mann-Whitney U test); and (2) an increase in the frequency of inhibitory post-synaptic currents (IPSCs) derived from GABAergic granule cells (GCs). Because LLDs drive most action potentials in MCs (Gire and Schoppa, 2009), the greater number of IPSCs were likely to be a secondary result of the enhanced LLDs, which excited GCs. Next, we employed a Go/No-go odor discrimination task to investigate behavioral effects of *Fmr1* KO. KO had no effect when mice were tasked with discriminating different monomolecular odors (e.g. ethyl acetate (EA) vs propyl acetate (PA)), but differences emerged for a more difficult task involving odor mixtures (Percentage of correct trials in Block 4 for EA versus 1:1 EA/PA mixture = $89 \pm 2\%$ for wild-type, $n = 7$; $57 \pm 2\%$ for *Fmr1* KO, $n = 7$, $p < 0.0001$ with Mann-Whitney U test). Thus, olfactory deficits in *Fmr1* KO mice appear to be specific to fine odor discrimination. As a potential explanation for disrupted odor discrimination, we propose that it could have been due to the *Fmr1* KO-induced enhancement of GC-to-MC inhibition. This inhibition is well-known to control bulbar gamma oscillations that need to be at a specific level for optimal odor discrimination (Lepousez et al., 2013).

Disclosures: P. Kuruppath: None. F.R. Pouille: None. N.E. Schoppa: None.

Poster

467. Olfaction: Behavior and Perception II

Location: SDCC Halls B-H

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Program #/Poster #: 467.23

Topic: D.04. The Chemical Senses

Support: NIH BRAIN Initiative Grant U19NS112953

Title: A behavioral paradigm for measuring perceptual distances between pairs of natural or artificial odors.

Authors: *S. CEBALLO¹, S. KARIMIMEHR^{1,2}, M. KARADAS¹, D. RINBERG¹;
¹NYU Langone Hlth., New York, NY; ²Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The perception of an external stimulus is the result of complex patterns of neuronal activity, and it is assumed that similar patterns of activity generate similar percepts. However, the features of neuronal activity that shape perception are still unknown. In this work, we developed an approach to manipulate specific features of neural activity and measure their impact on perceptual distance. We based our approach on a previously developed behavioral paradigm for measuring perceptual distances between multiple odor pairs in head-fixed mice using a delayed-match-to-sample task (Nakayama & Rinberg, 2022). We expanded it to measure the perceptual distances between pairs of artificial odors, which are optogenetically generated sequences of neuronal activity in the olfactory bulb (Chong et al., 2020). We made improvements in behavioral training, and combined both natural and artificial stimuli in single sessions. Additionally, we applied a modeling approach to better extract perceptual variables and monitor the behavioral state of the animal. Finally with our approach, we can now causally identify features of neural activity responsible for odor perception.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 468.01

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Autism R01

Title: Utilizing vagus nerve stimulation to reverse maladaptive plasticity in the inferior colliculus in a rat autism model

Authors: *Y. TAMAOKI¹, V. PASAPULA¹, T. DANAPHONGSE³, S. L. KROON¹, O. I. OLAJUBUTU¹, M. S. BORLAND⁴, C. T. ENGINEER²;

²TxBDC, ¹The Univ. of Texas at Dallas, Richardson, TX; ³Univ. of Texas, Dallas, Plano, TX;
⁴Univ. of Texas At Dallas, Plano, TX

Abstract: Receptive language deficits that are associated with abnormal auditory processing are often observed in individuals with autism spectrum disorders (ASD). Auditory cortex neurons in children with ASD respond slower and weaker compared to typically developing children. When children are prenatally exposed to valproic acid (VPA), an anticonvulsant medication used to control seizures, it increases the risk for ASD. Symptoms associated with ASD are often observed, including altered sensory processing and deficits in language development. Impairments in sensory processing are also seen in rodents prenatally exposed to valproic acid. As observed in humans, these rodents display deficits in social interaction as well as impairment in speech sound discrimination ability. These behavioral characteristics are accompanied by changes in cortical activity patterns. In the primary auditory cortex (A1), the normal tonotopic map observed in typically hearing animals is reorganized and degraded in VPA-exposed rats. In VPA exposed animals, not only is the auditory cortex physiology affected, but also neurons in the midbrain regions, such as the superior olivary complex, lateral lemniscus, and inferior colliculus (IC), have disrupted morphology. Developing a method to improve these neural deficits throughout the auditory pathway is needed. We have developed a new approach to drive robust, specific plasticity that enhances recovery after neurological damage. This strategy utilizes brief bursts of vagus nerve stimulation (VNS) paired with a sound presentation. The aims of this study are to 1) document differences in the multi-unit inferior colliculus response to sounds in VPA exposed rats in comparison to saline exposed control rats, and 2) investigate the ability of VNS paired with sounds to reverse the maladaptive plasticity in the inferior colliculus in VPA exposed rats. In these experiments, we test the hypothesis that VNS paired with speech sound and tone presentation, 300 times per day for 20 days, will reverse maladaptive plasticity and restore neural responses to sounds in VPA-exposed rats. Our results suggest that VPA rats displayed weaker responses to speech sounds in the IC and VNS-sound pairing is an effective method to enhance auditory processing ($p < 0.01$). VPA rats responded weaker to speech sounds compared to the control rats in the IC. VNS-sound pairing strengthened the IC response to both the paired sound and sounds that were acoustically similar. Insights derived from this study may influence the development of new behavioral and sensory techniques to treat communication impairments that result in part from a degraded neural representation of sounds.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 468.02

Topic: D.05. Auditory & Vestibular Systems

Support: Canadian Institutes of Health Research (CIHR) project grant
Natural Sciences and Engineering Council of Canada (NSERC) Discovery grant

Title: Hyperactivity of the pontine reticular nucleus underlies increased acoustic startle in female, but not male, *Cntnap2* knock-out rats

Authors: *A. ZHENG, K. E. SCOTT, A. L. SCHORMANS, R. MANN, B. L. ALLMAN, S. SCHMID;
Univ. of Western Ontario, London, ON, Canada

Abstract: Background: The contactin-associated protein-like 2 (*CNTNAP2*) gene encodes for the CASPR2 protein, which plays an essential role in neurodevelopment. Mutations in *CNTNAP2* in humans are associated with various neurodevelopmental disorders, including autism spectrum disorder and schizophrenia. Previous studies have consistently found that rats with loss of function of *Cntnap2* show increased acoustic startle response (ASR) and decreased sensorimotor gating, measured through prepulse inhibition (PPI), paralleling similar findings in autistic people and people with schizophrenia. The objective of this study was to investigate the neural basis of this altered auditory processing by conducting electrophysiological recordings from adult *Cntnap2* knock-out (*Cntnap2*^{-/-}) rats and wildtype littermates.

Methods + Results: Auditory brainstem recordings revealed no difference between genotypes, implicating changes in brainstem structures outside of the primary auditory pathway that mediate ASR and PPI, which are the pontine reticular nucleus (PnC) and pedunculo pontine tegmentum (PPTg), respectively. Multi-unit responses recorded from the PnC and PPTg *in vivo* of the same animals revealed sex-specific effects of loss of function of *Cntnap2* on PnC activity. Female *Cntnap2*^{-/-} rats showed increased PnC responsivity compared to female wildtypes, as indicated through increased firing rates, lower threshold and ES50 values, and longer response duration times. However, there were no genotypic differences in PnC responsivity between male wildtype and *Cntnap2*^{-/-} rats, even though both female and male *Cntnap2*^{-/-} rats showed increased ASR excitability by means of lower startle threshold and ES50 values. In contrast, we found no genotypic differences in PPTg activity in either females or males, as well as no genotypic differences in the inhibition of PnC firing rates, indicating that alterations in the firing rates of these brainstem structures are not responsible for decreased PPI in *Cntnap2*^{-/-} rats.

Conclusion: We conclude that the auditory processing changes seen in *Cntnap2*^{-/-} rats are associated with, but cannot be fully explained by, differences in PnC activity, and that loss of function of *Cntnap2* has differential effects depending on sex.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Program #/Poster #: 468.03

Topic: D.05. Auditory & Vestibular Systems

Support: NIH grant DC004450

Title: Electrical signaling in cochlear efferents driven by an intrinsic neuronal oscillator

Authors: *H. HONG, L. TRUSSELL;

Oregon Hearing Res. Ctr., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Efferent neurons are believed to play essential roles in maintaining auditory function. However, the lateral olivocochlear (LOC) neurons - the dominant population of auditory efferents - remain poorly understood. Here we present a synaptic and electrophysiological analysis of identified LOC neurons in juvenile and young adult ChAT-Cre:tdTomato mice (P17-42). LOC neurons were identified as tdTomato-positive neurons within the lateral superior olive. Ca²⁺ imaging performed with Cre-dependent expression of GCaMP6f revealed extremely slow (~0.1 Hz) waves of Ca²⁺ activity in the majority of LOC neurons (~90%). Using whole-cell patch-clamp recording from LOC neurons, bursts of Na⁺ spikes lasting for seconds were observed having the same frequency as the Ca²⁺ signal periodicity. These bursts were driven by an intrinsic oscillator dependent on L-type Ca²⁺ channels, and were not observed in prehearing mice (P9-11), suggesting an age-dependent mechanism underlying the intrinsic oscillator. Using optogenetic approaches, we identified ascending (T-stellate cells in the cochlear nucleus) and descending (pyramidal neurons in the auditory cortex) sources of synaptic excitation. Both inputs were mediated by Ca²⁺-impermeable GluA2-containing AMPA receptors, and lacked NMDA receptors. Some of the cortical inputs also engaged kainate receptors. Additionally, we identified potent inhibition originating in the glycinergic medial nucleus of trapezoid body (MNTB). Conductance-clamp experiments injecting realistic inhibitory and excitatory synaptic waveforms into LOC neurons revealed an unusual mechanism of electrical signaling, in which synaptic excitation and inhibition served to switch on and off the intrinsically generated spike burst mechanism, allowing for prolonged periods of activity or silence controlled by brief synaptic events. This novel electrical signaling mechanism allowing for protracted bursts of action potentials may be essential for effective exocytosis of the diverse transmitters, including peptides, released by LOC fibers in the cochlea.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 468.04

Topic: D.05. Auditory & Vestibular Systems

Support: NIH F32DC018211
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NIH R01DC018353

Title: Serotonergic Regulation of VIP interneurons and Auditory Learning

Authors: *C. G. SWEENEY¹, M. E. THOMAS¹, K. E. SMITH², A. STEWART³, L. G. VATTINO¹, C. P. MACGREGOR², A. E. TAKESIAN¹;

¹Massachusetts Eye and Ear/Harvard Med. Sch., Boston, MA; ²Massachusetts Eye and Ear, Boston, MA; ³Harvard Med. Sch., Boston, MA

Abstract: Auditory perceptual learning induces plasticity in the primary auditory cortex (A1), which can improve hearing perception, but the neural mechanisms that promote these changes are unclear. Neuromodulators such as serotonin (5-HT), acetylcholine and dopamine can trigger plasticity in the adult cortex. However, little is known about the actions of these neuromodulators within A1 circuits and their function in auditory learning. Here, we focused on 5-HT signaling in mouse A1, its cortical targets, and its effects on auditory perceptual learning. Cortical layer 1 (L1) is a major site for neuromodulatory projections, including the serotonergic raphe nuclei. Our work and others demonstrated that VIP (vasoactive intestinal peptide)-expressing interneurons in L1 robustly express the ionotropic 5-HT receptor, 5HT_{3A}-R. Additionally, they receive bottom-up thalamic input from auditory thalamus. This circuitry suggests that both sensory inputs and 5-HT may engage L1-circuits during learning. To understand how VIP interneurons are activated *in vivo* by sensory and behavioral stimuli, we expressed the calcium indicator GCaMP6f selectively in VIP interneurons and used *in vivo* 2-photon calcium imaging in awake mice to assess the response of these interneurons to a variety of sound stimuli as well as appetitive and aversive reinforcers that are known to activate serotonergic neurons. Our results reveal heterogeneous responses within the VIP population; many neurons were selectively activated by specific, complex sounds or behavioral cues. To understand the function of 5-HT release and VIP activation during auditory perceptual learning, we developed an appetitive Go/No-go auditory frequency discrimination task. Mice showed robust improvements in their perceptual thresholds over the course of three weeks of training. Ongoing fiber photometry studies are monitoring VIP interneuron activity and 5-HT release across perceptual learning using calcium sensor recordings in VIP neurons and signals reported by the fluorescent 5-HT sensor GRAB_{5HT}. Preliminary results show a prominent increase in 5-HT release during rewarded trials as the mice undergo associative learning. After associative learning, an analysis of single trial fluorescent 5-HT transients can discriminate ‘Hit’ versus other trial types in single trials. These ongoing works will help define the dynamics and function of 5-HT release and VIP interneuron activity during perceptual learning.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Program #/Poster #: 468.05

Topic: D.05. Auditory & Vestibular Systems

Support: F31 DC019292
R01 DC018284

Title: Vip signaling excites medial geniculate neurons in mice

Authors: *L. M. RIVERA-PEREZ, J. PATEL, M. T. ROBERTS;
Univ. of Michigan, Ann Arbor, MI

Abstract: The medial geniculate nucleus (MG), the thalamic relay center of the ascending auditory system, expresses high levels of vasoactive intestinal peptide (VIP) receptors. However, the sources of VIP signaling to the MG and the effects of VIP signaling on MG neurons remain largely unknown. Our lab uncovered that VIP neurons in the inferior colliculus (IC) project to the MG and express VIP mRNA. We therefore hypothesized that VIP modulates the excitability of MG neurons and that VIP neurons in the IC are an important source of VIP signaling in the MG. To test this hypothesis, we are using brain slice electrophysiology, pharmacology, and retrograde tracing in MG slices prepared from VIP-IRES-Cre x Ai14 and C57BL/6J mice of both sexes. Our data show that 2 μ M VIP applications elicit strong depolarization in most MG neurons, in some cases eliciting spikes. Currently, we are testing VIP receptor antagonists to determine the receptors that mediate this response. In addition, we are using Retrobead injections in the MG to determine whether there are additional sources of VIP signaling to the MG. The results of this project will uncover the sources of VIP signaling to the MG and provide mechanistic insights into the previously unappreciated role of VIP in modulating auditory processing in the tectothalamic pathway.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 468.06

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01 DC 019348
NSF Grant IOS 1652432

Title: Bilateral contribution of the inferior colliculus to auditory behavior and physiology

Authors: C. A. RAYMOND, T. T. ADEYELU, O. M. OGUNDELE, *C. C. LEE;
LSU Sch. of Vet. Med., Baton Rouge, LA

Abstract: The inferior colliculus (IC) is a major midbrain hub that is involved in the processing of auditory information. Excitatory and inhibitory projections from the IC target the medial geniculate body bilaterally and influence the processing of auditory information ascending to the auditory cortex. Although the ipsilateral pathways from the IC have been well studied, the

contralateral pathways have not been as fully investigated. Here, we assessed the role of the IC in the processing of bilateral sound stimulation using a chemogenetic approach. The IC in one hemisphere was injected with a floxed virus that expressed the DREADD receptor, hM4Di, in excitatory or inhibitory neurons of VGLUT2-CRE, VGAT-CRE, or wild-type mice. Following injections animals were assessed behaviorally and physiologically for responses to bilateral sound stimulation, with or without chemogenetic inhibition with the DREADD agonist, compound 21. We employed a modified speaker-swap, pre-pulse inhibition to acoustic startle test (SSwap-PPI) to assess the behavioral responses, and subsequently recorded neural responses of contralateral MGB neurons to the same stimuli. The behavioral and physiological responses were then correlated with injection site and recording locations. These experiments highlight the important bilateral contributions of the inferior colliculus to auditory behavior and physiology.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 468.07

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01DC009607

Title: Distinct temporal expression pattern of subplate markers in auditory cortex during postnatal development

Authors: *M. CHANG, S. NEHS, P. O. KANOLD;
Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Subplate neurons are one of the earliest generated cortical neurons receiving thalamic inputs. Subplate neurons are crucial for the establishment and refinement of thalamocortical axons and layer 4 during early circuit formation. Several molecular markers have been identified in subplate neurons and their respective functions have been studied in the forebrain. However, there is a lack of systemic and detailed studies on the temporal expression pattern of these genetic markers in the auditory cortex. In the light of different transgenic mouse lines, i.e., connective tissue growth factor (CTGF), dopamine receptor D1 (Drd1), and neurexophilin 4 (Nxph4) cre-lines, we crossed them with a td-tomato reporter line to characterize the reporter expression at different developmental time points in the auditory cortex. Our study suggested that the temporal expression profile of CTGF in the auditory cortex is similar to that in the somatosensory and visual cortex. However, the Drd1 expression profile in the primary auditory cortex is unlike the expression profile in the somatosensory and visual cortical region. Drd1 is expressed in the auditory cortex much later than in the other regions, starting around the second postnatal week. Similarly, Nxph4-expressing cells were found sparsely distributed after the first

postnatal week. These results indicate that subplate neurons are not a uniform cell population across the cortex and that subplate neurons expressing these genes may play a different role between the primary sensory cortical areas during development.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 468.08

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R21DC018327
NIH R21DC018327-S1
Hearing Health Foundation Emerging Research Grant
Klingenstein-Simons Fellowship Award in Neuroscience

Title: Mapping the deep: towards an input/output map of the descending auditory cortex

Authors: ***M. P. ARNOLD**^{1,2,3}, R. F. KRALL^{1,3}, C. N. CHAMBERS^{1,3}, H. J. MORFORD⁴, R. S. WILLIAMSON^{1,5,3,6};

¹Otolaryngology, ²Med. Scientist Training Program, ³Pittsburgh Hearing Res. Ctr., ⁴Neurosci., ⁵Neurobio., ⁶Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: The auditory pathway is comprised of a complex series of brain stations that are dedicated to the processing and interpretation of our sense of sound. Incoming sensory signals traverse an ascending hierarchy from the cochlea to the primary auditory cortex (ACtx), before being propagated brain-wide through networks of excitatory projection neurons to inform distinct behavioral outcomes. These neurons fall into three broad classes: intratelencephalic (IT), extratelencephalic (ET), and corticothalamic (CT). Of these classes, ET cells are unique as they form the only direct connection between the ACtx and myriad sub-cortical targets. Their unique organizational motifs place them in a privileged position to broadcast signals to multiple downstream targets simultaneously. However, the extent of axonal collateralization, downstream spatial organization, and upstream monosynaptic connectivity remains unknown.

To address these questions, we characterized the input/output circuitry of ACtx ET cells and compared their anatomical organization to that of IT and CT populations. Using intersectional viral tracing strategies involving both Cre- and Flp-recombinase, we drove selective expression of adeno-associated viruses in distinct populations of excitatory projection neurons, allowing us to characterize downstream organization with high spatial resolution and identify local and long-range synaptic input through monosynaptic rabies tracing.

Confirming prior reports, we found that ET neurons collateralize to non-lemniscal regions of the inferior colliculus and thalamus, as well as the lateral amygdala. Notably, ET cells that

collateralize to the thalamus primarily originated from deep layer 6, while amygdala-collateralizing ET cells were evenly distributed across both layers 5 and 6. Monosynaptic rabies tracing demonstrated widespread synaptic inputs to ET, IT, and CT populations. All three excitatory projection types received the bulk of their input (in varying degrees) from sensory cortices, including somatosensory and visual cortex, as well as higher-order regions in parietal and frontal cortex. Our ongoing experiments are focused on extending these findings to distinct ET organizational motifs. This work will provide an anatomical foundation for understanding how brain-wide interactions between distinct areas cooperate to orchestrate sensory perception, guide behavior, and become disrupted in perceptual and neurological disorders.

Disclosures: **M.P. Arnold:** None. **R.F. Krall:** None. **C.N. Chambers:** None. **H.J. Morford:** None. **R.S. Williamson:** None.

Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 468.09

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC017906
NIH Grant DC013826
NIH Grant MH117778
George Barth Geller Fund

Title: Deciphering the projection logic of the inferior colliculus

Authors: ***B. J. SLATER**¹, R. D. MOONEY^{2,1};
¹Dept. of Neurobio., Duke Univ., Durham, NC; ²Med. Ctr., Durham, NC

Abstract: The auditory midbrain, or inferior colliculus (IC), is a processing nexus and obligate waypoint in the ascending auditory system. The IC sends significant projections to the contralateral IC as well as the ipsilateral auditory thalamus, or medial geniculate body (MGB). Curiously though, unlike the projections from the cerebral cortex, which are almost exclusively excitatory, these collicular projections arise from both excitatory and inhibitory neurons, complicating our understanding of collicular output. Here we use identified neuronal markers coupled with detailed viral tracing to determine cell-type specific output of the IC. We made focal unilateral injections of an AAV expressing synaptophysin-GFP into the IC of various Cre driver mouse lines. Using this regime, we examined the postsynaptic locations and density of axonal projections from identified cell types in the IC to the ipsilateral MGB and/or the contralateral IC. We found that inhibitory (VGAT-expressing) and excitatory (VLGUT-expressing) IC neurons made extensive projections to both the contralateral IC and ipsilateral MGB, primarily in the lemniscal portions of each of these nuclei. Both Parvalbumin- and Somatostatin-expressing IC neurons, which include both excitatory and inhibitory neurons,

projected to the ipsilateral MGB, whereas only the parvalbumin-expressing neurons project to the contralateral IC. Interestingly, we found that *Foxp2*, which has been previously shown to be label corticothalamic neurons, identified putative excitatory IC cells that projected exclusively to the ipsilateral MGB. These initial steps to tease apart the complex circuitry of the IC have identified neuronal markers which define distinct subsets of IC projection neurons, paving the way for a more detailed functional dissection of collicular output.

Disclosures: **B.J. Slater:** None. **R.D. Mooney:** None.

Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 468.10

Topic: D.05. Auditory & Vestibular Systems

Title: A novel layer 4 corticofugal projection involved in cortico-thalamo-striatal sensory processing

Authors: ***A. BERTERO**, A. J. APICELLA;
UTSA, UTSA, San Antonio, TX

Abstract: In sensory cortices, the information flow has been thought to be processed vertically across cortical layers, with layer 4 being the major thalamo-recipient one which relays thalamic signals to layer 2/3, which in turn transmit thalamic information to layer 5 and 6 to then leave the cortex to reach subcortical and cortical long-range structures. Although several exceptions to this model have been described, neurons in layer 4 are still considered to establish only local (i.e., interlaminar and short-range) connections. Here, taking advantage of anatomical, electrophysiological, optogenetic techniques, we describe for the first time a long-range corticostriatal class of pyramidal neurons in layer 4 (CS-L4) of the mouse auditory cortex that receive direct thalamic inputs. The CS-L4 neurons are embedded in a feedforward inhibitory circuit involving local parvalbumin neurons and establish connections in the posterior striatum in yet another feedforward inhibitory thalamo→cortico(L4)→striatal circuit to potentially contribute to control the output of striatal spiny projection neurons. Here we propose a new wiring diagram that implemented the old one, in which layer 4 is not only involved in the transfer of thalamic input to the upper layer 2/3 but can exert a direct top-down control, bypassing intracortical processing, of subcortical structure such as the posterior part of the dorsal striatum posing a new conceptual cell element (CS-L4 neurons) for experimental and theoretical work of the cortical function. We also have established a new functional role of CS-L4.

Disclosures: **A. Bertero:** None. **A.J. Apicella:** None.

Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 468.11

Topic: D.05. Auditory & Vestibular Systems

Title: Supervised deep learning of anatomical tracing reveals conservations of efferent structural connections from the medial geniculate nucleus after deafening in the cat (*Felis catus*)

Authors: *S. SINGH¹, D. J. MILLER², R. GUPTA³, S. HAN⁶, S. YU³, D. MACGOWAN³, A. R. KHAN⁴, B. E. BUTLER⁵;

¹Evolution, Ecology and Behavior, Univ. of Illinois, Urbana-Champaign, Urbana, IL; ²Evolution, Ecology and Behavior, Univ. of Illinois, Urbana, IL; ⁴Biophysics, ⁵Psychology, ³Univ. of Western Ontario, London, ON, Canada; ⁶Evolution, Ecology and Behavior, Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Following hearing loss, auditory cortex undergoes crossmodal reorganization to facilitate visual and/or somatosensory over auditory perception, which may complicate the use of therapeutics like cochlear implants. While the exact details of this change between sensory modalities remains poorly understood, emerging work suggests that behavioral changes may be related to local microstructural reorganization within the supragranular layers, and may not involve the thalamus. To test this hypothesis, we developed a novel supervised deep learning mapping procedure to count anatomical connections identified by tracer injections to the medial geniculate body (MGB) following ototoxic deafening in the adult cat (*Felis catus*) (n=7; 6 females and 1 males, sex differences were not assessed). Our single cell instance identification rate of tracer-labeled neurons is 86.2% (SD = 4.2%) using the Nothing-New U-Net (Isensee et al., 2020) on 3,186 consensus annotated neurons. After identification, labeled neurons were coregistered to MRI scans and mapped into functional regions with CATLAS (Stolzberg et al., 2017), and we used Welch's T-test to examine regional differences in connection patterns. Our results indicate that the large-scale connectivity of the MGB to auditory and visual fields of the cerebral cortex remain largely unchanged following deafening. We discuss some of the caveats and statistical trends in our data that may be of further interest, including confidence intervals on cell identification and connectivity to the posterior auditory field (PAF), but provide evidence for the normal pattern of connections in all examined cases. This report is significant because it provides additional supporting evidence that the behavioral modification that accompanies crossmodal plasticity following hearing loss is likely a product of local cerebral cortical microstructure, chemistry, and signal timing over the loss of large-scale neural tracts, and narrows the range of therapeutic targets for improving technologies like cochlear implants.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 468.12

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC006877

Title: Activity dependent intrinsic plasticity regulates repetitive firing of superior olivary neurons prior to hearing onset

Authors: M. SINGH, F. H. THOMAS, T. A. BABOLA, S. D. CHERRY, R. VOGLEWEDE, *N. GOLDING;

Dept. of Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Neurons throughout all levels of the auditory system exhibit spontaneous firing that functionally links topographically related neurons. To understand how intrinsic firing patterns of central auditory neurons are regulated in the face of ongoing developmental changes in synaptic strength and dendritic morphology, we examined intrinsic firing during patch-clamp recordings in slices of the gerbil medial superior olive (MSO), a nucleus that conveys spatial cues for horizontal sound localization. Unexpectedly, most pre-hearing (<~postnatal day 12) MSO neurons exhibited trains of action potentials that exhibited both Na and Ca components: repetitive firing was eliminated both by the 10 μ M nifedipine as well as 50 μ M ICA123149, blockers of L-type (Cav 1.3) and persistent Na channels (n=XX). Ramp currents were adjusted to trigger spikes between 100 to 1000 ms after stimulus onset. While repetitive spiking was independent of excitation slope in pre-hearing neurons, subsequent blockade of persistent Na current in ICA123149 not only suppressed repetitive firing, but induced sensitivity to the rate of rise of transient firing, requiring excitation to reach threshold within XX ms (n=xx). Conversion of a repetitive to transient firing pattern could be induced by synaptic activity during the course of in vitro experiments. Trains of excitatory inputs to MSO neurons in gerbil brainstem slices were delivered prior to hearing onset (P3-P11). Subthreshold synaptic stimulation (trains of 10 monolateral stimuli at 100 Hz, repeated 20 times at 0.5 Hz) produced striking decreases in both input resistance and resting potential (xx to yy M Ω and xx to yy mV, respectively), and suppressed repetitive firing, typically within 5-10 min. and lasting for the duration of recordings (>30 min. up to 1 hr. post-stimulus). The decrease in repetitive firing was correlated with the magnitude of decrease in input resistance even when the resting potential was artificially matched to pre-stimulus values (R=xxx), with the latter relative decrease averaging 44 \pm 11% (n=8). These effects persisted for the duration of recordings (>30 min. post-stimulus, up to one hour). Activity-dependent changes in input resistance, resting potential and firing pattern were found to be dependent on type I metabotropic glutamate receptors and calcium influx through NMDA receptors and calcium-induced calcium release, acting through calcineurin. Taken together, our results show that action potential firing in pre-hearing neurons exhibit distinct properties from post-hearing neurons, and identify intrinsic plasticity mechanisms which provide feedback regulation of firing patterns.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 468.13

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC016905
Hearing Health Foundation - Emerging Research Grant

Title: Synaptic targets of unipolar brush cells in the dorsal cochlear nucleus

Authors: *C. T. CANDLER, T. S. BALMER;
Sch. of Life Sci., Arizona State Univ., Tempe, AZ

Abstract: The dorsal cochlear nucleus (DCN) receives acoustic signals from the auditory nerve, as well as non-auditory signals from various sources, to perform complex functions such as canceling self-generated sounds and localizing sounds relative to the head and body. Understanding how this cerebellum-like circuit processes information requires knowledge about how the different cell types in DCN are connected and how their synapses transform signals. Granule cells receive multimodal sensory signals and project parallel fiber axons to fusiform cells, the principal output neurons of the DCN. Unipolar brush cells (UBCs) also receive multimodal input and are presumed to project to granule cells and other UBCs. However, their location in the deep layer of DCN, relatively distant from the granule cell domains, suggests the possibility that they target additional cell types. Whole-cell patch-clamp recordings of fusiform cells and cartwheel cells were made in acute brain slices from P16-21 mice. UBCs that expressed channelrhodopsin-2 (ChR2) were optogenetically stimulated to investigate their connectivity and synaptic effects. Here our data shows that UBCs may synapse directly onto fusiform cells and cartwheel cells. Stimulation of UBCs evoked EPSC latencies averaging <4 ms in fusiform and cartwheel cells, in addition to the expected polysynaptic EPSCs. These latencies were similar to known monosynaptic EPSC latencies recorded in granule cells, the major target of UBCs, via the same UBC optogenetic stimulation. Postsynaptic responses in fusiform and cartwheel cells to electrical stimulation of parallel fibers facilitated, as expected, while UBC stimulation did not, suggesting that two separate pathways were activated. Confocal imaging of biocytin-filled cells revealed potential anatomically defined synaptic contacts between UBC axons and fusiform cells. Thus, UBC activity may have a more direct influence on DCN excitability than previously appreciated, and suggests that their role in auditory processing requires further examination.

Disclosures: C.T. Candler: None. T.S. Balmer: None.

Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 468.14

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC015508

Title: Changes in the auditory nerve transcriptome following noise exposure or conductive hearing loss

Authors: N. F. WONG¹, I. STARR², J. H. ENGEL³, D. DING⁴, S. MANOHAR⁴, R. J. SALVI⁴, O. GOKCUMEN², *M. A. XU-FRIEDMAN³;

¹Biol. Sci., Univ. At Buffalo, SUNY, Buffalo, NY; ²Dept. Biol. Sci., ³Univ. at Buffalo, SUNY, Buffalo, NY; ⁴Ctr. for Hearing & Deafness, Univ. Buffalo, Buffalo, NY

Abstract: Neurons of the spiral ganglion convey activity from the cochlea to the brain. Synapses formed by spiral ganglion neurons (SGNs) in the cochlear nucleus show physiological and structural changes during noise exposure and conductive hearing loss (CHL). However, it is not known how changes in gene expression contribute to these changes. To address this, we performed bulk RNA sequencing from SGNs of control, noise-exposed, and CHL mice. A large number of genes showed differential expression within 1 day of noise exposure or 2 days of CHL. Many of these genes play roles in synapse formation and activity. Surprisingly, after 7 days, expression returned to control levels despite continued noise exposure or CHL. This suggests that long-term synaptic changes resulting from NE or CHL may be due to downstream changes in protein expression. We examined protein markers associated with specific subtypes of SGN, and saw no changes in expression, suggesting that subtype identity is fixed shortly after the onset of hearing.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 468.15

Topic: D.05. Auditory & Vestibular Systems

Support: Czech Science Foundation GACR 19-09283S and GACR 21-17085S
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Title: Sensorineural hearing loss in mice with deleted GABAB receptor-associated KCTD12 proteins

Authors: *K. PYSANENKO¹, N. RYBALKO¹, S. SUCHANKOVA¹, D. ULRICH², M. KANG¹, N. JOVANOVIC¹, I. FILOVA³, A. MELICHAR¹, G. PAVLINKOVA³, B. BETTLER², T. RINALDI BARKAT², R. TURECEK¹;

¹Inst. of Exptl. Med. CAS, Prague, Czech Republic; ²Univ. of Basel, Basel, Switzerland; ³Inst. of Biotech. CAS, Vestec, Prague-West district, Czech Republic

Abstract: K⁺ channel tetramerization domain 12 (KCTD12) is a cytosolic protein that constitutively binds to inhibitory γ -aminobutyric acid type B receptors (GABABRs), increasing their surface expression and accelerating the kinetics of their G-protein-dependent responses. KCTD12 has been observed to be abundantly expressed in the mammalian auditory system, but its importance for auditory function remains unclear. Previous work has identified KCTD12 gene as a risk modifier for chronic tinnitus in humans. Here, we show that mice lacking KCTD12 exhibit suppressed otoacoustic emissions, elevated auditory thresholds, and reduced amplitudes of the auditory brainstem responses (ABRs). This suggested cochlear dysfunction in KCTD12^{-/-} mice further supported by the observation that direct electrical stimulation of the auditory nerve using cochlear implants elicited ABRs with similar thresholds in WT and KCTD12^{-/-} mice. Histological examination revealed no significant changes in the number or arrangement of outer and inner hair cells or spiral ganglion neurons in KCTD12^{-/-} mice. Behavioral testing showed significantly increased amplitudes of the acoustic startle reflex (ASR) and reduced inhibition of the ASR by gap-prepulses in KCTD12^{-/-} mice, suggesting the presence of auditory disturbances such as hyperacusis or tinnitus in these mice. Neurons of the central nucleus of the colliculus inferior in KCTD12^{-/-} mice showed increased spontaneous firing rates, steeper rate-level functions, as well as a striking shift in their characteristic frequencies toward lower values. Accordingly, excitatory neurons in the auditory cortex of these mice exhibited an altered distribution of their best frequencies as revealed by recording their electrical responses or by GCaMP6f meso-scale calcium imaging technique. These data indicate increased central gain and altered temporal processing of sensory information in KCTD12^{-/-} mice and support the importance of KCTD12 and thus proper GABAB signaling in processes underlying auditory function. In summary, deletion of KCTD12 has been shown to lead to peripheral and central auditory system disturbances and tinnitus-like behavior, suggesting that KCTD12 may represent a susceptibility gene for chronic tinnitus in mammals.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 468.16

Topic: D.05. Auditory & Vestibular Systems

Support: Centre of Reconstructive Neuroscience, registration number CZ.02.1.01/0.0./0.0/15_003/0000419

Title: Age-related changes in the neuronal morphology of inferior colliculus, medial geniculate body and auditory cortex in rats.

Authors: *J. SVOBODOVA BURIANOVA, J. SYKA;
Inst. of Exptl. Med. CAS, Prague, Czech Republic

Abstract: In the aging brain, neuronal numbers tend to decrease and neuronal trees simplify. However, such alterations are quite variable, depending on a given brain structure. Only few studies described morphological alterations in the upper part of the auditory pathway, i.e. inferior colliculus (IC), medial geniculate body (MGB) and the auditory cortex (AC). Here, we aimed to evaluate age-related changes in the Long-Evans rats with the advent of modern morphometric techniques. Using the Golgi-Cox method and Neurolucida software, we analyzed structure of neurons in the dorsal nucleus of IC (DIC), central cortex of IC (CIC), and external cortex of IC (EIC), in the dorsal and ventral divisions of MGB (MGB-D, and MGB-V, respectively), and pyramidal and non-pyramidal neurons in the AC of 3-month and 32-month old rats. In the IC, we observed alterations particularly in the DIC, where neurons in old animals showed decreased dendritic length and volume, and lower number of nodes and ends. In both divisions of the MGB, neurons in old animals had shorter dendrites and less spines. In the AC, we did not observe significant age-related changes in the non-pyramidal neurons in any evaluated parameter. In contrast, pyramidal neurons exhibited apparent age-related degradation of the neuronal tree. Their basal dendrites were shorter, occupied less volume, had fewer spines, and had fewer endings. However, the surface of the soma or the length or volume of apical dendrites did not decrease significantly in old animals. These findings demonstrate that particularly DIC is prone to age-related morphological alterations, and aging in MGB is apparent in both divisions. The non-pyramidal neurons in the AC remain intact in terms of neuronal morphology, while pyramidal neurons show significant degradation of branching complexity in basal dendrites.

Disclosures: J. Svobodova Burianova: None. J. Syka: None.

Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Program #/Poster #: 468.17

Topic: D.05. Auditory & Vestibular Systems

Support: UMass Faculty Research Grant
UMass Startup Funds

Title: The Contribution of 5-HT_{1A/2A} Receptors and GABAergic Neurons of the Pedunculopontine Tegmental Area to Sensorimotor Gating

Authors: *E. CORRELL, G. CASTELLANO, K. FENELON;
Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Sensorimotor gating is a fundamental pre-attentive process defined by the ability of sensory inputs to inhibit motor outputs. When reduced, sensorimotor gating is associated with disturbances in cognition and attention. Typically measured using the translational prepulse inhibition (PPI) of the startle reflex task, sensorimotor gating is affected in patients suffering from a variety of neuropsychiatric disorders including schizophrenia. Currently, the reversal of PPI deficits is routinely tested in disease experimental systems as pre-clinical trials of neurological drug efficacy. Yet, the cellular and circuit-level mechanisms underlying sensorimotor gating remain largely unclear, even under non-pathological conditions, therefore limiting therapeutic advances. Previous electrophysiological studies in rodents demonstrated that giant glutamatergic neurons of the brainstem Caudal Pontine reticular nucleus (PnC) mediate startle. The PnC receives inputs from the pedunculopontine tegmental region (PPTg), a midbrain structure that contains glutamatergic, cholinergic, and GABAergic neurons, and exhibits cytoarchitectural abnormalities in patients with PPI deficits. While general PPTg lesions markedly reduce PPI, how PPTg GABAergic neurons target the PnC startle circuit remains unknown. Additionally, it is unclear how PPTg GABAergic neurons are modulated by serotonin (5-HT) neurotransmission, which contributes to PPI and is affected in diseases with PPI deficits. Our study aims to determine how PPTg GABAergic neurons inhibit giant PnC neurons during PPI and how serotonin modulates the activity of these PPTg GABAergic neurons. Using vGAT-cre mice (N = 7), we performed tract-tracing, antibody staining, and confocal imaging to visualize how PPTg GABAergic fibers innervate the PnC *ex vivo*. Within PnC sections, we assessed whether PPTg GABAergic fibers are apposed to Gephyrin, a marker of inhibitory synapses. Then, we used an optogenetic approach to confirm the contribution of PPTg GABAergic neurons to PPI *in vivo*. The photo-inhibition of PPTg GABAergic neurons reduced PPI by 50%. Finally, we confirmed that PPTg GABAergic neurons express 5-HT_{1A/2A} receptors. Overall, our data show that PPTg GABAergic neurons express 5-HT receptors and form inhibitory connections with PnC neurons. We also provide strong evidence that PPTg GABAergic neurons contribute to PPI, which is critical to our understanding of the theoretical construct of sensorimotor gating.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Topic: D.05. Auditory & Vestibular Systems

Support: UMass Startup funds
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Title: Spatiotemporal identification of amygdala neurons active during sensorimotor gating

Authors: *W. HUANG, K. FÉNELON;
UMass Amherst, Amherst, MA

Abstract: Prepulse inhibition (PPI) of the acoustic startle reflex refers to the inhibition of a startle response when a weak stimulus (“prepulse”) is presented prior to an alarming stimulus (“pulse”). PPI is a standard operational measure of sensorimotor gating. As a hallmark of schizophrenia, PPI impairments are also found in other neuropsychiatric disorders and are associated with cognitive overload and attention deficits (Braff et al., 2001). Therapeutic advances are limited by the gap in our knowledge of the PPI underlying neuronal circuitry. In fact, the currently used dopaminergic-based antipsychotics have shown inconsistent effects on PPI in affected individuals (Frau et al., 2014).

Previous stimulation and electrophysiological studies showed that giant glutamatergic neurons located in the brainstem Caudal Pontine reticular nucleus (PnC) mediate the startle response (Lengenhöhl and Friauf, 1992). The PnC neuronal population includes both giant glutamatergic neurons and glycinergic neurons (Koch and Friauf, 1995; Rampon et al., 1996; Zeilhofer et al., 2005) which receive various glutamatergic inputs. We recently showed that the central nucleus of the amygdala (CeA) contributes to PPI by sending glutamatergic inputs to PnC glycinergic neurons (Cano et al., 2021). But the CeA neurons active during PPI remain unknown.

To answer this question, we used Cal-light, a calcium-dependent and blue light-sensitive method that enables the identification and manipulation of active neurons during a given behavior (Lee et al., 2017). By delivering Cal-light viral components to the CeA of wildtype mice (n = 3), we were able to identify a specific subset of CeA neurons (accounting for 13.1% of total neurons in the CeA) active during PPI, because upon calcium entry and in the presence of blue light, these neurons became green fluorescent. Since Cal-light targeted CeA neurons also expressed the inhibitory optogenetic tool Halorhodopsin sensitive to yellow light, photo-inhibiting these neurons with yellow light yielded a 25.1-52.8% reduction in PPI level measured at various interstimulus intervals between the prepulse and the pulse. Overall, our results confirm that CeA-PnC synapses contribute to PPI and Cal-light allows us to identify the CeA neurons involved, with high spatiotemporal resolution. Our findings provide critical insights toward identifying potential therapeutic targets for diseases associated with PPI deficits.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 468.19

Topic: D.05. Auditory & Vestibular Systems

Title: Short-latency non-lemniscal auditory inputs onto deep cortical layers

Authors: *A. M. KLINE¹, M. GARCIA¹, H. TSUKANO¹, K. ONODERA¹, M. R. KASTEN², P. B. MANIS², H. K. KATO¹;

¹Dept. of Psychiatry, ²Dept. of Otolaryngology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: How our brain integrates information across parallel sensory channels to achieve coherent perception remains a central question in neuroscience. In the auditory system, sound information reaches the cortex via two anatomically distinct pathways—the “primary” lemniscal pathway, which is thought to carry fast and accurate representations of sounds, and the non-lemniscal pathway, which is generally described as a slower integrator of multisensory information. However, the potential roles of the non-lemniscal pathway in fast sound processing remain unclear. In this study, we identified a short-latency (< 10 ms) input onto layer 6 (L6) of the secondary auditory cortex (A2), which was as fast as the lemniscal inputs to L4 of the primary auditory cortex (A1). We performed retrograde tracing and found that A2 L6 receives inputs from neurons along the non-lemniscal pathway: cochlear nucleus → external shell of the inferior colliculus (ECIC) → medial division of the medial geniculate nucleus (MGm) and the brachium of the inferior colliculus (BIC) → A2 L6. Using electrophysiological recordings, we confirmed the short-latency responses in these brain structures: 4-5 ms in ECIC and 5-7 ms in MGm/BIC. These anatomical and functional properties support a non-lemniscal origin of short-latency inputs that bypasses A1 and directly reaches the deep cortical layers of A2. Ongoing electrophysiology experiments in vitro and in vivo aim to understand how short-latency L6 input interacts with L4 input to shape sound representation in the auditory cortex and influence perceptual behaviors.

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Poster

468. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD R01DC017516
Foundation of Hope

Title: Experience-dependent gating of primary auditory cortex by frontal top-down inputs

Authors: ***H. TSUKANO**, H. K. KATO;
Dept. of Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Sensory stimuli lose their perceptual salience after repetitive exposure (“habituation”). We previously reported that daily passive sound exposure attenuates neural responses in the mouse primary auditory cortex (A1), and local inhibition by somatostatin-expressing neurons (SOM neurons) mediates this plasticity. In the current study, we further explored the source of top-down inputs that control SOM neurons to trigger habituation. We first conducted retrograde

tracing and found that A1 receives projections from the frontal cortical areas, including the orbitofrontal cortex (OFC). Interestingly, optogenetic activation of the OFC axon terminals suppressed A1 neuronal activity, suggesting a top-down inhibitory control of sensory representations. To investigate the plasticity of OFC top-down inputs during habituation, we performed two-photon calcium imaging of OFC axon terminals in A1 during daily passive exposure to tones. We found that tone-evoked activity of OFC axons was enhanced over days, suggesting their contribution to the attenuation of A1 sound responses. Finally, we examined the causal role of OFC in habituation by its pharmacological inactivation during chronic calcium imaging of A1 neural activity. Strikingly, acute muscimol infusion into OFC reversed the pre-established A1 habituation, indicating the requirement of the OFC in sensory habituation. Together, these results suggest the predictive gating of sensory activity by a global circuit mechanism recruiting the frontal top-down inputs.

Disclosures: H. Tsukano: None. H.K. Kato: None.

Poster

469. Structure and Function of Cortical Visual Circuits

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 469.01

Topic: D.06. Vision

Title: Neural responses in primary visual cortex of mice evoked by background stimuli and occlusion of the receptive field

Authors: *N. CUEVAS VICENTE¹, A. BROGGINI¹, A. TZANOU¹, I. ONORATO¹, C. URAN¹, M. VINCK^{1,2};

¹Ernst Strüngmann Inst. (ESI) for Neurosci. in Cooperation with Max Planck Society, Frankfurt am Main, Germany; ²Dept. of Neuroinformatics, Radboud Univ., Donders Ctr. for Neurosci., Nijmegen, Netherlands

Abstract: Animals are constantly exposed to sensory information making it challenging to observe the internally generated neural activity. A possible way to study generative neural activity is by presenting visual stimuli and omitting parts of the stimuli. Previous research in humans claims that under the occlusion of images, an imagery process acts as a filling-in of an image. Recent studies showed that neural activity occurs in the presence of occlusion of the receptive field (RF) and neural responses are driven by feedback connections. It remains unknown if this process is a filling-in/prediction or a response to salient stimuli. To further investigate that, we presented a set of visual stimuli with a mask occluding the RF while recording the neuronal activity of the primary visual cortex of mice in a head-fixed setup using laminar multi-channel probes. We compared neural activity for different types of stimuli (homogeneous luminance, static, moving, noise, etc.). Our results suggest that neurons can be driven by stimuli presented in the surround without direct activation of the RF. However, the responses of most neurons are not consistent with a fill-in/prediction of the RF stimuli. This

rather suggests other processes such as mismatch signaling/salience as the key driver of neural responses with occlusion.

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Poster

469. Structure and Function of Cortical Visual Circuits

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Program #/Poster #: 469.02

Topic: D.06. Vision

Title: Circuit mechanisms and role of interneuron subtypes in orchestrating cortical dynamics in V1

Authors: *I. ONORATO¹, A. BROGGINI¹, A. TZANOU¹, C. URAN¹, M. VINCK^{1,2};
¹Ernst Strüngmann Inst. for Neurosci. in Cooperation with Max Planck Society, Frankfurt, Germany; ²Dept. of Neuroinformatics, Donders Ctr. for Neuroscience, Radboud Univ., Nijmegen, Netherlands

Abstract: Primary visual cortex (V1) exhibits a well-described and reliable oscillation in the gamma range during visual stimulation. This oscillation represents a unique model to study basic principles of circuit mechanism underlying cortical dynamics. Gamma oscillations may result from a specific interaction between excitatory (E) and inhibitory (I) neuronal populations, as described by the pyramidal-interneuron network gamma (PING) model. It remains unclear how excitatory cells entrain the I cells and which circuit mechanisms are involved in this process. Furthermore, the contribution of specific I populations in the generation of the rhythm is under debate. Some studies suggest the involvement of somatostatin (SOM) interneurons (Veit et al., 2017), whereas others point at parvalbumin-expressing (PV) interneurons (Cardin et al., 2009) as the pacemaker of this cortical rhythm. To answer this question, we studied the role of functionally distinct sub-population of neurons in shaping cortical interactions in the mouse V1. We recorded a large (~5000) dataset of single units from mouse V1 using multi-channels laminar probes. The neurons were classified in distinct subpopulations of excitatory and inhibitory neurons. We examined their firing patterns and oscillatory properties in the presence of visual stimulation. Interneurons subtypes were identified by their increased response to optogenetic pulses. In a previous work (Onorato et al., 2020) we described a unique sub-type of excitatory neurons in primate V1 and hypothesized its dedicated role in entraining the network at high frequency. This cell type presented a strong bursting activity and high stimulus selectivity. Surprisingly, we identified a sub-type of excitatory cells with similar functional properties also in mouse V1, suggesting burst firing as a conserved circuit mechanism from mice to primate, critical to modulate the synchronization of neuronal networks, and ensuring a reliable recruitment of the postsynaptic neurons with high temporal precision. Furthermore, examining the synchronization properties of opto-tagged interneurons we made several striking

observations. PV neurons presented much stronger and narrower gamma phase locking than SOM interneurons, suggesting PV as the main element for gamma generation in the PING model. Our findings support SOM interneurons as a central element in modulating the cortical dynamics, critical to control bursting activity and tune the level of synchrony.

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Poster

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Topic: D.06. Vision

Support: NIH grant EY05253

Title: Luminance contrast changes on-off pathway dominance in human vision

Authors: *H. RAHIMI NASRABADI¹, V. MOORE-STOLL², J. TAN², S. DELLOSTRITTO², J. JIN¹, M. W. DUL¹, J.-M. ALONSO¹;

¹Dept. of Biol. Sci., ²SUNY Col. of Optometry, New York, NY

Abstract: Human vision relies on the function of two major ON and OFF pathways that process light and dark stimuli in visual scenes. In nature, stimuli lighter or darker than their local surround have different spatiotemporal properties and are differently affected by luminance changes throughout the day (Rahimi-Nasrabadi et al., 2021). Similarly, in animal models, ON and OFF pathways have different spatiotemporal properties and their response balance changes with the properties of the stimulus. Here, we demonstrate that, in human vision, the ON-OFF dominance estimated from the visual salience of light and dark stimuli is strongly dependent on a fundamental stimulus property - the luminance contrast. ON-OFF dominance was measured with an ON-OFF perimetry test that requires subjects to signal whether a target embedded in noise is located on the right or left hemifield. The target can be light or dark, have a contrast from 5 to 20%, and appear in 90 different positions within 5-30 degrees of eccentricity. The stimuli are shown on a head mounted display with an eye tracker (HTC VIVE Pro Eye, refresh rate: 90 Hz, maximum luminance: 110 cd/m²) and the appropriate optical correction for each subject. Measurements from 34 eyes of 34 subjects (age: 36 ± 18, 23-78 years old) reveal strong correlations between luminance contrast and ON-OFF dominance. The ON-OFF dominance was estimated by calculating the light/dark ratios for detection errors, missed targets, or reaction times. Luminance contrast was strongly correlated with the light/dark ratio of detection errors across all eccentricities (R²: 0.92, p = 0.001, n = 8 contrasts) and at different eccentricity ranges (R²: 0.86, p = 0.006 for 5-10 deg, R²: 0.94, p < 0.001 for 15-20 deg, and R²: 0.86, p = 0.007 for 25-30 deg). Luminance contrast was also strongly correlated with the light/dark ratio of missed targets (R²: 0.91, p = 0.002) and reaction times (R²: 0.89, p = 0.003) across all eccentricities. The

relations between visible contrasts and ON-OFF dominance were best fit with logarithmic functions ($a \log(x) + b$, where a/b are: 0.4/0.4, 0.5/0.2, 0.04/0.93 for detection errors, missed targets and reaction time, respectively). Based on these results, we conclude that high contrast stimuli are processed faster and more accurately by OFF than ON pathways in human vision. However, the OFF dominance reverses to ON dominance as the stimulus contrast decreases. Our findings may explain why patients without functional ON pathways have a pronounced loss of contrast sensitivity. They also demonstrate that clinical assessments of contrast sensitivity are more accurate with light than dark stimuli.

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Poster

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EY027361

Title: Challenging our theory of cortical map formation with simulations of binocular receptive fields

Authors: *S. NAJAFIAN¹, J. JIN¹, H. RAHIMI¹, F. OLIANEZHAD², J.-M. ALONSO¹;
¹Suny Optometry, SUNY optometry, NYC, NY; ²Suny Optometry, NYC, NY

Abstract: We have recently proposed a theory of cortical map formation that uses an afferent-density model to accurately replicate a large body of experimental measurements (Najafian et al., 2022). The model develops maps by sorting the thalamic afferents based on retinotopy, eye input and contrast polarity, then it makes the afferents converge in the cortex through axon arbor spread, and then it adjusts the afferent synaptic weights through visual experience. The model uses ON and OFF pathways with equal strength and afferent number to reproduce the combined topography of visual cortex for multiple stimulus dimensions such as spatial position, ocular dominance, ON-OFF polarity, receptive field structure, and orientation preference/selectivity. However, since the visual cortex is dominated by OFF afferents, the model should also replicate the cortical OFF dominance. The cortical OFF dominance could be simulated by feeding the cortex with more OFF than ON thalamic afferents and/or making the OFF thalamic afferents stronger. OFF/ON ratios in afferent number of 2-3 (or ON/OFF ratios in synaptic-strength of 0.5-0.7) generated 1.7-2.4 (or 1.3-7.8) more OFF- than ON-dominated cortical receptive fields. However, no combination of these ON/OFF ratios was able to replicate the bimodal distribution of ON-OFF receptive field polarity demonstrated in cat visual cortex for each eye (Wang et al., 2015). To replicate the bimodal distribution of ON-OFF polarity, the model had to make the

afferents with non-dominant polarity also 80% weaker than those with dominant polarity at each cortical point. In previous simulations, the model also adjusted the synaptic weight of afferents from the non-dominant eye to match the receptive field of the dominant eye. This synaptic adjustment improved the binocular receptive-field match of ON-OFF contrast polarity from $r=0.15\pm 0.08$ (embryonic primordial cortex) to $r=0.97\pm 0.01$ (mature cortex, $n=1600$ cortical pixels from 5 different simulations of 2×2 mm patches). However, in real brains, the binocular match of ON-OFF polarity is lower and closer to 0.7 (Olianezhad et al., 2022). We are currently investigating the parameter space needed to replicate these experimental measurements (e.g. implementing binocular visual experience, maximizing binocular retinotopic match). In conclusion, our theory of cortical map formation can accurately replicate the OFF dominance and bimodal distribution of ON-OFF polarity in visual cortex by strengthening OFF afferents and afferents with dominant polarity at each cortical point. However, replicating the ON-OFF binocular mismatches may require implementing binocular visual experience.

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Poster

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Topic: D.06. Vision

Title: Local and inter areal cell-type specific responses to optogenetic stimulation reveals distinct resonance properties of mouse visual cortex

Authors: *A. BROGGINI¹, I. ONORATO¹, A. TZANOU¹, C. URAN¹, M. VINCK^{1,2};
¹Ernst Strüngmann Inst. (ESI) for Neurosci. in Cooperation with Max Planck Society, Frankfurt am Main, Germany; ²Dept. of Neuroinformatics, Donders Ctr. for Neurosci., Radboud University Nijmegen, Netherlands

Abstract: Neural computation depends on inter-areal signal transformations, which are determined by the input-output (I/O) functions of individual neurons or also of network interactions. Due to intrinsic neuronal properties and inter-neuronal interactions, networks can show preferences for synaptic inputs in certain frequencies, e.g. in the form of low-pass filtering or resonance [Izhikevich 2003, Cardin et al 2009, Lewis et al 2021, Pike et al 2000]. High-density silicon-probe recordings were made from area V1 and V2 in awake mice. We used optogenetic stimulation (continuous (1s), pulses (5ms), sinusoids) with an opsin having fast kinetics (Chronos) to gain precise temporal control over specific cell populations and to study the I/O functions of (1) V1 neurons expressing the opsin, (2) excitatory and inhibitory V1 and V2 neurons not expressing the opsin. Additionally, we used channelrhodopsin (ChR2), a slower opsin, to compare the resonance properties that arise from the optogenetic activation. We determined the dependence of firing rates, phase-locking, coherence and power on stimulation

frequency. Spike-laser phase-locking increased steeply towards higher frequencies, indicating that opto-tagged excitatory neurons high-pass filtered optogenetic inputs. This was explained by the narrowing of phase distributions with frequency, suggesting a non-linear I/O transformation. Fast-spiking interneurons closely followed the phase-locking of excitatory neurons without exhibiting resonance. Surprisingly, non-opto-tagged excitatory neurons, both in V1 and V2, phase-locked predominantly to low-frequency optogenetic inputs. Next, we compared different measures of I/O transformations. Strikingly, opto-tagged excitatory neurons responded with similar firing rates to all optogenetic input frequencies. Likewise, spike-field coherence was relatively flat, indicating optogenetic inputs were reliably encoded at all frequencies. Thus, in the absence of synaptic filtering, neurons encode and respond to different frequencies very similarly, despite being more phase-locked to high frequencies. Using ChR2 prevents phase locking at high frequencies and creates a gamma frequency at the LFP spectrum. Together, these findings indicate a major difference between the filtering of synaptic and optogenetically-induced inputs. They further suggest that area V1 does not exhibit narrow band-pass resonance but rather increase of laser phase locking throughout frequencies without increase on the neuronal drive and reliability. Finally we suggest that locally as well as inter-areal synaptic communication is most effective at low frequencies.

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Poster

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Title: Sub-harmonics in human brainwaves: a network theory perspective

Authors: ***R. PHOGAT**¹, P. PARMANANDA¹, A. PRASAD²;

¹Indian Inst. of Technol. Bombay, Mumbai, India; ²Colorado State Univ., Fort Collins, CO

Abstract: The entrainment of human brainwaves to external stimulation is a well reported phenomenon. When exposed to a periodic visual stimulation at 10 Hz, our brainwaves exhibit an increment in power at the fundamental (10 Hz) and subsequent harmonics (20, 30, 40 Hz...) of this stimulation frequency. This kind of entrainment is well studied and widely reported. We report a less explored phenomenon of sub-harmonic entrainment, where, the same 10 Hz visual stimulation elicits a response at 5 Hz (sub-harmonic of 10 Hz) when the visual stimulus intensity is high. Interestingly, such a sub-harmonic entrainment was not observed with a 6 Hz stimulation of an equally high intensity. These results were primarily explored and explained using

bifurcation analysis in a Neural Mass Model (NMM) of the visual cortex. Along with this, we are currently exploring how the sub-harmonic entrainment changes the network connectivity and entropy production across various electrodes covering the cortex. This will help elucidate the network connectivity changes that take place during entrainment as compared to a resting state brainwave data.

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Poster

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Title: Sensory prediction error in cortical circuits develops across adolescence in a sex-specific manner

Authors: *J. M. ROSS^{1,2}, J. P. HAMM^{1,2,3};

¹Neurosci. Inst., Georgia State Univ., Atlanta, GA; ²Ctr. for Behavioral Neuroscience, Georgia State Univ., Atlanta, GA; ³Ctr. for Neuroinflam. and Cardiometabolic Diseases, Georgia State Univ., Atlanta, GA

Abstract: In natural environments, organisms are inundated with sensory information. The ability to process this information within the context of previously experienced stimuli is crucial to success. One theoretical framework for explaining this computationally complex task is predictive coding, in which brain networks continuously generate and test predictive models based on experience. Past work, using simple “oddball” sequences while simultaneously recording activity in adult mammalian sensory cortices, demonstrates that neurons exhibit reduced responses to repetitive stimuli known as “stimulus specific adaptation” (SSA) while rare stimuli evoke augmented neuronal responses, referred to as “deviance detection” (DD). These context-specific responses in adult mammalian cortex are putative signals of predictive processing and sensory prediction error, respectively, but it remains unknown the extent to which this emerges postnatally across adolescence. This could hold clues to the underlying neurobiology of predictive coding in cortical networks, as well as its significance for cognitive development. To assess adolescent development of SSA and DD, we employed two-photon calcium imaging of pyramidal cells (PYRs) in L2/3 of primary visual cortex (V1). We assessed stimulus-evoked activity of PYRs during visual oddball and many standards control sequences, which allows direct comparison of responses to the same visual stimulus in different contexts -

when the stimulus is expected (redundant), when it deviates from expectation (deviant), and when it is neither expected nor deviates expectations (control). Experiments utilized awake mice at distinct ages relevant to postnatal brain development: early adolescence (P28), late adolescence (P42), and adulthood (P84). At the population level, while both SSA and DD are evident in P28 females and persist into adulthood, SSA is absent in P28 males and DD is not apparent in males until adulthood. Interestingly DD in adult males is stronger than in adult females. This suggests that the developmental trajectory of prediction error signals in V1, and possibly the underlying mechanisms producing these signals, differs dramatically between males and females. Ongoing work aims to further characterize the postnatal development of predictive coding and understand underlying mechanisms that contribute to differential DD development between males and females. Altogether, this work may inform our understanding of how postnatal brain development contributes to SSA and DD and highlight sexual dimorphisms that may be relevant to neuropsychiatric conditions related to aberrant predictive coding.

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Poster

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Title: Characterizing the connectivity profiles of patch-seq derived types using morphological analysis of electron microscopy data

Authors: *E. M. JOYCE, M. MALLORY, C. M. SCHNEIDER-MIZELL, C. GAMLIN, M. CONSORTIUM, N. GOUWENS, S. SORENSEN, N. MACARICO DA COSTA, F. COLLMAN;
Allen Inst. for Brain Sci., Seattle, WA

Abstract: The diverse cell types of mammalian neocortex have been mapped systematically using a variety of molecular, electrophysiological and morphological approaches. Each modality offers new perspectives on the variation of biological processes underlying cell type specialization. Large scale electron microscopy (EM) data offers to add connectivity information to this perspective. Here we explore the relationship between the morphological and connectivity profiles of excitatory neurons in mouse visual cortex by comparing patch-seq data that contains electrophysiological, morphological, and transcriptomic (MET) data, to the IARPA MICrONS large scale EM dataset (available on microns-explorer.org). We performed a large-scale skeletonization of excitatory neurons within the EM data, converting more than 65,000 neurons

to a format that allows for direct comparison with light microscopy reconstructions. We fit and characterize classifiers that use features of neuronal morphology to categorize individual excitatory neurons into one of 16 MET types with an average accuracy of 86 +/- 6.2% as measured on a test set of patch-seq neurons. By applying these classifiers to the EM skeletons we can characterize the similarities and differences of pre-synaptic and post-synaptic connectivity profiles within and across excitatory MET types. Specifically, we examine whether there are individual interneurons which show selective connectivity preference for MET defined subclasses. These analyses shed light on which features of neuronal classification are coherent across data modalities and help orient molecular cortical cell-types into a circuit context.

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Poster

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Title: Mef2c-mediated neurodevelopmental dysfunction in cortical parvalbumin inhibitory interneurons

Authors: *C. WARD, E. SABRI, K. NASRALLAH, D. TRAN, P. E. CASTILLO, R. BATISTA-BRITO;

Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Central to understanding the role of inhibitory neurons in cortical network function, is identifying their contributions through a neurodevelopmental lens. To this end, we disrupted the expression of a critical regulator of neuronal maturation in interneuron precursors. Myocyte enhancer factor-2C (Mef2c) is a transcription factor linked to neurodevelopmental disorders that regulates the expression of genes involved in the differentiation, migration, survival, and synaptic function of excitatory neurons, yet little is known about its role in inhibitory neurons. Among the three major subtypes of cortical inhibitory interneurons, neurons expressing the calcium-binding protein parvalbumin (PV-INs) are highly enriched for Mef2c expression. We therefore examined whether embryonic removal of Mef2c in PV-IN precursors leads to signatures of impaired maturation in mouse cortical PV-INs. Using slice electrophysiology within the mouse primary visual cortex (V1), we found that this manipulation reduced both the

excitatory drive onto PV-INs and the inhibitory drive onto excitatory cells, suggesting a hyperexcitable cortical network. To further probe this possibility, we performed extracellular recordings within both V1 and the anterior cingulate cortex (ACC) of awake mice while monitoring behavioral state. We observed that mutant mice showed altered temporal patterns of network activity. In particular, mutant mice exhibited synchronous network events that were reflected as high amplitude fluctuations in LFP and synchronous spiking activity across the network, which were enriched during quiescence. These events were observed both in the V1 and ACC. Within V1, mutant animals displayed decreased visual responsiveness with weaker encoding of visual stimuli such as orientation selectivity. Finally, to understand the global effects of impaired PV-IN development, we performed behavioral phenotyping. Mutant animals displayed phenotypes such as paw clamping, increased locomotor activity, and impaired spatial memory. Together, our data show that early removal of Mef2c impacts the development of PV-INs, leading to abnormal circuit function and behavior.

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Poster

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Title: Excitatory inputs to somatostatin interneurons modulate context-dependence of the visual cortex

Authors: *C. CAMMARATA¹, T. M. HAWLEY¹, J. Y. LI¹, S.-X. LIM², M. R. TADROSS^{1,2}, L. L. GLICKFELD¹;

¹Neurobio., ²Biomed. Engin., Duke Univ., Durham, NC

Abstract: Interneurons are thought to play a central role in the behavioral-state dependence of primary visual cortex (V1). In particular, somatostatin+ (SOM) interneurons in layer 2/3 are driven by recurrent excitation of local Pyr cells and inhibit the dendrites of this same Pyr population, making SOM cells uniquely positioned to sample and regulate activity among these output neurons. SOM cells are implicated in the effects of behavioral state on visual responses, such as the elevation of stimulus responses observed during running compared to stationary periods, but the nature of this role remains controversial. Here, we used Drugs Acutely Restricted by Tethering (DART) to deliver an AMPA receptor antagonist, YM90K, selectively to SOM cells, attenuating their ability to sample local Pyr activity without preventing SOM cells from firing action potentials or from releasing inhibitory neurotransmitter onto Pyr cells. We

used two-photon calcium imaging to monitor the activity of SOM cells and nearby putative Pyr cells in response to visual stimuli. By tracking identified neurons across imaging sessions, we obtained within-cell comparisons of activity before and after DART-YM90K. Across six mice (five females and one male), we find that during stationary periods, reduction of glutamatergic input to SOM cells decreased visually-evoked SOM activity and increased Pyr responses, consistent with a disinhibitory role of SOM cells. However, during locomotion we saw not only elevated Pyr responses but, paradoxically, SOM responses were also higher following DART-YM90K. Thus, reducing AMPA input on SOM cells altered the relationship of SOM responsivity to Pyr responsivity: while under conditions of weak excitatory drive the ratio of SOM to Pyr activity was decreased by DART-YM90K, as may be expected from blocking excitatory transmission onto SOM cells, the rate at which SOM activity rose as Pyr activity increased was instead elevated. Our results suggest that reducing AMPAR input onto SOM cells enhances recurrent activity, lending support to the idea that regulating recurrent connections among Pyr cells is a key aspect of how SOM cells contribute to shaping visual responses. Moreover, this hints at a complex change in the operating regime of visual cortex circuits based on behavioral state that we will continue to probe in future work.

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Poster

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Topic: D.06. Vision

Support: Sheryl and Dan Tishman Postdoctoral Fellowship
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Title: Role of PV interneurons in the developmental emergence of the V1 sensory processing

Authors: *M. SALMI, E. SABRI, R. BATISTA-BRITO;
Albert Einstein Col. of Med. Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med. Dominick P. Purpura Dept. of Neurosci., Bronx, NY

Abstract: Sensory perception allows us to receive and integrate information from the environment, a process that starts right from birth. Indeed, newborns can interact with their environment and perform sensorimotor tasks soon after birth, without any previous experience of patterned sensory input. Understanding how neurons assemble into circuits that developmentally prepare to encode visual information accordingly to behavioral states is still poorly understood and is likely to provide critical insight not only into how these circuits function, but also how they malfunction in various neurological conditions. Parvalbumin (PV) fast-spiking expressing inhibitory interneurons (INs) are the main source of cortical GABAergic inhibition. The

maturation of PV INs has been shown to drive the timing of the critical period for visual plasticity, however their contribution towards in vivo sculpting of early sensory network activity remains largely unknown. More specifically how PV INs shape the network to support the emergent visual processing in V1 throughout the visual onset period (eye opening) has not been previously addressed. Here, we selectively disrupt PV INs function during embryonic development by removing Mef2c, an activity-dependent transcriptional factor, from PV INs progenitors. Longitudinal in vivo recordings in the murine V1 around the time of eye-opening (from P10 to P17), revealed that embryonic removal of Mef2c from PV INs leads to (1) an increase of spindle burst activity and network synchrony, as well as to (2) a decrease of visual responses and orientation selectivity in a state-dependent manner. Taken together our data show that early on PV IN progenitors shape the development and maturation of the V1 network activity and as consequences the proper development and maturation of the emergent visual sensory processing.

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Poster

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Support: NIMH R00-MH115082

Title: Pre-frontal influence in context processing in early visual cortex in mice

Authors: *G. BASTOS, J. T. HOLMES, A. M. RADER, J. P. HAMM;
Georgia State Univ., Atlanta, GA

Abstract: During sensory processing, the brain needs to account for the context of stimuli. This optimizes brain function, allocating energy to contextually deviant stimuli over repetitive - or predictable - stimuli. In the mouse visual cortex (V1), overall neuronal activity is augmented during a deviant stimulus, which is called “deviance detection” (DD), while in a predictable context (e.g. wherein stimuli are repetitive or appear in a regular sequence) V1 decreases its activation, a phenomenon called “response adaptation” (RA). This is hypothesized to be driven by higher areas of the cortex that are able to build predictions about contextually likely sensory stimuli to modulate early sensory processing, suppressing responses to predictable stimuli (RA) and amplifying responses to deviant stimuli (DD). However, there is not much consensus on the how frontal areas influence local processing, and whether RA and DD are interdependent. Here we combined 2-photon calcium imaging in V1 with local field potential (LFP) measurements of network-level synchrony to examine the circuit basis for context processing in mice. We sought to understand the role of prefrontal cortex in driving neuronal adaptation found in V1 during predictive processing. While past work has employed simple “oddball” paradigms, involving one

repeated stimulus interspersed with rare “deviants”, V1 response adaptation (RA) in this paradigm may involve both top-down modulation as well as simple stimulus specific adaption inherited from upstream regions (e.g. thalamus). To address this, we used a modified oddball paradigm called the “cascade oddball” which involves a highly predictable stimulus sequence, where the sequence itself is repetitive but the individual stimuli are not. We found that the anterior cingulate area (prefrontal area of mouse cortex, ACa), and V1 synchronize in the theta band (6-10HZ) during both runs. Importantly, the directionality of that synchrony is driven by ACC influence in V1 (but not V1 to ACa). This distinction was most pronounced during the cascade oddball, pointing to a bigger role of frontal areas in local processing when the prediction context is more complex. Further, V1 displayed DD and RA during the regular oddball paradigm, but only DD during the cascade paradigm. Suppression of V1 VIP neurons also disrupted the ACa/V1 synchrony and the typical DD responses during both paradigms. Together with our past work, these data suggest that “context” is communicated from ACa to V1 via VIPs, giving rise to augmented processing of deviant stimuli (DD). On the other hand, RA seen in the oddball paradigm mainly reflects adaptations in local neurons with stimulus preferences.

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Poster

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Support: R01EY028905
R21EY030291

Title: Corticotectal Neurons and Putative Fast Spike Interneurons: Distinct Properties of Two Types of Complex Cells in Layer 5 of Primary Visual Cortex

Authors: *C. SU¹, Y. BERESHPOLOVA¹, R. F. MENDES-PLATT¹, J.-M. ALONSO², H. A. SWADLOW¹;

¹Dept. of Psychological Sci., Univ. of Connecticut, Storrs, CT; ²SUNY Col. of Optometry, New York City, NY

Abstract: The receptive fields (RFs) of most neurons of primary visual cortex can be classified as simple or complex, either using the classical criteria of Hubel and Wiesel (1962) or by linearity of their responses to drifting gratings (F1/F0 ratios) (Skottun et al., 1991). Here, we investigate two types of cells found in visual cortical layer 5 of awake rabbits: (1) corticotectal (CTect) neurons, and (2) putative fast-spike inhibitory interneurons (suspected inhibitory interneurons, SINs). CTect neurons were identified by antidromic responses following electrical stimulation of the superior colliculus, and the SINs by their high-frequency response to electrical stimulation of the thalamus (3+ spikes at >600 Hz) and by short-duration spikes. All of the CTect

neurons ($n = 41$) and all SINS ($n = 21$) except one had complex RFs, as defined by nonlinear responses to a drifting grating ($F1/F0$ ratios of <1) presented at near-optimal orientation, spatial frequency, temporal frequency, and contrast. We studied 21 SINS, and the spatial RFs of all of them could be reliably mapped using sparse noise stimulation. 34 CTect neurons had RFs that could be reliably mapped using sparse noise stimulation and 7 CTect neurons could not be mapped. However, whereas most of the SINS (20/21) had spatial RFs consisting of highly overlapping ON and OFF subfields, the spatial structure of CTect neurons was heterogeneous, including a single ON or OFF subfield ($n = 14$), spatially separated ON and OFF subfields ($n = 10$), and spatially overlapped subfields ($n = 10$). Moreover, as is seen in cortical simple cells, the spatial structure of the CTect neurons with separated subfields was highly predictive of the preferred orientation when stimulating with the drifting grating (i.e., CTect neurons with vertically separated subfields tended to prefer horizontal gratings moving up and/or down, and those with horizontally separated subfields preferred vertical gratings moving right and/or left). Thus, such CTect neurons resemble simple cells, as defined by the spatial structure of the receptive fields, but they resemble complex cells as defined by the nonlinearity of their spatial summation (all $F1/F0$ ratios < 1). By contrast, the RFs of L5 SINS are mostly highly overlapped, and they can be classified as “complex” both by the spatial structure of their receptive fields and by the nonlinear spatial summation within their RFs. These results show that, even within the same cortical layer, differing cell types that display nonlinear spatial summation may display heterogeneity of spatial RF properties, some of which closely resemble the classical simple cells of Hubel and Wiesel. Supported by R01EY028905 and R21EY030291

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Poster

469. Structure and Function of Cortical Visual Circuits

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Title: Inhibitory connectivity across cell types in an electron microscopy reconstruction of a mouse cortical column

Authors: *C. M. SCHNEIDER-MIZELL¹, A. L. BODOR¹, J. BUCHANAN³, S. SESHAMAN⁵, L. ELABBADY¹, D. BRITTAIN⁴, S. DORKENWALD⁶, M. M. TAKENO², J. REIMER⁸, A. S. TOLIAS⁸, H. SEUNG⁷, R. REID³, F. C. COLLMAN¹, N. M. DA COSTA¹; ²Neural Coding, ¹Allen Inst. For Brain Sci., Seattle, WA; ³Neural Coding, ⁴Allen Inst. for Brain

Sci., Seattle, WA; ⁵Synapse Biol., Allen Inst., Seattle, WA; ⁷Princeton Neurosci. Inst., ⁶Princeton Univ., Princeton, NJ; ⁸Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Mammalian neocortex features a multitude of neuronal cell types, each with characteristic anatomical, molecular and functional properties. Synaptic connectivity powerfully shapes how each cell type participates in the cortical circuit, both in how a cell integrates input and distributes its output back to the network. However, comprehensively mapping synapses and connectivity across a large population of neurons remains difficult. Here, we used millimeter-scale volumetric electron microscopy to create a “core sample” of the full dendritic anatomy of neurons with cell bodies along a 100 micron column spanning all layers of mouse primary visual cortex, resulting in a proofread reconstruction spanning more than 1300 cells and 4.4 million synaptic inputs. Quantitative features were used to classify neurons into subclasses with differences not only in morphology, but synaptic properties such as input density and median synapse size. To relate these subclasses to synaptic connectivity, we further reconstructed extensive axonal arbors for the 160 inhibitory interneurons in the column, mapping more than 70,000 synapses onto columnar cells. While in aggregate, the connectivity corresponded well to a conventional understanding of the cortical circuit, the combination of single-cell connectivity and anatomically-defined subclasses revealed many surprises. We found diverse patterns of synaptic targeting, with both broad targeting and subclass-selective cells, including interneurons specifically targeting excitatory subclasses beyond laminar categories, as well as both expected and unexpected categories of disinhibitory specialists. Taken together, these data provide an unbiased synapse-level characterization of the cortical population and reveal new aspects of its connectivity, suggesting that activity is controlled by inhibitory neurons at multiple levels of precision, from specific targeting of narrow subpopulations to widespread output across many layers.

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Poster

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Title: An intracellular analysis of the origin of complex cells in mouse visual cortex

Authors: J. J. PATTADKAL¹, *N. PRIEBE²;

¹Neurosci., The Univ. of Texas at Austin, Austin, TX; ²Univ. Texas, Austin, Austin, TX

Abstract: Simple and complex cells represent two distinct stages of cortical visual processing in carnivores and primates that are thought to emerge from a circuit hierarchy. Orientation selectivity first emerges in simple cells from integration of thalamic relay cell inputs; nonlinear spatial invariance next emerges in complex cells from the integration cortical simple cells inputs. We examined this hierarchy in mouse visual cortex using in-vivo whole cell electrophysiology and cortical silencing via optogenetics (Li et al 2013, Lien and Scanziani, 2013, Barbera et al, 2021). We find that majority of the mouse V1 neurons, including both simple and complex cells, receive direct thalamic input (n = 28/36). In accordance with the hierarchical model, we find that the subset of neurons that did not receive any thalamic input exhibited greater nonlinear spatial invariance, consistent with complex cell behavior. For those neurons that did receive direct thalamic input we compared the orientation selectivity and spatial invariance of the direct thalamic input to the cortical input. We consider three models for emergence of complex cell orientation selectivity and spatial invariance. The thalamic input onto cortical complex cell could be orientation-selective and linear, suggesting a cortical emergence of complex cell behavior. The thalamic input could be non-linear but lack orientation tuning, in which case orientation selectivity in complex cells would emerge within the cortex. Lastly, the thalamic input to cortical cells could be both orientation-selective and non-linear, suggesting a thalamic basis for orientation selectivity and spatial invariance. We find that, in contrast to the hierarchical model, aggregate thalamic input onto complex cells exhibits spatial invariance. We assayed this spatial nonlinearity using both responses to drifting gratings as well as responses to contrast reversing gratings. Comparing the thalamic vs total response to drifting gratings, we find a high correlation in the two modulation ratios (correlation coefficient = 0.54). We next studied the orientation selectivity of the thalamic input and its origin. We find that the mean and modulated components of the thalamic input both exhibit comparable orientation selectivity, indicating that orientation selectivity in visual cortex originates from orientation-selective thalamic inputs. Together, we find evidence for non-linear and orientation-selective thalamic input that underlies the formation of complex cells in mouse V1. Our results demonstrate an alternate connectivity model in the mouse visual pathway forms the basis for generation of simple and complex receptive fields.

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Poster

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Title: Binocular receptive field match of ON and OFF afferents in primary visual cortex

Authors: *F. OLIANEZHAD, J. JIN, J. M. ALONSO;
SUNY Col. of Optometry, State Univ. of New York, New York, NY

Abstract: ON and OFF thalamic afferents from the two eyes converge in primary visual cortex to form binocular receptive fields. To investigate the differences in the binocular match across afferent types and receptive-field properties, we performed recordings with multielectrode arrays from cat visual cortex and measured cortical responses from the contralateral and ipsilateral eye to sparse noise stimuli and moving bars. Our findings demonstrate that, regardless of dominant afferent type, the cortical receptive fields from the two eyes are precisely matched in orientation preference ($r=0.96$, $n=541$, $p<0.00001$), direction preference ($r=0.58$, $n=541$, $p<0.00001$), orientation selectivity ($r=0.52$, $n=541$, $p<0.00001$), direction selectivity ($r=0.51$, $n=541$, $p<0.00001$), response latency ($r=0.45$, $n=495$, $p<0.00001$), and dominant contrast polarity ($r=0.5$, $n=92$, $p<0.00001$). However, whereas the binocular match for orientation/direction preference and selectivity is similar across the cortex, the binocular match for response latency and dominant polarity increases with cortical binocularity ($r=0.99$ and slope=12% for response latency; $r=0.99$ and slope=30% for polarity; $p=0.0002$ and $n=6$ binocularity bins for both). The binocular match was more accurate in OFF than ON spatiotemporal receptive fields (average binocular correlation: 0.72 ± 0.22 , $n=1,374$ for OFF receptive fields versus 0.58 ± 0.25 , $n=1,374$ for ON receptive fields, $p<0.00001$, Wilcoxon test) and more accurate than the monocular match between ON and OFF spatiotemporal receptive fields, which was similar in both eyes (contralateral: 0.48 ± 0.31 , $n=1,374$; ipsilateral: 0.46 ± 0.3 , $n=1,374$, $p=0.0499$, Wilcoxon test). On average, the binocular correlation mismatch was nearly one order of magnitude lower than the monocular ON-OFF correlation mismatch (correlation difference: 0.02 for contralateral-ipsilateral versus 0.14 for ON-OFF, $p < 0.00001$, Wilcoxon test). The binocular receptive field match calculated from ON-OFF subtraction was also more accurate in cortical sites tuned to horizontal than vertical orientations (0.45 ± 0.1 , $n=86$ cortical sites with 0 ± 45 degrees orientation versus 0.41 ± 0.09 , $n=70$ cortical sites with 90 ± 45 degrees orientation, $p=0.004$, Wilcoxon test, only correlation values >0.3 included in comparison). We conclude that the cortical receptive fields from the two eyes are exquisitely matched in stimulus preference and selectivity, response latency, and dominance contrast polarity. Moreover, the accuracy of the match is highest at binocular cortical regions dominated by OFF afferents tuned to horizontal stimulus orientations.

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Poster

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Topic: D.06. Vision

Support: VE 938/2-1

Title: Isolating the ongoing impact of specific cell-types onto recurrent circuits in-vivo

Authors: *D. ERIKSSON, C. RAMANATHAN, J. VEIT;
Albert Ludwig Univ. of Freiburg, Freiburg, Germany

Abstract: Recurrent circuits in the brain have the means to generate activity sequences and high-dimensional trajectories for sensory prediction, problem-solving, and motor output. However, recurrent circuits are inherently difficult to study. By reverberating previous activity states, they cause long-term correlations that undermine any mathematical scrutiny of the underlying physiology.

Here we asked whether it is possible to causally estimate the isolated impact of ongoing parvalbumin- (PV) and somatostatin-cell (SOM) inhibition onto principal cells (PC). These interneuron-types not only form some of the strongest loops in the brain, but the resulting dynamics - for example, gamma oscillations - are thought to govern sensory and cognitive processing. We aimed at extracting an “impact function” $T = IF(S,B)$, where T is the PC activity, S is the PV (or SOM) activity, and B is the background PC activity during inhibition of PV (or SOM) according to the triplet-principle (S, B, and T) described previously (Eriksson 2017, *Front. Neural Circuits*).

To this end we expressed eArch 3.0 (n=1 mice) or Jaws (n=7 mice) in PV or SOM cells. We recorded and manipulated activity using ultrathin side-light optical fibers attached to Neuropixels probes in the primary visual cortex of the awake head-fixed mouse. We registered the PV/SOM activity (S) before a brief (20ms), transient suppression of PV/SOM cells, as well as the activity of PC neurons before (T) and during (B) the inhibition.

As a proof of concept, we correlated the pre-suppression activity of each photo-tagged neuron (S) with the resulting activity modulation (T-B) of each target neuron. In contrast to the classical cross-correlation (between S and T) which has a positive peak due to the balanced excitation and inhibition, the impact function showed a negative correlation, revealing the suppressive influence of PV and SOM neurons on their targets.

We quantified how the impact function decayed with the distance between the source and target neuron. Consistent with spurious correlations from recurrent dynamics and common inputs, the classical cross-correlation resulted in a spatial decay that was 11-67.5% larger than that of the impact function. This reveals a new method to more accurately quantify computations in brain circuits, even if they are embedded in extensive recurrent networks.

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Poster

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Program #/Poster #: 469.18

Topic: D.06. Vision

Title: Feedforward and feedback contribution to contrast gain in mouse primary visual cortex and thalamus

Authors: *B. P. RUMMELL¹, A. TZANOU¹, C. URAN^{1,2}, M. SCHNEIDER^{1,2}, M. VINCK^{1,2};
¹Ernst Strüngmann Inst., Ernst Strüngmann Inst. for Neurosci., Frankfurt Am Main, Germany;

²Dept. of Neuroinformatics, Donders Ctr. for Neuroscience, Radboud Univ., Nijmegen, Netherlands

Abstract: Contrast is a driving feature of the visual system. In the primary visual cortex (V1), neurons respond to a dynamic range of visual contrasts through transformation of inputs they receive from the lateral geniculate nucleus (LGN). Typically, neurons in V1 increase their spike rate monotonically with increasing stimulus contrast, until they saturate, forming an S-shaped contrast-response function. We examined the functional role of LGN input in this process, by optogenetically inhibiting synaptic transmission of LGN thalamocortical inputs in V1 using the inhibitory opsin eOPN3. While presenting drifting gratings of different contrasts, we measured single-unit activity using Neuropixels simultaneously recorded in both V1 and LGN in awake, head-fixed mice. We found that responses to visual stimuli at intermediate contrasts in V1 neurons were reduced selectively during LGN terminal inhibition in a laminar dependent manner. Moreover, we observed subtle reductions in both evoked and spontaneous rates in recorded LGN neurons through inactivation of their presynaptic terminals in V1. Our results show that targeted inactivation of LGN inputs in V1 can act by decreasing the effective contrast of a stimulus, and provide support for a modulatory role of corticothalamic feedback in LGN.

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Poster

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R21EY030291

Title: Retinotopic scatter of cortical receptive fields is highly dependent upon cortical cell type in rabbit V1

Authors: *Y. BERESHPOLOVA¹, C. SU¹, R. F. PLATT¹, J. ALONSO², H. A. SWADLOW¹;
¹Dept. of Psychological Sci., Univ. of Connecticut, Storrs, CT; ²Biol. and Visual Sci., SUNY Optometry, New York, NY

Abstract: A detailed and accurate description of how the visual space is represented in the cortex at the level of local networks of neighboring excitatory and inhibitory neurons is crucial for understanding visual processing. In the visual cortex of cats and monkeys, neighboring excitatory neurons respond to similar stimulus positions in the retina and their retinotopic scatter is very small. Here we examine the retinotopic scatter of receptive fields (RF) of different cell classes in the deep layers of rabbit visual cortex. We compared the RF center positions of single cortical cells and the neighboring multi-unit activity (MUA, recorded on the same

microelectrode) using reverse correlation from sparse noise stimuli. MUA represents the average spiking of small neuronal population close to the vicinity of the microelectrode, and vertical penetrations through the cortical layers produced superimposed MUA RFs with little scatter in either size or position. Fast-spike suspected inhibitory interneurons (SINs), were identified by high-frequency burst of spikes (> 600 Hz) to thalamic electrical stimulation (Swadlow, 2003). They were recorded in all cortical layers, had complex RFs based on their linearity of spatial summation to optimal drifting gratings ($F1/F0 < 1$), and showed a strong spatial overlap of ON and OFF subregions. In layer 4 (L4), excitatory cells had simple RFs with $F1/F0 > 1$ and non-overlapping, spatially restricted ON and OFF subregions. In layer 6 (L6), corticogeniculate (CG) neurons were identified by antidromic activation from thalamus and also had simple RFs. We show that the retinotopic scatter in rabbit V1 is highly dependent on cortical cell type. Thus, in L4 and L6, putative fast-spike interneurons have RFs largely superimposed with the MUA RFs and display smooth retinotopic organization. However, putative excitatory neurons in the same layers (L4 simple and L6 CG cells) show less retinotopic precision and have RF centers 4 times more scattered. The differences in RF scatter between SINs and excitatory neurons were very pronounced and highly significant ($P < 0.001$ for L4, $P < 0.001$ for L6, Wilcoxon test for all comparisons). Moreover, among excitatory cells, the retinotopic scatter was significantly lower for OFF than ON RF subregions ($P < 0.01$). Since the average RF size of a geniculate input at the same eccentricity is 3.9 deg, the average RF scatter of excitatory neurons spanned from 0.7 (L4) to 1 (L6) geniculate RFs. We conclude that the retinotopic scatter is larger for excitatory than inhibitory neurons at the input layers of visual cortex, a finding that is consistent with their differences in thalamocortical convergence (Bereshpolova et al., 2020).

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Poster

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Support: CRSNG

Title: Differential monosynaptic feedback circuitry of the HVAs to V1 in mouse.

Authors: *G. LALIBERTE, A. FILION, D. BOIRE;
Anat., UQTR, Trois-Rivieres, QC, Canada

Abstract: The cortical treatment of sensory information relies on a feedforward (FF) stream that conveys information from the receptor to the primary visual cortex and subsequently to multiple surrounding high visual areas (HVAs), and feedback (FB) path from higher order areas projecting back to V1 and subcortical relays that provides contextual information to early stages

of cortical treatment of the information. Each HVAs demonstrate specific affinities to temporal and spatial frequencies and, as observed in primates, can be segregated by these preferences in a dorsal path, responsible for object localization, and a ventral path, for object recognition. FB projections to V1 originate mainly from the HVAs but also from multiple sensory, motor, or associative cortices. We hypothesize that HVAs might receive differential FB projections from cortices corresponding to their specific role in visual processing. Three HVAs (AL, LM and PM) were selected for their differential contribution to the dorsal (AL and PM) or ventral path (LM) and by their localization around V1, lateral (AL and LM) and median (PM). To investigate the afferent feedback connectome of these HVAs, ΔG -rabies was injected in HVAs. To further investigate the brain wide afferents that have monosynaptic contacts on HVAs neurons projecting to V1, envA- ΔG -rabies and Cre-expressing AAV were injected in the HVAs combined with Cre-dependent oG and TVA retrograde helper virus injected in V1. Labelled neurons were charted throughout the cortical mantle and registered to the neurons in the Allen Brain CCF atlas (Neuroinfo, MBF Biosciences). Results show high interconnectivity between HVAs. However, AL received abundant projections from other cortical sensory areas such as auditory and somatosensory related cortices while PM receives more projection from associative areas such as the anterior cingulate, orbital, and retrosplenial areas. Finally, LM received projections from other ventral path HVAs, as such as Po and Por but also numerous projections from PM and retrosplenial area. Interestingly, although AL and PM are both classified as dorsal visual path, AL received more projections from LM than from PM. Neurons with monosynaptic connections with HVA neurons projecting directly to V1 were less common. In every case, most of the input on these V1 projecting neurons are in the same HVA and surround the seed neurons. However, those neurons in AL also receive important projections from higher auditory areas and those in PM received input from retrosplenial area. These results demonstrate that these HVAs receive differential FB input, which could be highly locally processed before being conveyed to V1.

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Poster

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Title: Strong tuning for stereoscopic depth indicates orientation-specific recurrent circuitry in tree shrew V1

Authors: *S. TANABE, J. FU, J. CANG;
Univ. of Virginia, Univ. of Virginia, Charlottesville, VA

Abstract: Neurons in the primary visual cortex (V1) are tuned to the specific disparity between retinal images in the two eyes, which are thought to be the neural substrate for computing stereoscopic depth. Here, we study disparity tuning of V1 neurons in tree shrews. Tree shrews navigate arboreal 3D environments, and so presumably possess better depth computation than mice, a model used in recent disparity studies. Indeed, we find that tree shrew V1 neurons display highly selective responses to narrow ranges of disparity in random dot stereograms (RDSs). The strength of tuning had a median disparity-discrimination index (DDI) of 0.53 (n = 307), much higher than that of mouse V1 neurons (n = 181; median DDI = 0.42; Mann-Whitney U test, $p=6.1 \times 10^{-25}$). Surprisingly, neurons in both species showed similarly strong tuning to drifting sinusoidal gratings of varying interocular phase differences. In other words, tree shrew V1 neurons show consistently strong tuning to RDS and gratings, whereas mouse V1 neurons do not. The dissimilarity of disparity tuning between the two species occurred specifically when the stimulus was an RDS, which suggests that there is nonlinear integration of orientation signals occurring within the circuitry for disparity tuning. To understand the underlying mechanisms of these findings, we postulate a network model to reproduce this integration process. The network is composed of an excitatory population with disparity-energy model-like receptive fields, each paired with an inhibitory neuron. The neurons comprise an inhibition-stabilized network. The model reproduces the key observations in the two species with circuitries that are supported by their respective cortical organizations (columnar organization in tree shrew V1 and salt-and-pepper patterns in mouse V1). Finally, we validate the model by classifying the recorded population into regular-spiking and fast-spiking neurons and confirm their predicted disparity tuning to the two types of stimuli in both species. Together, our studies establish a solid foundation for using tree shrews in studying circuit mechanisms of disparity selectivity and raise an exciting possibility of how cortical columns could be uniquely important in computing stereoscopic depth.

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Poster

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Title: Electrophysiological characterization of binocular responses in the primary visual cortex of awake mice

Authors: *J. FU¹, S. TANABE², J. CANG^{3,2};

¹Neurosci. Grad. Program, ²Psychology, ³Biol., Univ. of Virginia, Charlottesville, VA

Abstract: The brain combines 2-dimensional images from the eyes to form a representation of 3-dimensional surroundings. This process, stereopsis, is a key component of depth perception and at the cellular level originates from disparity selective neurons in the primary visual cortex (V1). Although response properties such as ocular dominance and interocular matching have been reported as measures of binocular vision, disparity selectivity is unique in that it only emerges following binocular stimulation and subsequent integration of inputs from the two eyes, making disparity selectivity a more fundamental metric for assessing binocularity. To characterize the relationship between these multiple response properties, we performed electrophysiological recordings in the V1 of awake mice (n=22) while they viewed binocular grating stimuli via a polarized projector system. An ocular dominance index (ODI), difference between preferred orientation (ΔO) to assess interocular matching, and phase disparity selectivity index (PDSI) were calculated for every visually responsive neuron (n=275). We found no significant correlation between ΔO and ODI ($r(174)=-0.03$, $p=0.73$), PDSI and ODI ($r(176)=0.03$, $p=0.68$), or PDSI and ΔO ($r(174)=-0.08$, $p=0.27$), indicating that these response properties are likely computed via independent mechanisms. There was also no relationship between PDSI and the stimulus orientation at which the strongest phase disparity tuning was observed ($F_{(3,250)}=0.12$, $p=0.95$). Additionally, 92% of the cells displayed a binocular response that was suppressed below the response to the corresponding monocular stimulation through one of the two eyes, suggesting the presence of inhibitory activity elicited by binocular stimulation at non-preferred disparities. Finally, waveform analysis showed that fast-spiking, putative inhibitory interneurons, displayed overall weaker phase disparity tuning than broad-spiking neurons ($z=-2.29$, $p=0.02$). Together, these results serve as an in-depth characterization of binocular visual responses and warrant further exploration of the role played by inhibition in the neuronal circuitry generating binocular disparity selectivity.

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Poster

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Title: Viral-mediated gene expression restricted to GABAergic neurons and their subclasses in marmoset V1

Authors: F. FEDERER¹, J. L. BALSOR¹, A. INGOLD¹, D. P. BABCOCK¹, J. DIMIDSCHSTEIN², B. V. ZEMELMAN³, A. ANGELUCCI¹;

¹Univ. of Utah, Salt Lake City, UT; ²Broad Inst., Broad Inst., Cambridge, MA; ³Univ. of Texas at Austin, Austin, TX

Abstract: Advances in viral vector-based gene expression provide new opportunities to investigate inhibitory neurons (INs) in non-human primate (NHP) cortex. Here we have validated the selectivity of some GABA- and parvalbumin (PV)-specific AAV viruses in marmoset V1. For each virus we quantified the laminar distribution of infected cells, identified by the expression of the reporter protein tdTomato (tdT), as well as its colocalization with GABA+ or PV+, respectively, using immunohistochemistry (IHC). As GABA-specific vectors we injected AAV serotypes 1, 7 and 9 carrying the gene for tdT under the h56D promoter (Mehta et al 2019). The V1 laminar distribution of tdT-expression resembled that of GABA+ IHC, suggesting good viral specificity. Quantification of the percent of viral-infected cells colocalizing with GABA+ IHC showed types 1 and 9 have higher coverage and greater specificity (>85%) than type 7 (76%), but this varied by layer. For example, type 7 showed higher specificity in layer (L)4A-B (100%) and L4C (~95%), but lower specificity in L5 (~70%). Type 9, showed >95% specificity in L4A-C and 5, but the lowest specificity in L6 (~75%). In contrast, type 1 showed ~90% specificity in L6. Thus, individual serotypes could be used to optimally deliver transgenes to specific layers. As PV-specific vector we injected different volumes of AAV9-PHP.eB-S5E2.tdT (Vormstein-Schneider et al 2020). TdT laminar expression was nearly identical to that of PV+ IHC. Colocalization with PV+ IHC further revealed high viral specificity, but the degree of specificity depended somewhat on the viral injection volume. Smaller volumes (100-200nl) resulted in higher specificity (>95% across most layers), while volumes ≥300nl yielded lower specificity (though still >80-85%, depending on layer). We further utilized IHC staining to quantify the laminar distribution of GABA+ and PV+ cells in marmoset V1 for comparison with mouse V1. Overall PV neurons represent about 60% of GABAergic INs in marmoset V1. Both GABA and PV IHC expression peaked in L2/3 (34&33%, respectively) and 4C (31&39%, respectively), and their densities were also highest in these layers. This laminar distribution of PV+ cells is consistent with previous results in macaques and marmosets, but differs from that in mouse V1 in which PV cells represent 40% of GABAergic INs, peak in L4&5, and are much less numerous in L2/3 than in marmoset. These differences with mouse cortex, emphasize the importance of investigating IN function and connectivity in NHP cortex.

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Poster

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Topic: D.06. Vision

Title: A SYNERGISTIC CIRCUIT MECHANISM FOR PREDICTIVE PROCESSING IN VISUAL CORTEX

Authors: *S. FURUTACHI, A. D. FRANKLIN, T. D. MRSIC-FLOGEL, S. B. HOFER; Univ. Col. London, London, United Kingdom

Abstract: A SYNERGISTIC CIRCUIT MECHANISM FOR PREDICTIVE PROCESSING IN VISUAL CORTEX Shohei Furutachi¹, Alexis D. Franklin¹, Thomas D. Mrsic-Flogel^{1*} & Sonja B. Hofer^{1*1} Sainsbury Wellcome Centre, University College London, London, UK *Corresponding author Predictive processing has been proposed as a computational framework for prioritizing the unexpected (therefore relevant) over expected sensory input (i.e. already known, therefore less relevant). However, how and by which circuit-mechanisms predictive processing is implemented in sensory networks remain unclear. Using calcium imaging with optogenetic manipulations, we show that in mouse primary visual cortex (V1) local disinhibitory circuits and higher-order thalamic inputs cooperatively amplify the representations of unexpected sensory input in a prediction-error dependent manner. Violation of animals' predictions by an unexpected visual stimulus caused response facilitation preferentially in L2/3 neurons that responded highly selectively to that stimulus. Concomitantly, both vasoactive-intestinal-peptide-expressing (VIP) inhibitory interneurons and input from the pulvinar, a higher-order thalamic nucleus, strongly responded to unpredicted visual stimuli. Optogenetic silencing of these populations reduced visual responses of V1 L2/3 cells only to unexpected but not expected visual stimuli. Conversely, when both populations were activated simultaneously, they acted cooperatively to strongly facilitate the activity of a specific subset of V1 cells, mimicking the prediction-error dependent facilitation. In contrast, stimulating either pulvinar or VIP cells alone had opposing but minor effects on visual responses. These data demonstrate that the brain prioritizes unpredicted sensory information by selectively amplifying the salience of sensory responses through the synergistic interaction of thalamic input and neocortical disinhibitory circuits.

Disclosures: S. Furutachi: None. A.D. Franklin: None. T.D. Mrsic-Flogel: None. S.B. Hofer: None.

Poster

469. Structure and Function of Cortical Visual Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 469.25

Topic: D.06. Vision

Support: Grant No. 2018YFA0701400
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Title: Multiple Foveolar Representations in Awake Macaque Monkey Imaged in 7T MRI

Authors: *Q. MEIZHEN¹, J. WANG², Y. GAO², Y. LIU³, X. ZHANG², J. HU⁴, A. W. ROE⁵;
¹Zhejiang Univ. Interdisciplinary Inst. of Neurosci. and Technol., Zhejiang Univ.

Interdisciplinary Inst. of Neurosci. and Technol. (ZIINT), Hangzhou, Zhejiang Province, China;

²Zhejiang Univ. Interdisciplinary Inst. of Neurosci. and Technol., Hangzhou, China; ³Zhejiang Univ. Interdisciplinary Inst. of Neurosci. and Technol., Zhejiang, China; ⁴Interdisciplinary Inst. of Neurosci. and Technol., ⁵Interdisciplinary Inst. of Neurosci. & Technol., Zhejiang Univ., Hangzhou, China

Abstract: Introduction: A common tenet of neural sensory representation is that species-specific behaviors are reflected in specialized brain organizations. In humans and nonhuman primates, the central one degree of vision is processed by a retinal structure called the foveola. This structure comprises a high density of photoreceptors and is crucial for primate-specific high acuity vision, color vision, and gaze-directed visual attention. Here, for the first time, we have first direct determined the precise cortical location of the foveolar center. **Methods:** Functional EPI images (0.6x0.6x1mm³ acquired in 7T MRI with a custom 16 channel RF coil) were acquired from two macaque monkeys trained to fixate within a 1 deg window. fMRI data were screened for runs with precise fixations. Representations of vertical and horizontal meridian, and foveolar, foveal, and para-foveal arcs were mapped to derive a full cortical map of the central 7 degrees. Foveola-specific stimuli were presented. Custom FreeSurfer methods transformed slice data to surface view. **Results:** Within V1, quantification of cortical magnification factors revealed values consistent with previous studies and provided novel data within the central 2 degrees. Focal foveolar activations were observed at the lateral focus of V1/V2, V2/V3, V3/V4 as well as within PIT; activations were consistent with separate representations in ventral and dorsal fields. These foveolar activations formed a previously unknown ringed network of foveolar representations which encircled a substantial area of cortex. **Discussion:** Our results show that the use of ultrahigh field fMRI mapping and foveolar stimuli in well-trained monkeys enable precise mapping of foveolar cortical locations. The most novel finding is the presence of a unique cortical region surrounded by a ‘ring’ of foveolar representations on the lateral operculum. Given that these multiple areas have different spatial resolutions (small to large receptive field sizes) and complexity (local to global feature specificity), we suggest this region constitutes a higher order cortical specialization for foveolar vision in primates.

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Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 470.01

Topic: D.06. Vision

Support: National Science Center, Poland (2020/39/D/NZ4/01881)
Foundation for Polish Science (MAB/2019/12)

Title: The Basal Forebrain modulates single neuron receptive fields properties in rat's primary visual cortex

Authors: *A. FOIK¹, J. PŁACZKIEWICZ¹, K. KORDECKA¹, D. C. LYON²;

¹Intl. Ctr. for Translational Eye Research, Inst. of Physical Chem. PAS, Warsaw, Poland; ²Anat. and Neurobio., UC Irvine, Irvine, CA

Abstract: There is a strong need to understand how our brain processes visual information and how different brain centers interact with each other. In this project, we investigate the Basal Forebrain's role in the modulation of cortical activity. The Basal Forebrain (BF) is the primary source of cholinergic input into the visual cortex. We propose that cholinergic modulation coming from BF will significantly impact single neuronal responses of the primary visual cortex (V1) in Long-Evans rats. The primary goal of this project is to understand the Basal Forebrain's role in the processing of the visual information in the V1 and its effect on the visual system activity. To answer this question, we will use cutting-edge techniques like viral tracing and optogenetic stimulation of neuronal circuits paired with electrophysiological recordings in the primary visual cortex. Precisely, we inject the modified Rabies virus (RV) into the V1 to retrogradely trace inputs coming from the Basal Forebrain. The RV carries opsins to allow for both activation and inhibition of infected cells in the Basal Forebrain. We hypothesize that the manipulation of the BF changes the selectivity of single cells in the V1. Indeed, our preliminary results confirm this hypothesis. The most prominent observation in our experiment is a significant change in firing rate when showing various directions of drifting gratings at optimal Spatial Frequency (SF), Temporal Frequency (TF), and Size. However, this change was different for different layers (depth) in the cortex. All layers tend to have a lower firing rate; however, the cell around layer IV tend to have an increased firing rate. Perhaps the most important result is the change in the optimal stimulus size. Most cells showed decreased optimal stimulus size, and the difference between conditions tends to be stronger in the upper layers than in the deep layers of the V1. Our preliminary results suggest that the Basal Forebrain plays a substantial role in the modulation of the center-surround interactions. These results align with the previous data from Lyon Lab (Lean et al., 2018), showing that the main inputs from BF are coming into the GABAergic cells in the V1.

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Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

Location: SDCC Halls B-H

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Program #/Poster #: 470.02

Topic: D.06. Vision

Support: BioTechMed-Graz Young Researcher Groups Grant Program.

Title: High-resolution 7T fMRI reveals the Visual Sensory Zone of the Human Claustrum

Authors: *A. COATES^{1,2}, D. LINHARDT³, C. WINDISCHBERGER³, A. ISCHEBECK^{1,2}, N. ZARETSKAYA^{1,2};

¹Univ. of Graz, Graz, Austria; ²BioTechMed-Graz, Graz, Austria; ³Med. Univ. of Vienna, Vienna, Austria

Abstract: The claustrum is a brain area situated between the putamen and insula that is interconnected with most regions of the cortex. While much is known about the structure of this area, its function is still debated, with hypotheses ranging from multisensory integration to its central role in consciousness (Crick & Koch, 2005). Due to the claustrum's thin shape, it is difficult to image in human participants using conventional neuroimaging techniques, which hinders testing these hypotheses empirically. In this study, we utilised high resolution ultra-high-field (7 Tesla) functional magnetic resonance imaging (fMRI) to test whether it is possible to measure claustrum activity in human subjects in vivo. Research on primates indicates that the claustrum contains functionally specific visual and auditory zones that are activated in response to stimulation of the corresponding sensory modalities (Remedios, Logothetis & Kayser, 2010). We therefore presented naturalistic video stimuli either as visual only, auditory only or as audiovisual conditions whilst participants carried out a central fixation task. We found that a ventral region in both left and right claustrum had significantly higher BOLD activation when comparing visual vs. auditory stimulation conditions. The location of this region was consistent across participants and approximately corresponded to the expected location of the visual sensory zone in primates. There was no increase in activity when comparing auditory vs. visual stimulation. Our study shows that activity from the visual claustrum zone can be reliably measured with ultra-high-field fMRI non-invasively in human participants. Our findings thus enable further investigations of claustrum function in humans.

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Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

Location: SDCC Halls B-H

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Program #/Poster #: 470.03

Topic: D.06. Vision

Support: Canada Research Chair Program
Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC)
IVADO-Apogée fundamental research project grant
Foundational Questions Institute (FQXi) and Fetzer Franklin Fund

Title: Unconscious processing of complex affective stimuli is mediated by cortical and subcortical neural synchrony: An MEG case study of affective blindsight

Authors: *V. HADID¹, A. PASCARELLA², T. LAJNEF³, M. SAHRAOUI³, J. O'BYRNE³, D. K. NGUYEN⁴, K. JERBI⁵, F. LEPORE⁶;

¹Dept. De Sci. Biomédicales, Univ. D, Laval, QC, Canada; ²Italian Natl. Res. Council, Rome, Italy; ³Univ. de Montréal, Montréal, QC, Canada; ⁴Ctr. Hospitalier de l'Université de Montréal, Montréal, QC, Canada; ⁵Univ. de Montréal, Outremont, QC, Canada; ⁶Psychology, Univ. Montreal, Montreal, QC, Canada

Abstract: Introduction Individuals with lesions to primary visual cortex (V1) are sometimes able to recognize affective stimuli even when they are presented to their blind hemifields, i.e. in the absence of visual awareness. To date, this phenomenon known as affective blindsight has only been reported for emotional faces. Here, we investigated the rare case of a patient (SJ) who, following complete resection of their right V1, exhibited blindsight for complex affective scenes. We probed for the first time the neural cortical and subcortical dynamics associated with this extraordinary ability. Methods *Patient:* SJ's resection left no spared V1 islands in their right hemisphere resulting in a complete left homonymous hemianopia with no visual awareness. *Experimental design:* A 3-alternative forced-choice affective discrimination paradigm was assessed during the scanning session. We presented 300 pictures of unpleasant, neutral and pleasant natural scenes selected from the International Affective Picture System which we controlled for arousal and salience. Data were acquired using a 275-channel whole-head MEG system. *MEG analysis:* MEG data preprocessing and source reconstruction were conducted. Time-frequency and granger causality analyses were assessed to probe the local and long-range neural synchrony between cortical and subcortical structures. Results SJ's unique neurological condition allowed unprecedented insights into the mechanisms of conscious versus unconscious visual processing. Behaviorally, we showed that SJ's RTs across conditions varied in similar ways for seen and unseen pictures, suggesting that patients with V1 lesions can potentially process higher-order complex features in the absence of visual awareness. Moreover, we demonstrated that unconscious processing of complex affective stimuli is mediated by fast-acting subcortical and subcortico-cortical neural synchrony. Specifically, we showed that (1) high-gamma activity was increased in visual awareness and (2) was modulated for affective-specific differences in conscious and unconscious processing; and (3) unconscious affective processing was supported by fast thalamo-amygdalar and thalamo-extrastriate pathways. Conclusion The present work characterized for the first time the temporal dynamics of the thalamo-amygdalar and thalamo-extrastriate functional pathways in a patient with affective blindsight. This study provides new insights on blindsight and furthermore reveals the surprising potential for complexity in unconscious, "intuitive" processing—extending even to the emotional appraisal of diverse naturalistic objects and scenes.

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Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 470.04

Topic: D.06. Vision

Support: NIH R01 EY025219

Title: Effects of Optogenetic Activation of Corticogeniculate Feedback on Rhesus Macaque (Macaca mulatta) Lateral Geniculate Nucleus Neurons

Authors: *S. MAI¹, A. J. MURPHY², F. BRIGGS¹;

¹Dept. of Neurosci., ²Neurosci. Grad. Program, Univ. of Rochester, Rochester, NY

Abstract: In primates, vision is the primary sense used to interact in and navigate the physical and social environment. The feedforward visual pathways, originating from the retina through the dorsal lateral geniculate nucleus (LGN) to primary visual cortex (V1), have been extensively studied in primates and are characterized by segregated magnocellular, parvocellular, and koniocellular parallel visual information processing streams. The functional role of the corresponding feedback pathway, mediated by corticogeniculate (CG) neurons that link V1 to the LGN, is less clear. Although CG neurons in primates are also organized into parallel streams that map onto the feedforward streams. Here, we set out to investigate the effects of CG feedback on LGN neurons' spatial and temporal receptive field properties through the use of optogenetics. We were specifically interested to determine whether activation of CG feedback generated different effects among LGN neurons per stream. A subpopulation of CG neurons in layer 6 of V1 was optogenetically activated while electrophysiological recordings were obtained from LGN neurons in anesthetized monkeys during the presentation of flashed spots and gratings, drifting gratings varying across multiple parameters, and white noise m-sequence stimuli. To selectively express the optogenetic cation channel channelrhodopsin2 (ChR2) along with the fluorescent marker mCherry in the CG neurons, a genetically modified rabies virus (SAD-B19ΔG-ChR2-mCherry) was injected into the LGN, where it traced the axons of the CG neurons in a retrograde manner. Optogenetic activation of CG feedback resulted in reductions in LGN neuronal response latencies and increases in response precision with no change in most spatial receptive field properties. These effects were largely consistent across LGN neuronal types. However, differential effects of CG feedback activation on extraclassical surround suppression were observed across LGN types, consistent with previous reports (Andolina et al., 2013; Geisert et al., 1981; Jones et al., 2012; Murphy & Sillito, 1987). These results further demonstrate the role of CG feedback in regulating the timing and precision of LGN responses and also support the idea that CG feedback acts upon LGN neurons in a stream-specific manner.

Disclosures: S. Mai: None. A.J. Murphy: None. F. Briggs: None.

Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

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Program #/Poster #: 470.05

Topic: D.06. Vision

Support: NIH Grant EY015387
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NIH Grant MH082174

Title: Center-surround mechanisms and corticogeniculate feedback

Authors: *A. N. SANCHEZ, H. J. ALITTO, W. USREY;
Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: Neurons in the lateral geniculate nucleus (LGN) process and relay visual signals from the retina to primary visual cortex (V1). V1, in turn, gives rise to feedback pathways terminating in the LGN. Although much is known about the anatomical organization of corticogeniculate (CG) feedback, relatively less is known about its role during sensory processing. Because CG feedback can provide monosynaptic excitation and disynaptic inhibition to LGN neurons, the effects from CG feedback may be complex. We have previously shown that the filtering of retinal spike trains by the LGN depends upon both stimulus size and contrast. Specifically, the likelihood that a retinal spike is transmitted to the cortex is reduced by both large stimulus sizes (e.g., extraclassical suppression) and high stimulus contrasts (e.g., contrast gain control). Thus, current work is focused on understanding how CG feedback may contribute to these two processes in the LGN. To investigate the influence of CG feedback on visual processing in the LGN of alert primates, we infected V1 neurons (n = 2 female macaque monkeys) with either Jaws or mDLX-channelrhodopsin and reversibly silenced CG neurons while recording from individual parvocellular LGN neurons. Our results demonstrate that CG feedback has a spatial organization that differentially modulates geniculate responses to stimuli within the classical and extraclassical receptive fields. Importantly, the extent of effects is dependent upon the presence of extraclassical suppression: extraclassical suppression is most robust for off-center LGN neurons and minimal or non-existent for on-center neurons. Among off-center neurons, CG feedback suppresses responses to stimuli that extend into the extraclassical surround and facilitates responses to optimal size stimuli. Among on-center neurons, CG feedback suppresses response to optimal size stimuli. Ongoing analysis is investigating how stimulus contrast influences the effects of cortical feedback. These results require revision to our current view of corticothalamic circuitry and have important implications for understanding the dynamics of thalamocortical interactions.

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Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

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Program #/Poster #: 470.06

Topic: D.06. Vision

Support: NEI Grant EY022577

Title: Complementary input/output organization of visual corticothalamic pathways

Authors: ***R. M. CASSIDY**, A. V. MACIAS, W. NUÑEZ LAGOS, G. UGORJI, E. M. CALLAWAY;

The Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Corticocortical projections in the visual system facilitate the hierarchical processing of sensory information. In addition to these direct connections, visual cortical areas are extensively and reciprocally connected to the pulvinar nucleus of the thalamus. Whether these cortico-thalamo-cortical pathways provide a parallel channel for sensory transmission between cortical areas or whether they make reciprocal connections depends on the input/output relationships of the pulvinar. In this study, we systematically mapped the brain-wide inputs to seven different projection populations in the pulvinar. Using G-deleted rabies in adult male and female mice, we traced inputs to populations of pulvinar neurons projecting to each of seven higher visual areas (HVAs). HVAs were uniquely mapped using intrinsic signal imaging, and post-mortem sections were aligned to these functional maps. This comprehensive study revealed circuit motifs that were common across target cortical areas. Consistent with a feedforward relay, “driving” cortical inputs from layer 5 (L5) predominantly originate from primary visual cortex (V1), regardless of the target HVA. L5 inputs were also located in other HVAs, but they were notably absent from the target HVA, consistent with the “no strong loops” hypothesis (Crick & Koch, 1988). Unlike L5 inputs, “modulator” layer 6 (L6) inputs were distributed and overrepresented in the target HVA. These findings establish complementary connection rules for the two cortical pathways to the pulvinar, where L5 inputs avoid reciprocal connections and support feedforward trans-thalamic relays, and L6 inputs are biased toward reciprocal connections, reminiscent of the feedback from V1 L6 to the dorsal Lateral Geniculate Nucleus.

Disclosures: **R.M. Cassidy:** None. **A.V. Macias:** None. **W. Nuñez Lagos:** None. **G. Ugorji:** None. **E.M. Callaway:** None.

Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

Location: SDCC Halls B-H

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Program #/Poster #: 470.07

Topic: D.06. Vision

Support: K99 EY030550
R01 EY022951

Title: Corticocortical and higher-order thalamocortical pathways make distinct contributions to activity in higher-order cortex

Authors: *G. T. NESKE¹, J. A. CARDIN^{1,2,3};

¹Neurosci., ²Kavli Inst. for Neurosci., ³Wu Tsai Inst., Yale Univ., New Haven, CT

Abstract: Many fundamental sensory, motor, and cognitive operations of the brain depend on intricate communication patterns among multiple regions of the neocortex. Such interactions provide the basis for the specialized stimulus feature selectivity of higher-order cortical neurons and promote the flexible information flow required for context-dependent behavior. The precise sensory- and behavioral-state-dependent contributions of the multiple corticocortical afferents impinging on the neurons within higher-order cortical areas are not well understood. Additionally, the thalamocortical projections from the higher-order thalamus that often mirror these corticocortical connections are rarely considered in accounts of higher-order cortical processing. Here, we address these questions by studying the impact of multiple types of long-range afferents innervating a higher-order visual cortical area in the mouse, area PM. Specifically, we consider the corticocortical inputs from primary visual cortex (V1) and secondary visual cortex (LM) and the higher-order thalamocortical inputs from the lateral posterior nucleus of the thalamus (LP). Using 2-photon calcium imaging of PM cortical neurons in awake, behaving mice, we find that these neurons spatially integrate visual stimuli, monotonically increasing in responsiveness with stimulus size and exhibiting a higher sensitivity to coherent motion compared with V1 neurons. Compared with V1 neurons, PM neurons are also more strongly modulated by behavioral state variables (i.e. locomotion, pupil dilation, and facial motion). Using 2-photon calcium imaging of axon terminals in PM, we find that while V1-to-PM, LM-to-PM, and LP-to-PM inputs convey diverse information about visual stimuli, LP-to-PM projections convey stronger state-related signals than either of the corticocortical projections. Optogenetic suppression of transmitter release from these different projections revealed distinct effects on the neuronal responses of PM neurons. In ongoing work, we are using projection-type-specific chronic ablation techniques to further characterize the causal impact of these different projection types. Furthermore, we are combining soma-targeted and axon-targeted calcium indicators to simultaneously image axons and cell bodies in PM in order to more precisely identify contributions from each afferent. Our results provide novel insight into the synaptic foundations of corticocortical communication and suggest both complementary and distinct roles for cortex and higher-order thalamus in flexible sensory processing.

Disclosures: G.T. Neske: None. J.A. Cardin: None.

Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

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Program #/Poster #: 470.08

Topic: D.06. Vision

Support: DFG-KR 4062/4-1

Title: Cortical tangential insertions of high-density electrodes can capture populations of connected thalamo-cortical axons and cortical neurons

Authors: *J. SIBILLE^{1,2,3,4}, C. GEHR^{1,2,3,4}, J. KREMKOW^{1,2,3,4},

¹Neurosci. Res. Ctr., Charite Universitätsmedizin Berlin, Berlin, Germany; ²Bernstein Ctr. for Computation Neurosci., Berlin, Germany; ³Inst. for Theoretical Biol., Humboldt-Universität, Germany; ⁴Einstein Ctr. for Computat. Neurosci., Berlin, Germany

Abstract: A prerequisite to a better understanding of cortical computation requires a better monitoring of a significant portion of the cortical neuronal population's inputs and outputs. In particular, measuring the activity of neighboring thalamo-cortical axons, when they activate the different neurons in the cortex, would allow a better understanding of how incoming sensory activity is embedded in the ongoing cortical activity *in vivo*. To address such a question, we propose tangential insertions of high-density silicone probes (Neuropixels) in the layer 4 of the cortex to capture simultaneously, a population of thalamo-cortical axons together with a population of synaptically-connected neighboring cortical neurons. Pharmacological injections in both the cortex (muscimol) and the thalamus (tetrodotoxin) confirm the possibility to distinguish the electrophysiological signatures of thalamo-cortical axonal projections next to classical cortical neurons. Observing individual electrophysiological signatures of thalamo-cortical axonal projections within the layer 4 permits to measure their axonal and dendritic responses, which both exhibit a large morphological extend (respectively 303 and 448 μm , $n = 135$). Within a single recording, we report up to 120 synaptically connected pairs between 25 different thalamo-cortical axons to numerous excitatory ($n = 170$) and putative inhibitory ($n = 83$) neurons. Overall, such a methodological refinement - in both anesthetized and awake animals - give a first grasp at the possibility to measure incoming spikes from a population of neurons in the thalamus into a portion of the cortical neuronal population.

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Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

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Program #/Poster #: 470.09

Topic: D.06. Vision

Support: NIH Grant NS118960
NIH Grant NS100016

Title: Synaptic properties of parallel corticothalamic pathways from postrhinal to pulvinar

Authors: *J. B. ZALTSMAN¹, R. D. BURWELL², B. W. CONNORS¹;

¹Neurosci., ²Cognitive, Linguistic and Psychological Sci., Brown Univ., Providence, RI

Abstract: Nearly all sensory information passes through the thalamus en route to the cerebral cortex. The cortex in turn provides robust projections to the thalamus, and descending corticothalamic (CT) axons vastly outnumber thalamocortical (TC) axons. CT pathways allow the cortex to exert a considerable influence on the thalamus, and thus on its own inputs. Studies of CT pathways from primary sensory areas and the prefrontal cortex have suggested motifs of anatomy and physiology; they also raise new questions: Does every cortical area have a similar pattern of feedback to the thalamus? How are diverse CT pathways relevant to vastly different types of behavior? The parahippocampal cortex (postrhinal cortex, POR, in rodents), is a polymodal association area and the principal source of visual information to the hippocampus. The POR is heavily interconnected with the pulvinar nucleus of the thalamus (lateral posterior nucleus in rodents). Here, we utilize cortical layer-specific Cre- mouse lines, optogenetics, *in vitro* electrophysiology, as well as retrograde tracers and histology to investigate the functional properties and anatomy of top-down projections from POR to pulvinar. Anatomically, we observe retrogradely labeled CT cells in layers 5 and 6 of POR, consistent with the projection layers of other CT systems studied thus far. To investigate the electrophysiology of these projections, we are using established cell type-specific Cre- mouse lines (Rbp4-Cre and Ntsr1-Cre) and optogenetics to record from pulvinar cells while stimulating the presynaptic terminals of CT cells. We find that Rbp4-Cre-targeted layer 5 cells evoke a depressing response in pulvinar cells, while Ntsr1-Cre-targeted layer 6 cells evoke a facilitating response, again consistent with other CT systems. Our work suggests that the structure and physiology of CT circuits are largely conserved across systems with very different behavioral functions. POR and pulvinar are reciprocally connected, and we are also asking whether there are differences in the intrinsic physiological properties of “closed loop” pulvinar cells, i.e. POR-projecting pulvinar TC cells that receive CT inputs, versus “open loop” pulvinar cells, i.e. POR-projecting pulvinar TC cells that do not receive input.

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Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

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Program #/Poster #: 470.10

Topic: D.06. Vision

Support: NIH-U01NS122040

Title: Characterization of the morphological and synaptic properties of terminals in koniocellular versus magnocellular/parvocellular input-recipient laminae in the lateral geniculate nucleus of the tree shrew (*Tupaia belangeri*)

Authors: *F. SCIACCOTTA, A. ERISIR;
Univ. of Virginia, Charlottesville, VA

Abstract: In the primate visual system, 3 functionally distinct parallel processing streams extend from retina through thalamus and to cortex: magnocellular, parvocellular and koniocellular pathways. Tree shrews (*Tupaia belangeri*) provide an advantageous model to study koniocellular pathway in isolation because, while magno and parvocellular pathways remain mixed in laminae (L)1, 2, 4, and 5 of the lateral geniculate nucleus (LGN), L3 and L6 receive axons only from koniocellular retinal ganglion cells, in addition to a glutamatergic input from the superior colliculus (SC). To reveal how synaptic circuitries in koniocellular (K) vs. magno/parvo (M/P) input-recipient laminae differ, we analyzed the morphology and connectivity of synaptic boutons in 3 datasets of terminal cross-section areas: 1) all synaptic terminals, 2) terminals with pale mitochondria (presumed retinal), and 3) terminals labeled for VGluT2 (presumed retinal across L1-L6 and potentially from SC in L3/6). Firstly, morphometric and immunolabeling analysis revealed that the synaptic circuitry in L6 was devoid of large terminals with pale mitochondria. The mean terminal bouton area in L6 was significantly smaller than those in L3 ($0.49\mu\text{m}^2$ vs. $0.93\mu\text{m}^2$); VGluT2+ terminals in L6 were also smaller than in L3 and L1/2 ($0.67\mu\text{m}^2$ vs. $1.27\mu\text{m}^2$ and $1.72\mu\text{m}^2$), suggesting that K input-recipient L3 and L6 may carry on distinct functions. This analysis also revealed that L3 terminals with pale mitochondria were smaller than those in L1/2 (Mann-Whitney, $p < .05$); VGluT2+ terminals in L3 were also significantly smaller than in L1/2 (Mann-Whitney, $p = .01$), providing evidence for the morphological uniqueness of M/P vs. K axon terminals in LGN. An examination of postsynaptic targets of VGluT2+ terminals in M/P- and K-recipient laminae revealed that, while VGluT2+ boutons in L1/2 preferentially targeted vesicle-filled profiles (presumed interneurons) 12% of the time, they did so 30% and 27% of the time in L3 and L6, respectively, suggesting that K pathways may engage inhibitory circuitry more prominently than M/P pathways. To reveal how SC axon boutons may have contributed to differences that emerged between M/P and K laminae, we examined anterogradely labeled terminals after a tracer injection in the SC. We found that SC-originated boutons had smaller cross-section areas than VGluT2+ terminals in K-input laminae (Mann-Whitney, $p < .0001$). Together, these results provide evidence that the synaptic circuitry in K laminae of the tree shrew LGN is morphologically distinct, engages inhibitory local circuitry more prominently, and uniquely utilizes SC modulation.

Disclosures: F. Sciacotta: None. A. Erisir: None.

Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 470.11

Topic: D.06. Vision

Support: NIH NEI EY025219 to F.B
NIH Grant EY013588 to W.M.U.

Title: Morphological evidence for multiple distinct channels of corticogeniculate feedback originating in mid-level extrastriate visual areas of macaque monkeys

Authors: *M. ADUSEI¹, E. M. CALLAWAY², W. USREY³, F. BRIGGS¹;

¹Univ. of Rochester, Univ. of Rochester, Rochester, NY; ²Salk Inst., Salk Inst., La Jolla, CA;

³Univ. of California, Davis, Univ. of California, Davis, Davis, CA

Abstract: In primates, a hallmark of early visual pathways is strict functional and anatomical segregation of parallel processing streams. Geniculocortical projection neurons in the dorsal lateral geniculate nucleus of the thalamus (LGN) include magnocellular, parvocellular, and koniocellular cells that convey distinct visual information. Physiological and morphological evidence suggests that corticogeniculate neurons in primate primary visual cortex (V1) are also organized into parallel streams that map onto the feedforward streams. When V1 is damaged, residual visual responses remain in extrastriate cortex in a phenomenon known as blindsight. Blindsight may in part be mediated by koniocellular neurons in the intercalated layers of the LGN that project directly to extrastriate cortex, bypassing V1. Following long term V1 lesions, some MT-projecting LGN neurons are also found in the magnocellular and parvocellular layers and co-express calbindin and parvalbumin, which are neurochemical markers for magnocellular and parvocellular LGN neurons. Hence it is likely that residual visual abilities following V1 damage rely on information channeled through surviving neurons in all three feedforward parallel processing streams. In this study, we investigated whether corticogeniculate neurons are also present in extrastriate visual cortex. If the visual response preferences of corticogeniculate neurons match those of their LGN target neurons, we predict that corticogeniculate neurons in extrastriate areas will also be organized into parallel streams with most of the cells sharing properties similar to koniocellular LGN cells. Detailed analysis of multiple morphological metrics of corticogeniculate neurons in areas MT, MST and V4 revealed distinct subclasses of corticogeniculate neurons, suggesting that extrastriate corticogeniculate feedback could involve separate information channels. Interestingly, the majority of corticogeniculate neurons observed in each extrastriate area were spiny stellate cells, which may project to koniocellular LGN neurons, consistent with the notion that koniocellular LGN neurons make more direct projections to extrastriate cortex. Overall, our findings suggest that direct reciprocal connectivity between the LGN and each visual cortical area is the rule, i.e., connections to “primary” sensory thalamus are not limited to primary sensory cortex. Extrastriate corticogeniculate neurons are therefore positioned to influence feedforward V1-bypassing signals transmitted directly from the LGN to extrastriate visual cortex, with several implications for blindsight.

Disclosures: M. Adusei: None. E.M. Callaway: None. W. Usrey: None. F. Briggs: None.

Poster

470. Brain Areas and Circuits Connected to the Visual Cortex

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 470.12

Topic: D.06. Vision

Support: NIH F31 EY032332
NIH R01 EY025219

Title: Rules of connectivity between ferret corticogeniculate and lateral geniculate nucleus neurons

Authors: *A. J. MURPHY¹, F. BRIGGS²;

¹Neurosci. Grad. Program, Ctr. for Visual Sci., ²Ernest J. Del Monte Inst. for Neuroscience, Ctr. for Visual Sci., Univ. of Rochester, Rochester, NY

Abstract: The early visual systems of highly visual mammals are characterized by parallel streams of visual information processing from retina, through dorsal lateral geniculate nucleus (LGN), to primary visual cortex (V1). In carnivores, these streams are called X, Y, and W. Connectivity between individual cells from retina to LGN and LGN to V1 is like-to-like, with cells in one area projecting to cells in the next area sharing similar physiological characteristics and receptive field locations. Corticogeniculate feedback neurons, located in layer 6 of V1 with axons that project to the LGN, are physiologically and anatomically diverse. In primates, these neurons have physiological characteristics that correspond with the feedforward parallel streams. While rules of connectivity have been established in the feedforward direction, corresponding connectivity rules for feedback circuits have not been explored. We hypothesized that CG-LGN connections in carnivores would be between neurons with similar physiological characteristics, supporting the existence of parallel streams in the feedback direction. Using acute electrophysiological recordings from V1 and LGN in anesthetized female ferrets, we found functionally connected CG-LGN neuronal pairs using spike train cross correlations, and then analyzed these pairs for several physiological characteristics including tuning similarity and receptive field overlap. Our results indicate several factors that predict connectivity between CG and LGN neurons, supporting stream-specific organization of feedback circuits.

Disclosures: A.J. Murphy: None. F. Briggs: None.

Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 471.01

Topic: D.07. Visual Sensory-Motor Processing

Title: Visual saccade remapping may not be a cortical function, but rather cerebellar; a new theory of spatial localization and calibration

Authors: *M. RIGGLE;

Causal Aspects, Charlottesville, VA

Abstract: Visual saccade remapping is where neurons in the superior colliculus (SC) or the cortex remap their receptive fields prior to a saccade. The current remapping theory is that the frontal eye field (FEF) calculates these remapped fields for the upcoming saccade by using a corollary discharge from the SC, and that this remapping contributes to visual stability across saccades. However, we show some consistency problems with this current theory, and propose a

new consistent and generalizable theory. We suggest that the remapping appearing in the SC is calculated and stimulated by the oculomotor cerebellum (OMC) [There must be a sub-cortical source for remapping because it occurs in the absence of forebrain commissures (Dunn2010 doi.org/10.1152/jn.00675.2009) - a critical but ignored finding]. Then post-saccade, with that remap stimulation in the SC, the SC can produce an error vector for the OMC (the error is the difference between the calculated remapped retinotopic position and the actual retinotopic position of the visual object). That error vector is passed from the SC to the Inferior Olive of the cerebellum for correcting the calculations of the OMC. One important part of these calculations is the OMC control of the eye muscles to steer and stop a saccade; termed saccade adaptation. However, saccade adaptation must be more than just muscle tuning, rather adaptation corrects all calculations in the OMC - including the calculated remapping of retinotopic positions. A necessary part of remapping are conversions to/from retinotopic SC and spatiotopic (body/world) coordinates; these conversions are supported by calculating (in the cerebellar vermis) the head orientation in an earth reference frame (Yakusheva2007 doi.org/10.1016/j.neuron.2007.06.003). This remap functioning of the OMC implies that the cortex does not separately calculate a remap, and thus a corollary discharge from the SC is unneeded. Furthermore, the OMC theory generalizes to the other localizing senses where auditory and somatosensory targets correctly appear in the SC by the cerebellum for error feedback in localization. To support this novel theory, we show studies providing data inconsistent with the current theory, but which are consistent with and supporting of the proposed theory. Those studies include: the two studies mentioned above; OMC and SC error signals in saccade adaptation; particular saccade and gaze saccade adaptation scenarios; and saccades to moving targets. We propose a theory, using OMC calculations and error feedback from the SC, that unifies visual saccade remapping with a broader view of saccade adaptation and spatial localization.

Disclosures: M. Riggle: None.

Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 471.02

Topic: D.07. Visual Sensory-Motor Processing

Support: ISF grant

Title: Two-phase extra-retinal input into monkey's V1: the effect of fixational saccades on population responses

Authors: *Y. NATIV, T. BOUHNİK, H. SLOVIN;
The Leslie and Gonda (Goldschmied) Multidisciplinary Brain Res. Ctr., Bar-Ilan Univ., Ramat Gan, Israel

Abstract: During natural viewing the eyes incessantly move, scanning the visual scene, which leads to a continuous image shift over the retina. Yet, even during fixation periods, miniature eye movements displace the retinal image across tens of foveal photoreceptors. Such fixational eye movements (FEM) come in two primary flavors: slow ocular drifts interspersed with occasional faster microsaccades (MSs). Thus, as far as the retina is concerned, stationary stimuli do not exist during natural viewing. Despite the continuous FEM, our visual perception is stable. This paradoxical phenomenon known as ‘the problem of stabilization’ suggests the existence of an extra-retinal input to correct the image motion and produce perceptual stability. Here we aimed to investigate the existence of an extra-retinal input into the primary visual cortex (V1) during MSs. We used voltage-sensitive dye imaging (VSDI) in two adult macaque monkeys to measure and characterize the spatio-temporal neural population activity in V1 related to MSs performed during fixation, in the absence or presence of a visual stimulus. VSDI enables to measure the population response at a high spatial (meso-scale) and temporal (ms) resolution. After validating the algorithm for MSs detection, we aligned the VSD signal from V1 of the two behaving monkeys on MSs onset. Interestingly, the VSD signal revealed that MSs, in the absence of a visual stimulus, induce a spatio-temporal modulation in V1 population response. This modulation was comprised of two phases: an early suppression followed by enhancement of the neural response. Interestingly, this modulation exhibited a non-homogenous spatial pattern: foveal regions showed mainly the enhancement transient, whereas more parafoveal regions showed a suppression that was followed by a delayed enhanced neural activation. In addition, we found that neural synchronization increased during this modulation. We then compared the MSs modulation in the presence and absence of visual stimulus within stimulated and non-stimulated sites at the imaged cortical area. Our results present the spatio-temporal effects of MSs in V1 and reveal a nonhomogeneous modulation. These results suggest that two distinct extra-retinal sources can be involved in visual and perceptual stabilization.

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Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 471.03

Topic: D.07. Visual Sensory-Motor Processing

Support: Duke Institute for Brain Sciences Incubator Award

Title: Frontal eye field neurons predict the use of a Discriminative but not Bayesian model for visual continuity across saccades

Authors: D. SUBRAMANIAN¹, J. M. PEARSON², *M. A. SOMMER³;

¹Neurobio., ²Biostatistics and Bioinformatics, ³Biomed. Engin., Duke Univ., Durham, NC

Abstract: Saccades displace the retinal image, yet the visual system distinguishes saccade-induced displacement from external object displacement to perceive a stable world. How does it resolve this uncertainty? Bayesian models propose that priors can be used to resolve sensory uncertainty. In two rhesus macaques, we used a Saccadic Suppression of Displacement (SSD) task to test whether priors are used to compensate for sensory uncertainty caused either by 1) motor-induced noise due to the saccade, or 2) external noise added to the image. We found that priors were used to compensate for motor-induced uncertainty in a Bayesian manner. For external image noise, however, prior use was anti-Bayesian. That is, prior use decreased with increasing noise. Decreasing prior use was explained by a discriminative, neural network mode. We recorded the activity of 90 single neurons in the Frontal Eye Field (FEF) across two monkeys while they performed the SSD task. The main analyses compared the activity session-by-session to Bayesian and Discriminative behavior in the motor- and image-noise version of the tasks, respectively. We found that FEF activity predicted Discriminative but not Bayesian behavior. These results show a dissociation between Bayesian and Discriminative models at the computational and neuronal levels and set the stage for understanding how they interact for perception across saccades.

Disclosures: **D. Subramanian:** None. **J.M. Pearson:** None. **M.A. Sommer:** None.

Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

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Program #/Poster #: 471.04

Topic: D.07. Visual Sensory-Motor Processing

Support: NIH Grant EY027373

Title: Frontal cortical neurons form a distributed basis for the top-down control of sensory-motor function

Authors: ***S. W. EGGER**, S. G. LISBERGER;
Neurobio., Duke Univ. Sch. of Med., Durham, NC

Abstract: Frontal cortical neurons exert top-down control over sensory-motor transformations. In the context of the smooth eye movements made in pursuit of moving objects, the strength, or gain, of motor output is flexibly adjusted according to behavioral context and stimulus features. While causal experiments implicate the smooth eye movement region of the frontal eye fields, or FEFsem, as crucial to gain control, how neurons in FEFsem implement the computation is not clear. We probe the relationship between FEFsem and behavioral gain by manipulating target speed, motion reliability, and stimulus timing while measuring their corresponding effects on neural and behavioral responses. Behavioral gain was strongly affected by motion reliability, modestly affected by target speed, and weakly affected by timing. The effect of these manipulations on FEFsem neurons, however, was heterogeneous. The firing rate of individual

neurons over time varied considerably, including neurons with a burst of firing limited to the onset of visual motion, neurons with sustained firing, and neurons with responses that increased throughout the duration of pursuit. Given the relatively weak effect of time on behavioral gain, we sought a data-driven method to identify neurons with sustained responses. We therefore used unsupervised clustering methods to group neurons according to their temporal response profiles. After identifying neurons with sustained responses, we compared the effects of target speed and motion reliability on the response of these neurons to the behavioral gain. Surprisingly, however, the response of neurons with sustained firing could not control the behavioral gain because their responses were more strongly affected by the target speed than motion reliability. In fact, no cluster of neurons could uniquely specify gain across target speeds, motion reliabilities, and time. Instead, a linear summation across neurons with different temporal response profiles was required to accurately predict the behavioral gain. These results argue against the idea that frontal cortical neurons are organized into specialized subpopulations that contribute uniquely to the flexible control of behavior. Instead, the combination of mixed stimulus selectivity with the diversity of temporal response profiles forms a distributed basis through which specific contributions to top-down control can be rendered.

Disclosures: S.W. Egger: None. S.G. Lisberger: None.

Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

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Program #/Poster #: 471.05

Topic: D.07. Visual Sensory-Motor Processing

Support: Canadian Institutes of Health Research (CIHR) grant FDN-143212 to M. Petrides
Natural Sciences and Engineering Research Council of Canada (NSERC) to K.
Drudik
Fonds de recherche - Santé (FRQS) to K. Drudik

Title: Spatial probability maps and morphological examination of the superior parietal sulcus in the human brain

Authors: *K. DRUDI, V. ZLATKINA, M. PETRIDES;
Neurol. and Neurosurg., Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada

Abstract: The superior parietal sulcus (SPS) is the defining sulcus of the superior parietal lobule (SPL), separating the anterior from the posterior part of the SPL (Economo, Koskinas 1925, *J. Springer*). The pattern of morphological variability of the SPS was examined to provide criteria for its identification. In addition, the morphological variability and spatial extent of the SPS was quantified by means of spatial probability maps in the Montreal Neurological Institute (MNI) standard stereotaxic space. Thus, the aim of the present investigation was to provide an anatomical framework to facilitate the development of anatomico-functional correlations within

the SPL. Forty MRI scans (80 hemispheres) were randomly selected from the International Consortium for Brain Mapping (Mazziotta et al. 2001, *Phil. Trans. R. Soc.*). The sample consisted of 24 males (mean age 24.6 years, SD 3.18) and 16 females (mean age 24.6 years, SD 4.33) who had no history of neurological and/or psychiatric illness, and gave informed consent. Identification of the SPS was based on criteria established in recent atlases of the sulcal morphology of the human cerebral cortex in MNI stereotaxic space (Petrides 2018, *Elsevier Academic*). Voxels of the SPS were manually identified in all three-planes of section, on individual *in-vivo* MRI brain volumes, using an interactive imaging software package called Display (MacDonald 1996, *McConnell BIC*). In addition, the morphological variability and spatial extent of the SPS were quantified by means of volumetric and surface spatial probability maps. The SPS was identified in all hemispheres and the morphological patterns of the sulcus were classified into two primary morphological patterns. A sulcus was categorized as a Type I SPS when it was a single sulcus, separating the anterior from the posterior part of the SPL (75% of all hemispheres, 72.5% of left hemispheres, 77.5% of right hemispheres). A sulcus was identified as a Type II SPS when it was found as a complex of multiple sulcal segments (22.5% of all hemispheres, 22.5% of left hemispheres, 22.5% of right hemispheres). These two morphological patterns were subdivided based on whether the single SPS or the SPS complex remained distinct or merged with surrounding parietal sulci. The SPS or SPS complex merged most frequently with the superior postcentral sulcus, followed by the anterior and posterior rami of the intraparietal sulcus, and least frequently with a medial precuneal sulcus. The present study established consistent morphological patterns, and quantified these patterns in a common anatomical space, to facilitate structural and functional analyses associated with the SPL.

Disclosures: K. Drudik: None. V. Zlatkina: None. M. Petrides: None.

Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

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Topic: D.07. Visual Sensory-Motor Processing

Support: The Vision: Science to Applications (VISTA)
the Canada Research Chairs Program
NSERC Research Grant

Title: Functional Brain Networks for Egocentric and Allocentric Memory-guided Reaching

Authors: *L. MUSA¹, A. GHADERI², Y. CHEN⁴, J. CRAWFORD³;

¹Ctr. for Vision Research, Psychology, ²Ctr. for Vision Res., ³Ctr. for Vision Research, Psychology, Biol. and Kinesiology, York Univ., Toronto, ON, Canada; ⁴Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada

Abstract: The location of a remembered reach target can be encoded in egocentric and/or allocentric reference frames. While the differences in the cortical activation of these two representations have previously been identified (Chen et al., 2014; Neggers et al., 2006), differences in the functional organization of brain networks have not been described. Chen et al. (2014) demonstrated that allocentric and egocentric reach mechanisms use partially overlapping but distinct cortical substrates, that differ in directional selectivity of the target during the memory delay and response. Higher activation in dorsal brain areas (the parietofrontal cortex) was characteristic of egocentric reaching, while allocentric task also involved activity in ventral brain areas (inferior temporal gyrus and inferior occipital gyrus). It is thus expected that the size and connectivity of those functional brain networks will differ, reflecting more widespread connectivity between dorsal and ventral brain areas in the allocentric task. Here, we performed a secondary analysis of an event-related fMRI design to distinguish human brain networks involved in these two forms of representation. The paradigm consisted of three tasks with identical stimulus display but different instructions: egocentric reach (remember absolute target location), allocentric reach (remember target location relative to a visual landmark), and a nonspatial control, color report (report color of target). The properties of brain networks involved were analyzed using graph theory to derive the major network hubs, the efficiency of the network and the clustering between the nodes. The difference in the network hubs of allocentric and egocentric brain networks were identified after subtracting the correlation matrices and applying a sparsity threshold of 25%. The egocentric task had a relatively stronger activation hub in the left intraparietal lobule, while the allocentric task had a relatively stronger activation hub in the left cuneus. Consistent with previous finds, the egocentric task more strongly recruited parietal brain networks. Despite significantly higher clustering of brain areas in the egocentric task, we observe similar global efficiency in the allocentric task. Lower clustering in the allocentric task may be due to decreased modularity and increased interaction between dorsal and ventral visuomotor regions.

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Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

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Program #/Poster #: 471.07

Topic: D.07. Visual Sensory-Motor Processing

Support: NSERC to SD
NSERC to DYPH

Title: Time-course of implicit adaptation varies with type of feedback

Authors: *S. D'AMARIO, J. E. RUTTLE, B. M. 'T HART, D. Y. P. HENRIQUES;
York Univ., Toronto, ON, Canada

Abstract: People constantly adapt their movements to their changing circumstances, which is mostly handled by our automatic, unaware, or implicit motor adaptation systems. While the time course of these implicit processes is thought to be slow, there is surprisingly little evidence for this. Here, I have tested the effects of various kinds of feedback of the unseen hand motion on the speed of implicit learning in visuomotor rotation adaptation. Three groups adapted to a 45° rotation: 1) “Continuous”, the hand-cursor was continuously visible 2) “Terminal”, which used impoverished visual feedback by only seeing a static cursor at the end of each reach 3) “Cursor jump”, the cursor was aligned with the hand in the start, then jumped 45° mid-reach to show the source and nature of the perturbation on each trial. All groups completed the same rotation schedule and alternated their training trials with testing trials: no-cursor reaches with exclusion instructions to probe implicit adaptation. This allowed us to measure the rate of implicit adaptation at a fine temporal resolution. For “terminal” and “cursor jump” groups, we hypothesized a decrease in the magnitude and possibly rate of implicit adaptation. In overall adaptation, the continuous group showed a higher learning rate and thus reached asymptote faster (16 trials to saturation vs 55 for terminal and 61 for cursor jump). Overall adaptation was surprisingly smaller for the continuous group with an asymptote of 26° vs 37° for terminal and 33° for cursor jump. However, the continuous group showed more implicit learning with an asymptote at 22° and the slowest rate of change requiring 22 trials to hit asymptote vs 6 for terminal and 11 for cursor jump. Our results indicate that despite a lower final amount of adaptation, implicit learning saturates faster with changed visual feedback. It also confirms implicit adaptation is faster than usually assumed and suggests there may be useful feedback-dependent mechanisms which can increase the amount and rate of implicit learning.

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Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 471.08

Topic: D.07. Visual Sensory-Motor Processing

Support: NSERC Grant 418589

Title: On the effects of acute exercise on implicit adaptation

Authors: *L. ARSENAULT-LEVESQUE, E. DE LA FONTAINE, P.-M. BERNIER;
Dept. de kinanthropologie, Univ. de Sherbrooke, Faculte des Sci. de l'activite Physique,
Sherbrooke, QC, Canada

Abstract: Sensorimotor adaptation is an important process by which motor commands are recalibrated to ensure accuracy in the face of changes in sensory contexts or throughout ageing (Shadmehr et al., 2010). Recent studies have found that aerobic exercise improves this process

(Mackay et al., 2021; Neva et al., 2019). For instance, Neva et al. (2019) exposed participants to a 45° visuomotor rotation and found that acquisition was improved if preceded by an acute bout of aerobic exercise. Still, it is well known that in such paradigms, adaptation is driven both by an explicit process, through the use of cognitive strategies such as re-aiming (Taylor et al., 2014), as well as an implicit process, driven by sensory prediction errors (SPE) (Mazzoni & Krakauer, 2006). Given that exercise is known to benefit cognitive functions (Chang et al. 2012), it is likely that enhanced adaptation was attributable at least in part to the explicit component, leaving open the question as to whether exercise also improves implicit adaptation. The aim of this study was to test this hypothesis, using a paradigm known to isolate implicit adaptation driven by SPE. In a within-subject design, participants (n=13) had to reach toward a target in front of them, while being pseudo randomly exposed to CW or CCW 30° visuomotor rotations. Implicit adaptation was assessed by the involuntary bias in hand direction opposite to the error's direction that follows a rotated trial, called post rotation bias (PRB). Participants carried out the task for 180 trials before exercise (PRE) and 180 trials after exercise (POST). The exercise bout consisted of 20 minutes of moderate intensity cycling (65-75% of maximal heart rate), the same intensity that has previously been shown to benefit cognitive and sensorimotor adaptation tasks (Chang et al. 2012; Neva et al., 2019). Results revealed robust PRBs in directions opposite to that of the perturbation in both the PRE and POST conditions. Critically, however, their magnitude was not significantly modulated by exercise (PRB mean difference = 0.036°, p = 0.873, effect size = 0.045). Further analyses revealed that movement vigor (RT+MT) was significantly reduced in a step-wise function following exercise (mean difference = -22.178 ms, p < 0.001, effect size = 1.604), indirectly confirming that exercise impacted motor function. These results suggest that an acute bout of moderate exercise does not improve implicit adaptation, at least not as measured through the PRB method.

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Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

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Program #/Poster #: 471.09

Topic: D.07. Visual Sensory-Motor Processing

Support: DFG Grant EXC 2050/1 – Project ID 390696704

Title: Neuronal correlates of adapting to vs. ignoring changes in visuomotor delay under different attentional sets

Authors: *G. VIGH, F. QUIRMBACH, J. LIMANOWSKI;
Technische Univ. Dresden, Dresden, Germany

Abstract: In this fMRI study, we asked whether the processing of visuomotor delay and, crucially, changes therein, depends on current behavioural demands; i.e., the current attentional

task set. We used a data glove to measure participants' movements in a virtual reality setting. Thus, participants were able to control a virtual hand, which showed their real hand movement - however, always with some temporal delay. The visuomotor delay changed repeatedly and unpredictably in a roving oddball fashion (between 100 and 600ms). Participants performed continuous, simple grasping movements, aligning the rhythm of the movement with the rhythmic oscillation of the fixation dot. Crucially, participants had to either match the target rhythm with the delayed virtual hand (i.e., visuomotor adaptation) or their real, unseen hand (i.e., ignoring delays and their changes). This effectively induced an attentional task set, which we expected to influence the processing of visuomotor mismatches, and their changes. Thus, we expected a stronger BOLD correlation with delays and 'oddballs' in visuomotor regions (i.e., the fronto-parietal reach circuit, the temporoparietal cortex, and the cerebellum) in the virtual > real hand task. A preliminary behavioural analysis (N=10; data collection of N=30 in progress) showed that participants adapted to delays only in the virtual hand task, while ignoring delays and changes in the real hand task (T=6.33, p<0.001). Visuomotor delays correlated mainly with activity in the dorsal and ventral premotor (T=13.80, p<0.001) and the temporoparietal cortex (T=12.51, p<0.001), and in the cerebellum (T=5.61, p<0.001). Of these regions, activity in the right ventral premotor cortex (T= 4.96, p<0.001) and the left cerebellum (T= 6.43, p<0.001) more strongly correlated with delay in the virtual > real hand task. Furthermore, transitions between delays correlated with activity in the bilateral anterior insula (T=7.45, p<0.001) and the lateral occipitotemporal cortex (T=6.82, p<0,001). These preliminary results support previous findings of differential BOLD correlation with visuomotor transitions vs delays; and show that the neuronal processing of visuomotor mapping depends on its relevance for the task at hand.

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Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

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Program #/Poster #: 471.10

Topic: D.07. Visual Sensory-Motor Processing

Support: NIH Grant 5R01HD089952-05

Title: The impact of proprioceptive deficit on the accuracy of force scaling and force direction matching in chronic stroke survivors

Authors: *K. OH, W. Z. RYMER;
Shirley Ryan AbilityLab, Chicago, IL

Abstract: Several recent studies highlighted the importance of proprioception in stroke rehabilitation, as the severity of proprioceptive loss is one of the strongest predictors of the variation in rehabilitation outcomes. The authors' recent study reported that chronic stroke survivors with proprioceptive deficit showed inaccurate initial reaching movement trajectory.

These results imply that proprioceptive deficit might be related to the quality of feedforward motor control. It needs to be further determined, however, if such inaccurate initial reaching movement was potentially due to the inaccurate control of force magnitude or force direction. Thus, this study seeks to determine if the quality of proprioception after stroke significantly impairs the accuracy of the control of force magnitude or force direction. Thirteen chronic stroke survivors with hemiparesis participated in two separate tests: a force magnitude matching test and a force direction matching test. For the force magnitude matching test, the participants were holding the end effector of a programmable haptic device and were asked to exert 10%, 30%, and 50% of their isometric MVC force with and without visual feedback of force magnitude. For the force direction matching test, the participants were trained to generate contact forces in four cardinal directions with visual feedback of force magnitude and direction. After this training, each blindfolded participant's hand was passively moved to a random position and was asked to reproduce the contact forces in the same four cardinal directions as trained. The quality of proprioception was assessed to determine if each blindfolded participant could detect a passive movement of the elbow joint at 2 deg/s, could touch the nose with the finger, and could mimic the elbow joint angle of the opposite or same side with the paretic side of the upper limbs. Each error in the force magnitude and force direction was then correlated with the proprioceptive measures. Five out of 13 participants could not recognize the passive movement with their eyes closed, which indicated impaired proprioception. No significant effect of hemiparesis and the quality of proprioception on the error in force magnitude matching was found. Interestingly, a statistically significant correlation between the error in force direction and the quality of proprioception was found. This study highlights the importance of somatosensory training poststroke, as proprioceptive loss is potentially related to the distorted spatial body map and inaccurate predictive motor control, which humans heavily rely on when they plan their voluntary movements and calculate required muscular forces.

Disclosures: K. Oh: None. W.Z. Rymer: None.

Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 471.11

Topic: D.07. Visual Sensory-Motor Processing

Support: ORF-RE Grant RE-09-112
CIHR Operating Grant MOP 106662
Heart and Stroke Foundation of Canada Grant-in-Aid G-13-0003029
Alberta Innovates Health Solutions Team Grant 201500788

Title: Directional and general impairments in initiating motor responses after stroke

Authors: ***K. PARK**¹, M. CHILVERS², T. A. LOW³, S. DUKELOW³, S. H. SCOTT⁴;
¹Queen's Univ., Queen's Univ., Kingston, ON, Canada; ²Univ. of Calgary, ³Univ. of Calgary,
Calgary, AB, Canada; ⁴Queen's Univ., Queens Univ., Kingston, ON, Canada

Abstract: Visuospatial neglect (VSN) is a disorder characterized by an impairment of spatial attention. Clinical assessments designed to diagnose VSN are reliable and valid but commonly do not assess reaction time (RT). Reaching studies have found individuals with VSN can exhibit delayed RT towards the contralesional side of space. Despite this, only a few studies have examined RT impairment for individuals with VSN beyond reaching left and right. Further, understanding impairment after VSN becomes increasingly important as it has been associated with poor recovery and functional outcomes. Using an 8 target centre-out reaching task, we quantified visuospatial ability in 265 individuals with stroke (162 right hemi-damaged (RHD), 103 left hemi-damaged (LHD); days since stroke (mean, range): 11, 1-59). Individuals with stroke were recruited if they were above 18 years of age and had a first time reported ischemic or hemorrhagic stroke. Exclusionary criteria were previous stroke, non-stroke related neurological disease (e.g. Parkinson's Disease), upper extremity musculoskeletal injury, or unable to understand task instructions. To assess RT in each direction we developed two measures: RT Asymmetry to quantify RT variability (direction of the slowest RT) and RT General to quantify the fastest response. Impairment was defined as scores greater than 95% of controls. The Behavioral Inattention Test (BIT) was used to determine the presence of VSN. The Functional Independence Measure (FIM) was used to quantify functional outcomes. Almost half of individuals with RHD had impaired RT (48%). 34% were impaired in RT Asymmetry, commonly to stimuli on the left. A similar proportion (32%) had impaired RT General, highlighting longer RT in all directions. Less individuals with LHD had RT impairment (30%). Of note, impaired RT Asymmetry (24%) was greater than impaired RT General (12%). Impaired RT was common for individuals who failed the BIT (95% RHD, 56% LHD) but also evident in many individuals that passed the BIT (35% RHD, 28% LHD). The FIM was weakly correlated with RT General impairment. Lesion analysis identified distinct cortical regions associated with impaired RT Asymmetry and RT General. Impaired RT Asymmetry was associated with right inferior white matter tract lesions, whereas impaired RT General was associated with right temporal lesions. We demonstrated almost half of individuals with stroke have RT impairment that could range from a limited direction to all directions, and these were associated with specific lesions. Most individuals that failed the BIT had impaired RT but there was also a substantial proportion that passed the BIT with RT impairment.

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Poster

471. Sensorimotor Transformation: Eye Movements and Reaching

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 471.12

Topic: D.07. Visual Sensory-Motor Processing

Support: Solve ME/CFS Initiative Ramsay Award
CIHR #PJT-175063
NSERC Create Dependable Internet of Things Applications program

Title: Cognitive-motor performance and associated brain activity shows differences in individuals with Post Acute Sequelae of SARS-CoV-2 (PASC) or Myalgic Encephalomyelitis (ME)

Authors: H. AHUJA¹, S. BADHWAR², M. LITOIU¹, H. EDGELL², *L. E. SERGIO²;
¹Information systems and technology, ²Sch. of Kinesiology and Hlth. Sci., York Univ., Toronto, ON, Canada

Abstract: Our research examines rule-based eye-hand coordination and associated brain activity. Such cognitive-motor integration (CMI) tasks are used in everyday activities where a rule is used to align the required motor output to the guiding visual information. Previously we have observed alterations in brain network activity associated with complex movement control in youth and young adults with a history of concussion, as well as older individuals affected by dementia. Here we examine skilled performance and brain activity (electroencephalography, EEG) in adults experiencing prolonged symptoms following COVID-19 infection (PASC) and those diagnosed with ME for more than a year. Based on recent findings of reduced oxidation affecting physical energy and cognitive health, we hypothesized that these groups would have impaired CMI performance and EEG differences relative to healthy controls. Participants were tested on two visuomotor transformation tasks using a Samsung tablet running BrDI™ software, while wearing a Muse2™ headband. They slid their finger from a central target to one of four peripheral targets. In Standard condition one, the spatial location of the target and finger-to-cursor relationship were in alignment (standard visuomotor control). In CMI condition two, participants viewed the targets on the top half of the tablet while moving their finger on the blank bottom half of the tablet, and the cursor feedback was 180° reversed so that the required hand motion and hand location was decoupled from guiding visual information. We observed that the patient groups displayed slower reaction time (RT) in CMI performance ($p=0.032$), and a greater change in RT in going from standard visuomotor control to CMI ($p=0.027$), relative to controls. We found no group differences in movement execution variables. The task-related EEG waveforms were converted into two-dimensional (2D) image-based scalograms which allowed us to extract the temporal and spatial aspects of the EEG signals. The scalogram images were fed to a multi-channel convolutional neural network with long short-term memory (CNN-LSTM) which was used to classify the difference between the EEG waveforms of healthy and patient groups. We observed that the model was able to differentiate between the EEG waveforms of the two groups. These data suggest that neural activity associated with complex, rule-based movement control is affected by both PASC and ME in such a way that affects planning of such movement. Further, we demonstrate the utility of consumer-available EEG measurement systems to assess task-related brain activity in healthy and patient populations using an AI approach.

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Poster

472. Cerebellum: Sensorimotor

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 472.01

Topic: E.02. Cerebellum

Support: ANR-19-CE16 SynPredict
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NIH R01 NS118401

Title: Cerebellar interneuron activity during evoked whisking

Authors: *M. COSTREIE¹, G. DIANA¹, Z. PIWKOWSKA-ZVONKINE¹, F. BENDER¹, G. MONERON¹, C. SAINT-CLOMENT¹, A. W. HANTMAN², J. M. CHRISTIE³, D. A. DIGREGORIO¹;

¹Neurosci., Inst. Pasteur, Paris, France; ²Cell Biol. and Physiology, Neurosci. Ctr., Univ. of North Carolina Chapel Hill, Chapel Hill, NC; ³Physiol. and Biophysics, Max Planck Florida Inst., Aurora, CO

Abstract: Fine-tuning of temporally precise behaviors by the cerebellum likely depends on the functional diversity of its cell types. Molecular layer interneurons (MLIs) inhibit Purkinje cells (PCs) thus shaping the sole channel of output from the cerebellar cortex. Anatomically distinguishable MLI classes include those in the superficial molecular layer that preferentially target PC dendrites and those in the deep molecular layer that preferentially target PC somata. Whether these MLI classes perform layer-specific computations that aide sensorimotor processing is not clear. To address this question, we used in vivo two-photon calcium imaging in mice to measure the activity of MLIs in either the outer or inner third of the molecular layer during spontaneous whisking as well as whisker deflections resulting from brief air-puff stimuli to the ipsilateral whisker pad. GCaMP8f-expressing MLIs in lobule Crus I were imaged at 30 Hz through a cranial window while animals were awake and alert but not locomoting. Air-puff stimuli widely engaged the MLI ensemble. Trial-averaged calcium responses in MLIs displayed heterogeneous rise times ranging from 32.9 ms to 79.8 ms (IQR = 46.9 ms; n = 466 cells) and a mean peak amplitude of $18\% \pm 0.3$ ($\Delta F/F$). Interestingly, deep-layer MLIs responded to the air-puff with a shorter delay, exhibiting a peak response that was ~20% faster than MLIs in the superficial layer (p-value = 0.003, Mann-Whitney test). These results are consistent with rapid and narrower time windows of activity of deep-layer MLIs. Calcium transient amplitudes of deep- and superficial-layer MLIs were similar and appeared to represent change in whisker angle or velocity rather than the absolute angle of deflection. Extracellular electrophysiological recordings from putative MLIs revealed a brief, high-frequency response that immediately preceded whisker protraction, suggesting that MLIs may also encode either passive whisker deflection or a motor command. Our results suggest layer-specific sensorimotor processing by MLI classes tuned to kinematics rather than simply position.

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Poster

472. Cerebellum: Sensorimotor

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 472.02

Topic: E.02. Cerebellum

Support: ANR-18-CE37-0006-01 WalkingCrossingNeurons
ANR-17-EURE-0017

Title: Acquisition of a complex locomotor task: activity of cerebellar molecular layer interneurons and paw dynamics

Authors: A. ANDRIANARIVELO¹, H. STEIN², J. GABILLET¹, C. BATIFOL¹, N. CAYCO GAJIC², *M. GRAUPNER¹;

¹SPPIN, Univ. Paris Cité, CNRS, Paris, France; ²Dept. d'Études Cognitives, École Normale Supérieure, Univ. Paris Sci. et Lettres, Paris, France

Abstract: The cerebellum is a key brain region for motor coordination and learning. By processing sensorimotor information, it predicts and refines movements. However, the contribution of the cerebellar cortex neurons in the learning of a challenging motor task remains poorly understood. To study the implication of the cerebellar cortex microcircuit in motor coordination and learning, we combined behavioral analyses, electrophysiology and two photon calcium imaging of the molecular layer interneurons (MLI) network which exerts a strong inhibitory control over the activity of cerebellar cortex output neurons, the Purkinje cells. We implemented a challenging locomotor task where we trained the mice to walk on a motorized treadmill with rungs over multiple days while simultaneously recording the activity of the same MLI population in lobulus simplex using 2 photon calcium imaging and cell-attached recordings. We used high-speed behavioral video recordings to extract individual paw and rung locations, which were subsequently divided into swing and stance phases. When studying individual paws movement patterns during the task acquisition, we found that mice performed fewer, longer strides and increased the duration of the stance phase during learning. Muscimol inactivation of lobulus simplex during the task execution affects individual paw movement parameters such as increasing the number of swings executed and altering different stride parameters over days. These results point to a causal contribution of the lobulus simplex in the execution and learning of the task. MLIs exhibit pronounced locomotion-related activity: a global increase in the MLI population upon locomotion onset and rich dynamics during walking. We use individual spiking recordings to investigate stride related dynamics of MLI activity. Our results have the potential to shed light

on the involvement of the cerebellar interneurons in generating coordinated movement in mammals.

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Poster

472. Cerebellum: Sensorimotor

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Program #/Poster #: 472.03

Topic: E.02. Cerebellum

Support: NIH NS116854
AHA Grant 829841

Title: Synaptic mechanisms underlying sensorimotor computations in the cerebellar corticonuclear circuit

Authors: *M. R. HOLLA¹, S. T. BROWN², I. M. RAMAN³;
¹Northwestern Univ., ³Northwestern University, ²Northwestern Univ., Evanston, IL

Abstract: The cerebellum coordinates movement by predicting relationships among ongoing sensory inputs and motor commands. We previously found that an air puff applied to the whisker pad in mice evokes a synchronous, short-latency, brief suppression of simple spikes in crus I/II Purkinje (Pkj) cells, associated with an increase in firing by the cerebellar nuclei and enlarged whisker protraction. This suppression is correlated with the degree of predictability of sensory input, raising the question of the synaptic mechanisms that underlie discrimination of predicted and unpredicted input. Notably, simple spike suppression precedes any excitation, although puff-activated granule cells provide monosynaptic excitation (via parallel fibers, pfs) and disynaptic inhibition (via molecular layer interneurons, MLIs) to Pkj cells. *In vitro* experiments show that the combination of strongly facilitating EPSCs and stable, non-facilitating IPSCs in Pkj cells yields a changing I/E ratio that can be dominated by inhibition only at the onset of a train of stimuli. To test whether this synaptic mechanism underlies the representation of sensory input *in vivo*, we made single-unit recordings from Pkj cells in awake head-fixed *Gabra6-ChR2* mice. Much like puffs, optogenetic activation of granule cells reliably evoked a 2-4 ms suppression of simple spikes (change in rate, -21.0 ± 3.7 sp/s) in 27/40 Pkj cells. When optogenetic stimulation was followed by a puff 25 ms later, the puff no longer suppressed spiking, but instead evoked an elevation of 27.0 ± 18.1 sp/s, N=7); when the puff came 200 ms later, suppression remained (-27.8 ± 10 sp/s, same 7 cells). The results are consistent with the idea that Pkj cells *in vivo* are sensitive to the relative facilitation of pfs and MLIs. When granule cells are unfacilitated, as is likely for unexpected sensory input, their activation can transiently inhibit Pkj cells. However, when the I/E ratio is shifted towards excitation by recent activation of granule cells, sensory input does not suppress Pkj cell simple spikes. The data suggest a synaptic mechanism

underlying the discrimination of unexpected (initial) and expected (repeated) sensory input and allow a predictive signal to emerge in the cerebellar cortex.

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Poster

472. Cerebellum: Sensorimotor

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 472.04

Topic: E.02. Cerebellum

Support: Wellcome Trust 224668/Z/21/Z

Title: What Purkinje cells tell the cerebellar nuclei: insights from monosynaptic paired recordings in behaving mice

Authors: *M. HAUSSER¹, H. STABB², M. VARADI², D. COHEN³, D. KOSTADINOV², M. J. R. BEAU²;

¹Wolfson Inst. for Biomed. Res., UCL, London, United Kingdom; ²Wolfson Inst. for Biomed. Res., Univ. Col. London, London, United Kingdom; ³The Gonda Brain Res. Ctr., Bar-Ilan Univ., Ramat Gan, Israel

Abstract: To understand cerebellar function, it is crucial to elucidate how cerebellar output is generated. This remains an open question: the interplay between the Purkinje cells (PCs) - the output neurons of the cerebellar cortex - and the nuclear cells (NCs) - the ultimate output neurons of the cerebellum - remains poorly understood. PCs are GABAergic, and they could influence NCs either by directly inhibiting them or by entraining them (e.g. via disinhibition or rebound firing). Which of these two modes prevails during different behaviours, and how this depends on PC synchrony, remains controversial.

We are addressing this problem using Neuropixels silicon probe recordings in which we target monosynaptically connected pairs of PCs and NCs. We performed these recordings in mice executing different behaviours, including a forelimb motor coordination task (wheel steering), locomotion, and reward prediction during Pavlovian conditioning. Using a combination of cell type-specific optogenetic tagging, prior knowledge about the firing properties of cerebellar cell types, and post-hoc 3D atlas registration of our recording sites, we identified PC and NCs in simultaneous recordings from lobule simplex and interpositus nucleus. We used cross-correlations to infer monosynaptically connected pairs of PCs and NCs, and developed novel analysis strategies to disentangle direct inhibitory connections from confounding correlations caused by the synchrony of neighbouring PCs.

We found that during rhythmic licking, wheel steering, and locomotion, PCs and NCs tend to discharge out of phase from each other, suggesting that PC inhibition predominates in many contexts. We observe enhanced synchrony of PC simple spikes during specific step cycle phases in locomotion, and are currently exploring its impact on the firing pattern of NCs. Complex

spikes have a surprising effect on nuclear firing: unexpectedly, nuclear cells were not inhibited but rather excited concomitantly with complex spikes, suggesting that the same climbing fibre can excite both a PC and its downstream NC targets. These recordings reveal the direct impact of Purkinje simple spikes and complex spikes on their target nuclear neurons, and shed light on the long-debated signal transformations taking place along the cerebellar output pathway during behaviour.

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Poster

472. Cerebellum: Sensorimotor

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Program #/Poster #: 472.05

Topic: E.02. Cerebellum

Support: NIH R01NS112917
NIH R01NS092623
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Title: Information transmission in the cerebellum: the role of rate and synchrony during smooth pursuit eye movements

Authors: *D. J. HERZFELD¹, M. JOSHUA², S. G. LISBERGER¹;

¹Neurobio., Duke Univ., Durham, NC; ²The Edmond and Lily Safra Ctr. for Brain Sci., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: A common mechanism for information transmission in neural circuits is the coherent modulation of neuron firing rates. However, information may also be encoded in the precise timing of spikes, where the synchronous firing of multiple units could then faithfully transmit relevant temporal information to downstream populations. We address this issue for a specific neural circuit by recording simultaneously from pairs of cerebellar Purkinje cells (PCs) in monkeys and isolating the contributions of rate and synchrony for control of motor behavior. The floccular complex of the cerebellum is crucial for the performance of smooth pursuit eye movements, allowing us to assay how changes in PC rates and synchrony contribute to pursuit behavior. We recorded from 32 pairs of well-isolated PCs, definitively identified by the presence of post-climbing-fiber pauses, in three rhesus monkeys. To ensure that we could measure spiking synchrony across simultaneous units with high accuracy, we deployed “FBP” - a spike sorter designed specifically to resolve temporally and spatially overlapping extracellular spikes. The likelihood that two simultaneously recorded PCs fired together with millisecond precision was relatively small, with a less than 10% increase relative to chance. The absence of synchrony was consistent across a much larger population of 118 putative PC pairs, where one or both units lacked climbing-fiber responses. We also tested whether PCs preferentially synchronize during

execution of pursuit eye movements. As simultaneously recorded PCs tended to have very similar preferred directions of pursuit (mean angular distance of 13.4°), changes in their firing rates tended to be strongly correlated. While the likelihood of observing synchronous spikes between PC pairs was modulated with pursuit, it could be almost wholly accounted for by changes in firing rate. Using the measured millisecond correlations between simultaneous units, we simulated a population of 40 PCs, allowing us to assay the impact of the correlations in our data on a recipient cell in the cerebellar nucleus. The presence of the observed weak synchrony in the PC population resulted in only a 6% change in the input to a downstream neuron. Further, the rate responses of PCs could predict the firing of downstream units in the vestibular nucleus well (>0.90 VAF) based on PC rate signals alone without the need for precise timing of PC spikes. Our results suggest that the synchronous activity of PCs is unlikely to contribute substantially to motor behavior. Rather, coherent changes in the firing rate of PCs are relayed faithfully to downstream neurons.

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Poster

472. Cerebellum: Sensorimotor

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 472.06

Topic: E.02. Cerebellum

Title: Simple spike patterns and synaptic mechanisms encoding unpredicted sensory inputs by Purkinje cells and the cerebellar nuclei

Authors: ***S. BROWN**¹, M. MEDINA², C. VAAGA², M. HOLLA², I. RAMAN²;
¹Univ. Col. London, London, United Kingdom; ²Northwestern Univ., Evanston, IL

Abstract: Tactile stimulation of the whiskers of awake head-fixed mice elicits a transient, short-latency, 2-4 millisecond synchronous suppression of crus I/II Purkinje cell simple spike probability followed by an elevation in firing rate that correlated with whisker position (Brown and Raman, 2018). The sequence of suppression preceding elevation is paradoxical, given that feedforward inhibition of Purkinje cells by molecular layer interneurons (MLIs) follows direct excitation from parallel fibers (PFs). We therefore investigated the biophysical and circuit-level determinants of this pattern of activity. *In vivo* dendritic and somatic whole-cell recordings from anesthetized mice confirmed that the sensory-evoked suppression is driven by feedforward inhibition (N=6). In cerebellar slice recordings, disynaptic inhibitory postsynaptic currents (IPSCs) remained stable during trains of stimuli to PFs (5 stimuli at 50 Hz; IPSC5/IPSC1 = 1.1), while excitatory postsynaptic currents (EPSCs) strongly facilitated (EPSC5/EPSC1 = to 3.8), demonstrating that relative inhibitory strength is greatest at train onset when PFs are unfacilitated. To test how sensory-evoked suppression changes under naturally varying levels of PF facilitation, we made *in vivo* extracellular recordings from awake mice that experienced both passively applied and actively sensed stimulation of the whiskers. Suppression of simple spikes

was strong when whiskers were contacted passively by a bar, but weak with active whisking against the bar (-44 ± 6 sp/s vs -18 ± 5 sp/s). We next tested whether suppression was modulated by predictability, movement, and/or sensory input strength. Passive and active whisker deflections preceding suppression were similar, suggesting a minimal role for sensory strength. Simple spike suppression was strongly evoked by air puffs even during spontaneous movements, suggesting a limited regulation by movement. Suppression was greatly reduced, however, by conditions that rendered sensations predictable. With unpredicted stimuli, responses of cells in the cerebellar nuclei (CbN) inversely correlated with Purkinje responses, but with predicted stimuli, they were largely decoupled. Data and modeling suggest that, owing to short-term synaptic plasticity, synchronous suppression of crus I/II Purkinje simple spikes encodes the onset of sensory anomalies, disinhibiting the CbN to elicit motor responses.

Disclosures: **S. Brown:** None. **M. Medina:** None. **C. Vaaga:** None. **M. Holla:** None. **I. Raman:** None.

Poster

472. Cerebellum: Sensorimotor

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 472.07

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01-DC002390
NIH Grant R01-DC018061

Title: Cerebellar internal models accurately detect errors introduced by small disturbances with a marked transition in encoding

Authors: ***O. ZOBEIRI**¹, R. L. MILDREN², K. E. CULLEN³;

¹Johns Hopkins Univ., McGill Univ., Baltimore, MD; ²Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; ³Dept. of Biomed. Engin., The Johns Hopkins Univ., Baltimore, MD

Abstract: The ability to distinguish sensory self-generated (reafference) vs. external (exafference) information is fundamental for ensuring accurate motor control as well as cognition. For example, the same vestibulo-spinal reflex responses that are essential during passive perturbations would be counterproductive during active movements. Similarly, the ability to discriminate between 'self-generated' and 'external' sensory stimuli is essential for perceptual stability. In this context, our recent work has shown that the vestibular system distinguishes between reafference and exafference. Specifically, the brain computes an internal forward model of the predicted sensory consequences of the motor command. By comparing this prediction with the actual sensory feedback, the brain then calculates the 'sensory prediction error' (SPE). During normal active movements SPEs will be negligible, whereas when large perturbations are applied SPEs will be substantial. Our prior studies have shown that the vestibular nuclei neurons responsible for postural reflexes and perception are markedly

suppressed when head motion is actively produced. In contrast, these neurons demonstrate robust responses to unexpected motion. To date, however, experiments have only considered the encoding of large perturbations. This raises the question of how neurons respond to small perturbations within versus outside the range of natural variability. To address this, we recorded responses of Purkinje cells as well as their target neurons in the rostral Fastigial Nucleus (rFN) while we randomly applied assistive or resistive head perturbations during a fraction of active head movement trials. The smallest level of perturbation changed head velocity within the band of natural variability, while the largest level doubled or halved head velocity. We found that the rFN neurons robustly responded to perturbations across levels. Notably, even the smallest perturbation increased neuron sensitivity to values comparable to the passive condition. Interestingly, while we could not detect responses to small perturbations in individual Purkinje cells due to their greater variability, we could detect responses at the population level (~40 cells) that could explain the observed responses in rFN. Taken together, our findings suggest that the population of Purkinje cells detects small SPE and transmits this information to deep cerebellar to cancel reafference. This computation in turn, serves to ensure accurate motor control and perceptual stability. These findings have provided new insight into how motor learning can occur in new contexts, even in the presence of trial-to-trial variability.

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Poster

472. Cerebellum: Sensorimotor

Location: SDCC Halls B-H

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Program #/Poster #: 472.08

Topic: E.02. Cerebellum

Support: NIH NS116854

Title: Purkinje cell and cerebellar nuclei responses to trains of regular and irregular somatosensory stimuli

Authors: *S. GOLDSTEIN, S. T. BROWN, I. M. RAMAN;
Neurobio., Northwestern Univ., Evanston, IL

Abstract: The cerebellum is involved in tasks that require tracking time intervals shorter than one second, raising the question of how absolute and/or relative timing is represented in the activity of cerebellar neurons. When repetitive air puffs are applied regularly to the whisker pad of awake mice, Purkinje cell (Pkj) simple spike (SS) and complex spike (CxS) responses change over the course of stimulation (Zempolich et al., 2021). To address the question of whether these changes result from the repetition or the regularity of stimuli, we recorded from Pkj and cerebellar nuclear (CbN) cells in awake, head-fixed mice. We applied 900-1800 air puffs to the whisker pad in either a regular train, with an inter-puff interval of 300 ms, or an irregular train, with variable intervals uniformly distributed over 100-500 ms, and a mean of 300 ms. Consistent

with previous results, 22 Pkj cells responded to puffs with a transient elevation in CxS probability, generally peaking between 15-75 ms. This response was preceded by a transient, well-timed, short-latency (<10 ms) decrease in SS firing probability. CbN cells showed a correspondingly well-timed increase in firing probability. Differences in spiking patterns evoked by the irregular train became evident, however, when responses were binned by inter-puff interval (50-ms windows). Plotting the magnitude of SS suppression and CxS elevation vs. inter-puff interval revealed a U-shaped relation, with maxima at both short and long intervals and a minimum at intervals near the mean (300 ms). The magnitude of the change in CbN spiking also varied, but generally increased with longer inter-puff intervals. Puff evoked whisker movements also increased linearly with longer inter-puff intervals ($r^2=0.9$), consistent with sensitivity to CbN output. Notably, the changes in SS, CxS and CbN spike probability after a mean puff interval of 300 ms in the irregular train each closely matched the changes evoked by the 300-ms interval in the regular train. The variation in Pkj and CbN cell responses across different inter stimulus intervals provides a basis to examine how these signals emerge synaptically and how they may be used by the cerebellum to extract information about absolute and relative timing.

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Poster

472. Cerebellum: Sensorimotor

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Topic: E.02. Cerebellum

Support: Wellcome trust/DBT India alliance
DAE core funding

Title: Purkinje neurons control swim kinematics via eurydendroid cells in larval zebrafish

Authors: *V. AGARWAL, S. NARAYANAN, S. CHINTA, V. THIRUMALAI;
Natl. Ctr. for Biol. Sci., Bangalore, India

Abstract: The cerebellum is involved in motor control and coordination. Its circuitry is well conserved among vertebrates. It receives excitatory inputs from various sensory as well as pre-motor centres which converge onto Purkinje neurons (PNs) in the cerebellum. PNs send inhibitory inputs to eurydendroid cells (ECs) (in zebrafish) which are the major output cells of the cerebellum. PNs exhibit correlated activity with the motor output of animals and disruption of PN activity impairs motor output. Therefore, we sought to test how activity of PNs affects cerebellar output which in turn leads to changes in motor output. We use larval zebrafish as our model organism to answer these questions. We started by simultaneously recording cerebellar output (EC activity) and fictive swims of the fish. We found that activity of ECs is also correlated with swims of the animals. As multiple PNs converge onto single cerebellar output cells (ECs), we hypothesized that when PNs fire synchronously there will be a small window

when ECs are relieved from inhibition and have higher probability of firing. This will entrain the activity of ECs with respect to PN activity. To test this idea, we expressed channelrhodopsin (ChR2), a light activated cation channel, fused with mCherry specifically in PNs. We developed protocols to change population activity patterns of PNs by activating them using blue light. We successfully activated them in synchronous and asynchronous manner by using high frequency pulse train and long continuous light stimulus respectively. Next, we recorded the activity of ECs while activating PNs using different protocols. Finally, we recorded motor output (swims) of the animals while simultaneously manipulating PN activity optogenetically. During these experiments optic flow was presented to induce swims reliably. Our results show how modulation of PN activity cascades down to their post synaptic partners and finally to motor behaviors.

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Poster

472. Cerebellum: Sensorimotor

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 472.10

Topic: E.02. Cerebellum

Support: DFG Research Unit 1847 “The Physiology of distributed computing underlying higher brain functions in non-human primates”
Okinawa Institute of Science and Technology Graduate University

Title: Multidimensional cerebellar computations for flexible kinematic control of movements

Authors: A. MARKANDAY¹, *S. HONG², J. INOUE¹, E. DE SCHUTTER², P. THIER¹;
¹Dept. of Cognitive Neurol., Hertie Inst. For Clin. Brain Res., Tübingen, Germany; ²Okinawa Inst. of Sci. and Technol., Okinawa Inst. of Sci. and Technol., Okinawa, Japan

Abstract: Maintaining the precision of our movements critically depends on how well our movements adapt to continuously changing environments and states of the body. This function, captured by the term sensorimotor adaptation, is known to rely on the cerebellum. However, its circuit-level implementation remains poorly understood. More specifically, it has remained unclear how representations of expedient information on the input side are transformed into useful output. To address this central question, we recorded from mossy fibers (MF, network input, n=117) and Purkinje cells (PC, output, n=151) from two rhesus monkeys performing a repetitive saccade task. In this task, the fast repetition of saccades induced a fatigue-based decline in the velocity and a compensatory adaptive elongation of saccade duration such as to keep saccade amplitude nearly constant. In the MF and PC simple spike (SS) firing data, we identified multidimensional, manifold-like structures, which reflected representations of individual movement parameters in terms of the manifold geometry and dynamics. The PC

manifolds were distinguished from the ones of MF by their higher dimensionality, much more selective representations of movement parameters, and drastic changes over the course of fatigue-induced adaptation. Complex spikes, carrying error feedback signals, modulated the PC manifolds in error context-specific fashion, predicting the changes in the future movements. Surprisingly, we found that a simple feed-forward network (FFN) model could accurately simulate the MF-to-PC transformation. Our FFN model revealed that PC SS manifolds could be led back to their MF input through substantially expanding small variabilities in the latter. Overall, our results suggest that the cerebellum performs multidimensional adaptive computations by encoding several kinematic parameters concurrently, accommodating a flexible control of sensorimotor adaptation, and taking information on the prevailing type of error into account. Thereby, the cerebellar computations ensure that movements remain precise despite variable contextual requirements.

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Poster

472. Cerebellum: Sensorimotor

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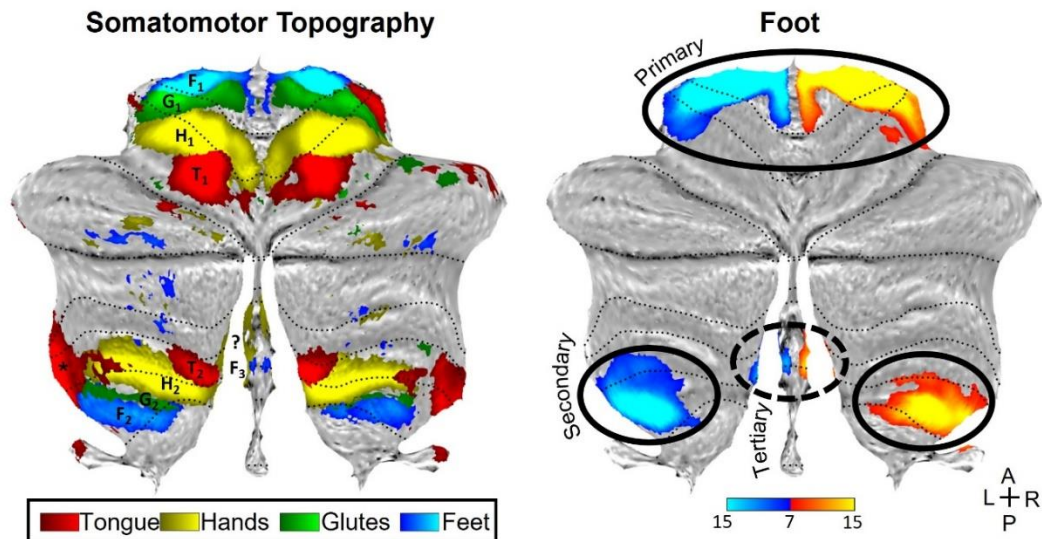
Title: A Third Somatomotor Representation in the Vermis of the Human Cerebellum

Authors: *N. SAADON GROSMAN¹, P. A. ANGELI¹, L. M. DINICOLA¹, R. L. BUCKNER^{1,2};

¹Harvard Univ., Cambridge, MA; ²Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Seminal neurophysiological studies in the 1940s discovered two somatomotor maps in the cerebellum - an inverted anterior lobe map and an upright posterior lobe map. Both maps have been confirmed in the human using non-invasive neuroimaging with additional hints of a third map near to the cerebellar vermis. We sought direct evidence for a third somatomotor map by using intensive, repeated functional MRI (fMRI) in people performing movements across multiple body parts (tongue, hands, glutes and feet). An initial discovery sample (N=4, 4 sessions per individual including 576 separate blocks of body movements) yielded evidence for the two established cerebellar somatomotor maps, as well as evidence for a third discontinuous foot representation near to the vermis. When the left versus right foot movements were directly contrasted, the third representation could be clearly distinguished from the second representation in multiple individuals. Functional connectivity from seed regions in the third somatomotor representation confirmed anatomically-specific connectivity with the cortex, paralleling the

patterns observed for the two well-established maps. All results were prospectively replicated in an independent dataset with new individuals (N=4). These collective findings provide direct support for a third somatomotor representation in the vermis of the cerebellum. A third somatomotor representation, together with spatially close regions linked to cognitive and affective networks, is consistent with a complete map of the cerebrum in the posterior lobe of the cerebellum and is inconsistent with a discontinuity between cognitive and motor representations. The third somatomotor representation also has implications for understanding the specific organization of the cerebellar vermis where distinct zones appear functionally specialized for somatomotor and visual domains.



Three somatomotor representations detected in a group-averaged flatmap of the cerebellum. (Left) Winner takes-all map of movements averaged across 8 participants is displayed for 4 body parts. A clear topography is evident in the primary (T1-H1-G1-F1) and secondary cerebellar representations (T2-H2-G2-F2). (Right) A contrast map of right (red) versus left (blue) foot movements, averaged across participants is displayed. Note that there are three separate representations (black circles) of the foot, with a tertiary representation (dashed) falling within the vermis, spatially discontinuous with the secondary representation. Color bars represents mean beta values. L: Left; R: Right; A: Anterior; P: Posterior.

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Poster

472. Cerebellum: Sensorimotor

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Support: NIH R01 HD040289 (Bastian, Cowan)
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Title: The cerebellar contribution to human feed-forward and feedback visuomotor control

Authors: *D. CAO^{1,2}, M. G. T. WILKINSON^{1,2}, A. J. BASTIAN^{3,4}, N. J. COWAN^{1,2};
¹Dept. of Mechanical Engin., ²Lab. for Computat. Sensing and Robotics, ³Johns Hopkins Univ.,
Baltimore, MD; ⁴Kennedy Krieger Inst., Baltimore, MD

Abstract: Damage to the cerebellum can cause ataxia, a condition associated with impaired movement coordination. Our prior work showed that people with ataxia (CBAs) have only modest deficits in visuomotor feedback control, allowing them to respond appropriately to visual perturbations. It remains unclear how other elements of motor control are affected by ataxia. A popular hypothesis is that the cerebellum is important for feedforward movement control through its contribution to an inverse internal model of the body, i.e., the computation of necessary motor commands to realize desired states. The primary goal of this study is to investigate how feedforward (inverse internal model) control is impacted by the severity of ataxia. We also examine how feedback control is affected by ataxia severity. Subjects tracked a 2D, pseudo-random green target cursor using a white cursor representing their hand position in virtual reality. We simultaneously applied a second, uncorrelated pseudo-random 2D disturbance to their hand position cursor. This allowed us to dissect the contributions of feedforward control (i.e., hand response based purely on the target cursor) from feedback control (i.e., hand response to the perceived error) in this tracking task. We computed different feedforward and feedback model structures to explain the performance of CBAs (n = 19) and age-matched controls (n = 15). For both groups, a pure gain with time delay accurately modeled feedforward control, though CBAs showed a significantly greater time delay in this computation (245 vs. 185 ms). This suggests that ataxia leads to greater time delay, rather than a substantially altered internal model for this task. Feedback control was modeled by an integrator with a gain and time delay in both groups, but with CBAs showing significantly greater time delay (144 vs. 123 ms), and significantly smaller gain (1.0 vs. 1.3). Modeling suggests this decrease in gain by CBAs helps to maintain stability robustness. We investigated if a “preview” of the target trajectory could be used to enhance feedforward performance. While subjects tracked the green target, we showed 500 ms of the future target trajectory in blue. Both groups reduced tracking error significantly with the preview, and the benefit was comparable in magnitude. This suggests that both groups can effectively utilize preview information during feedforward and feedback control. Our results suggest that cerebellar ataxia increases the delay in both feedforward and feedback control but does not structurally change either element for this tracking task. Feedforward control can be improved by providing CBAs with a visual prediction of where they will be asked to move.

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Poster

472. Cerebellum: Sensorimotor

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Topic: E.02. Cerebellum

Support: NIH Grant R01 HD040289

Title: Learning a sequential 3D movement in cerebellar ataxia using temporally specific reinforcement

Authors: *R. A. MOONEY¹, D. CAO¹, P. A. CELNIK¹, A. J. BASTIAN^{1,2};
¹Johns Hopkins Univ., Baltimore, MD; ²Kennedy Krieger Inst., Baltimore, MD

Abstract: Most actions, whether as simple as drinking from a glass, or as complex as a gymnastics floor routine, involve sequential movements. Learning to execute these movement sequences, or relearning to after neurological injury, relies on interacting behavioral mechanisms that engage distinct neural circuits. Reinforcement learning is a fundamental mechanism, driven by binary signals (e.g., success or failure), that requires an individual to explore different actions to maximize rewards. Yet, binary feedback after a sequential movement can be difficult to interpret - it does not tell the individual which segment of the movement was faulty. Providing segment-specific feedback, either during the movement (temporally specific) or after the entire sequence is complete (terminal) could alleviate this 'credit assignment' problem. First, we examined whether temporally specific or terminal feedback leads to better learning of a sequential movement. Healthy young participants (n = 12 per group) learned a 3-dimensional sequential arm movement in virtual reality over four consecutive days. They were asked to make a continuous arm movement that traversed four invisible segments marked by a starting point, three via points and an end point. Their hand position was represented by a virtual ball. For each trial, we calculated the average distance error between their hand position and the invisible segments. Reinforcement was given via binary feedback based on whether each segment's error was above or below the average error of the previous five trials. On each day, five trials with no binary feedback were performed before and after 60 trials of reinforcement training. Both groups improved performance on the whole movement by the end of day four, though the temporally specific group learned more than the terminal group, reducing error by ~75% versus ~50%, respectively. We suspect that the closer temporal proximity of feedback to execution explains superior learning in the temporally specific group. Next, we determined whether temporally specific reinforcement could be used to train the same sequential movement in people with cerebellar ataxia (n = 18) and older age-matched controls (n = 15). Importantly, we found that both groups were able to reduce error by ~50% by the end of day four. Thus, providing segment-specific reinforcement in close temporal proximity to execution leads to learning of a sequential movement and can be leveraged to train arm movements in people with ataxia. Our results have important implications for designing effective reinforcement paradigms to optimize motor learning in clinical rehabilitation contexts.

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Poster

472. Cerebellum: Sensorimotor

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Topic: E.02. Cerebellum

Support: R37 HD081168

Title: Preferential gating of self- and other-generated input in the infant rat cerebellum

Authors: *A. RICHARDSON¹, G. SOKOLOFF², M. S. BLUMBERG²;

¹Univ. of Iowa, ²Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA

Abstract: The integration of incoming sensory stimuli with outgoing motor commands is one of many critical functions performed by the cerebellum. The cerebellum uses this integrated sensorimotor information to coordinate the execution of fine motor behavior and motor learning. In adults, studies of cerebellar integration of sensorimotor signals focus on the interaction between the Purkinje cells in the cerebellar cortex and the neurons in the deep cerebellar nuclei. These interactions begin early in development when the cerebellum is starting to distinguish exafferent signals (i.e., externally generated sensory signals) and reafferent signals (e.g., self-generated sensory signals). This distinction is central to the cerebellum's ability to develop internal models of movement. We have hypothesized that infant rats develop internal models through extensive exposure to both exafferent stimuli in the nest, and reafferent stimuli arising from the myoclonic twitches that occur during active (REM) sleep. To test this hypothesis, we recorded extracellular activity in the cerebellar cortex and interpositus nucleus in rats at postnatal day 12. Pups cycled freely between sleep and wake, and their twitch-related activity was compared with the activity evoked by peripheral stimulation of the limbs and whiskers. Pups were also recorded under light urethane anesthesia to isolate exafferent activity. In unanesthetized pups, whereas Purkinje cells responded to both exafferent and reafferent input, neurons in the interpositus nucleus responded preferentially to reafferent input from twitches. In contrast, when pups were tested under urethane, neurons in the interpositus nucleus now responded to exafferent stimuli, suggesting that urethane acts on Purkinje cells to disinhibit the interpositus nucleus. Thus, Purkinje cells receive both exafferent and reafferent input, while neurons in the interpositus nucleus receive only reafferent input due to selective inhibition during exafferent stimulation. Previous work from our lab shows that the inferior olive and lateral reticular nucleus convey corollary discharge signals associated with the production of twitches. We therefore suggest myoclonic twitches may provide a mechanistic basis for the developing cerebellum's ability to distinguish reafferent and exafferent input.

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Poster

472. Cerebellum: Sensorimotor

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Title: A Cerebellar Brain-Machine Interface

Authors: *R. RANGWANI^{1,2}, A. ABBASI², A. FEALY², T. GULATI^{2,1};

¹Dept. of Bioengineering, Henry Samueli Sch. of Engin., UCLA, Los Angeles, CA; ²Ctr. for Neural Sci. and Med. (CNSM), Departments of Biomed. Sci. and Neurol., Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Brain-machine interfaces (BMIs), or neuroprosthetics allow use of the neural activity for controlling external device. Impressive clinical applications in humans and non-human primates have demonstrated that activity of sensorimotor neocortical areas can be decoded as a control signal to replace lost motor function by controlling prosthetic devices. However, activity in subcortical sites, such as cerebellum (Cb), have received relatively little attention to implement direct neural control. This is surprising as studies have shown that Cb neurons (both in the Cb cortex and deep cerebellar nuclei) are tuned to limb position, velocity, duration and muscle activity during voluntary arm movements. BMI control through such sites, if viable, is also a lucrative option when sensorimotor neocortical areas are injured due to events such as stroke. We validated the use of direct Cb neural activity in a neuroprosthetic task in healthy and stroke models, and compared it to primary motor cortex (M1) direct control in healthy animals. We recorded single-units and local-field potentials (LFPs) from drivable polytrodes in the Cb as well as microelectrodes in M1 while adult healthy Long-Evans rats (n=7) performed a neuroprosthetic task using the M1 or Cb activity. We also conducted Cb BMI experiments in a cohort of M1-stroke injured animals (n=3), to test viability of Cb neural activity for BMI application with M1 injured. These animals underwent a photothrombotic stroke in M1. During the task, rats exerted control over the angular velocity of a water tube by modulating activity of a subset of experimenter defined Cb neurons, classified as ‘direct’ neurons and using a simple linear decoder (Gulati et al, Nat Neuro 2017; Kim et al, Cell 2019). Our results show that efficient direct neural control from Cb is possible and is not different from M1-driven control when compared through reductions in the proportion of unsuccessful trials and in time to reward. We observed that direct Cb neurons developed robust modulation which was not different from M1-driven BMIs. Strikingly, similar robust learning was seen in rats recovering from stroke that had their forelimb motor function compromised. We also saw extensive indirect task-related modulation in Cb. We also found band-limited oscillations emerged in LFPs of these regions which modulated task-related neural spiking with learning. We are currently analyzing how the local indirect activity predicted BMI direct activity in M1 and Cb driven sessions, and if this local modulation in Cb BMI sessions was different in healthy versus stroke animals. This work demonstrates feasibility of direct control using Cb activity when M1 is stroke-injured.

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Poster

472. Cerebellum: Sensorimotor

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Title: Aging-related declines in Purkinje cell firing contributes to motor deficits in aging mice

Authors: *E. FIELDS¹, M. KERN², A. HUANG², A. WATT^{1,2};
¹Integrated Program in Neurosci., ²Biol., McGill Univ., Montreal, QC, Canada

Abstract: The economic burden resulting from an aging population has led to the idea that we are facing an “aging epidemic.” While cardiovascular and neurodegenerative disease are well-known causes of decline and death in aging individuals, reductions in motor coordination, impaired gait, and balance deficits increase during aging, and limit a person’s quality of life and independence. Preventing and delaying motor decline is thus imperative to improving the quality of life of our aging population. The cerebellum is critically involved in motor coordination. Cerebellar Purkinje cells fire spontaneous action potentials at high frequencies which is disrupted in several animal models of ataxia, and rescuing Purkinje cell firing rate deficits in mouse models of ataxia has been shown to improve motor coordination (Cook, Fields & Watt, 2021). This suggests that high frequency firing of Purkinje cells is important for normal cerebellar function. We wondered whether cerebellar alterations contribute to aging-related motor decline. To address this, we measured motor coordination in healthy C57Bl/6J mice across their adult lifespan, from young to old adult, and observed a progressive age-related decline. We next performed loose cell-attached recordings from Purkinje cells to measure spontaneous action potential firing in acute cerebellar slices from young and old adult mice. We observed an age-dependent reduction in Purkinje cell firing rates across adult lifespan, suggesting that Purkinje cell firing might contribute to the decline in motor coordination we observed. To determine whether Purkinje cell firing alterations directly contribute to motor dysfunction in aging, we virally delivered designer chemogenetic receptors to Purkinje cells in order to modulate their action potential activity. We found that chemogenetically reducing firing rates of Purkinje cells in the anterior cerebellar vermis with inhibitory (Gi) DREADDs led to a decrease in motor coordination in young mice, suggesting that Purkinje cell firing output directly modulates motor coordination, and that lowered firing is associated with a reduction in motor coordination. Taken together, our data suggest that aging-related decreases in Purkinje cell firing rates contribute to declining motor coordination observed in aging, and that targeting these physiological changes may lead to future therapeutic avenues to improve motor aging.

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Poster

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Support: NINDS Grant R01NS050808
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Title: Mechanism underlying caffeine-Induced motor dysfunction in episodic neurological disorders

Authors: *H. SNELL, A. VITENZON, J. A. VERA, K. KHODAKHAH;
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Episodic Ataxia type 2 (EA2) is caused by loss-of-function mutations in the CACNA1A gene, which encodes the pore forming subunit of the P/Q type voltage gated calcium channel. Patients exhibit baseline ataxia and attacks of dyskinesia triggered by consumption of caffeine or ethanol, or by physiological or emotional stress. Here we investigate the mechanism underlying caffeine induced-attacks. Using the tottering mouse, a well characterized mouse model of EA2, we conducted behavior, and in-vivo and in-vitro electrophysiology, and found that caffeine increases levels of phosphorylated calmodulin in the cerebellum of tottering mice via a CK2 dependent mechanism. This causes erratic firing of cerebellar Purkinje cells by decreasing calcium activated potassium (SK) channel current. ShRNA knock down, or block, of CK2 prevented this erratic firing and attacks. At concentrations that induce attacks, caffeine is known to block presynaptic A1 adenosine receptors, which increases the release of neurotransmitters such as glutamate. Thus, we hypothesized activation of mGluR1 receptors on Purkinje cells led to the increased irregularity and attacks of dyskinesia. Indeed, fiber photometry data suggest caffeine increases the release of presynaptic glutamate at the granule cell-Purkinje cell synapse. Additionally, block of A1 adenosine receptors alone induced attacks of dyskinesia and increased Purkinje cell firing irregularity. Through co-immunoprecipitation, we identified novel interaction between mGluR1 and CK2. Lastly, the block of mGluR1 receptors significantly decreased the percent of caffeine-induced attacks, and prevented caffeine-induced Purkinje cell irregularity of firing and increase in phosphorylated calmodulin. These data establish the ground-work for the development of therapeutics to treat caffeine-induced attacks in EA2 patients.

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Poster

472. Cerebellum: Sensorimotor

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Support: Research Grants Council of the Hong Kong Special Administrative Region (CityU 11102618)
Research Grants Council of the Hong Kong Special Administrative Region (CityU 11102120)

Title: Ataxia is caused by suppressed expression of a circadian clock gene, *Bmal1*, in cerebellar Purkinje cells

Authors: *E. H. WONG, S. YANG;
Neurosci. department, City Univ. of Hong Kong, Kowloon Tong, Hong Kong

Abstract: Altered expression of circadian clock genes is considered to be a critical factor for ataxia, a neurological disease of uncoordinated movement. To date, a causal relationship of the Brain and Muscle ARNT-Like 1 (*Bmal1*), a circadian clock gene, with ataxia remains unknown. To investigate a specific role of *Bmal1* in ataxia, *Bmal1*-deficient mice are selected for various motor skill assays such as rotarod, elevated beam, irregular ladder, and footprint tests. *Bmal1*-deficient mice show ataxia-like behaviors which are similar to those of the established drug-induced ataxia mice with 3 acetylpyridine (3AP). The fact that 3AP-intoxicated mice lack *Bmal1* in the cerebellum further demonstrates a close involvement of *Bmal1* with ataxia. Unlike wildtype (WT) mice showing better motor skills in the daytime, chemical and genetical *Bmal1*-deficient mice show no diurnal motor activity and learning. Moreover, the viral administration of a *Bmal1*-containing AAV in cerebellar Purkinje cells alleviates the behavior signs of ataxia, which suggests a critical role of abnormal expression of Purkinje cells' *Bmal1* in ataxia manifestation.

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Poster

472. Cerebellum: Sensorimotor

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Support: NIH Grant R01MH112168

Title: Cerebellar microRNA-206 regulates Purkinje cell excitability and sensorimotor gating

Authors: *M. P. HEYER¹, M. ISHIKAWA¹, J. E. EVANGELISTA², A. MA'AYAN², G. FENG³, P. J. KENNY¹;

¹Neurosci., ²Pharmacol. Sci., Icahn Sch. of Med. at Mount Sinai, New York, NY; ³Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: The cerebellum is historically known for its roles in motor learning and coordination, but emerging evidence implicates this highly conserved region in more complex behaviors related to cognition, affect, and reward. Furthermore, cerebellar dysfunction is increasingly linked to the pathogenesis of schizophrenia, autism, and other neurodevelopmental disorders. The cerebellum shares extensive reciprocal connectivity with the neocortex, basal ganglia, and hindbrain nuclei, and is thought to convey sensory experiences that shape cortical development and function. Nevertheless, the genes, cells, and circuits that govern cerebellar interactions with higher-order brain circuits are poorly understood. Using brain-wide *in situ* hybridization, we find that a schizophrenia-associated microRNA, miR-206, is specifically enriched in cerebellar Purkinje cells. Mice with a targeted deletion of miR-206 exhibit impaired pre-pulse inhibition, an endophenotype of schizophrenia, stress-induced hypolocomotion, reduced extinction of fear memories, and sex-dependent cognitive deficits. Pre-pulse inhibition impairments were recapitulated by conditional deletion of miR-206 in Purkinje cells, and viral rescue of miR-206 expression in Purkinje cells reversed these deficits, suggesting that altered cerebellar output in the adult underlies these behavioral abnormalities. Consistent with this possibility, spontaneous firing frequency was increased and pacemaking channel current was decreased in Purkinje cells after miR-206 deletion. High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation after miR-206 deletion in Purkinje cells revealed potential target mRNAs in pathways related to neuronal excitability, glutamate signaling, and schizophrenia and bipolar disorder. Together, these findings suggest that miR-206 regulates cerebellar Purkinje cell function and downstream sensorimotor, cognitive, and affective behaviors relevant to schizophrenia and other neurodevelopmental disorders.

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Poster

472. Cerebellum: Sensorimotor

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Topic: E.02. Cerebellum

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Office of Naval Research (N00014-15-1-2312)
National Science Foundation (CNS-1714623)

Title: The neural input to the cerebellum during saccadic eye movements

Authors: *M. FAKHARIAN¹, P. HAGE⁴, E. SEDAGHAT-NEJAD², J. PI³, R. SHADMEHR⁵;
¹Johns Hopkins Univ., ³Biomed. Engin., ²Johns Hopkins Univ., Baltimore, MD; ⁴Biomed. Engin., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; ⁵Dept Biomed. Eng, Johns Hopkins Univ. Dept. of Biomed. Engin., Baltimore, MD

Abstract: The inputs that the cerebellar cortex receives are transformed into predictions by Purkinje cells (P-cells), which in turn play a critical role in control of movements. These inputs come in two forms: mossy fibers and climbing fibers. While the climbing fibers produce complex spikes that can be readily identified electrophysiologically, identification of mossy fibers is more difficult. As a result, little is known regarding the information that mossy fibers provide to the cerebellum.

Here, we used silicon probes in the marmoset oculomotor vermis (lobules VI and VII) and recorded activities of n=186 mossy fibers and n=193 climbing fibers, many of them simultaneously, during saccadic eye movements. The high-density electrodes provided multiple views of a single spike, demonstrating that a complex spike was orderly transformed from a slow waveform in the P-cell dendritic tree in the molecular layer, to a faster waveform near the cell body, and finally a weaker waveform along the P-cell axon in the granule layer. In the granule layer, we consistently observed a unique tri-phasic spike waveform in the shape of “m” followed by a negative after wave, which is thought to arise from the interaction of the mossy fibers with the rosettes that constitute synapses on the granule and Golgi cells.

The majority of the mossy fiber inputs were nearly silent during fixation and produced a burst at saccade onset with a tuning that was aligned to a specific saccade direction and amplitude. These inputs resembled activities of neurons in the caudal superior colliculus, coding for a specific saccade. In contrast, other mossy fibers were tonically active during fixation and then paused for saccades in all directions and amplitudes. These inputs resembled activities of neurons in the rostral superior colliculus, which are suppressed during all saccades. While the distribution of preferred saccades among mossy fiber inputs was very broad, there was increased representation for horizontal saccades.

Whereas the climbing fiber inputs to P-cells were highly organized, responding to visual cues and movements towards the contralateral space, the bursting mossy fiber inputs that arrived in close proximity to these P-cells represented movements in all directions. Taken together, the results suggest that P-cells receive a teaching signal (climbing fiber input) from a specific region of the visuomotor space, but that teaching signal can be associated with movements that activate mossy fibers in any direction or amplitude.

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Poster

472. Cerebellum: Sensorimotor

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 472.21

Title: WITHDRAWN

Poster

473. Reaching Movement Selection and Strategy in Health and Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 473.01

Topic: E.04. Voluntary Movements

Support: NSERC RGPIN/05408
NSERC RGPIN/05345
FRSQ fellowship to C.A. Canaveral

Title: Vestibular contributions to reach trajectory selection during simulated body motion

Authors: S. HACHÉ, C. A. CANAVERAL, L. VADNAIS-CUDDIHY, P. CISEK, *A. M. GREEN;
Univ. Montreal, Montreal, QC, Canada

Abstract: Daily activities involve coordinating voluntary limb movement with body motion. In line with this, vestibular signals have been shown to contribute to both spatial and dynamic aspects of reach planning and execution. However, less is known about how they contribute to action selection. Recent studies have shown vestibular influences on goal selection for eye movements (Rincon-Gonzalez et al., 2016) and effector choice for reaching (Bakker et al., 2017). Here we explored how vestibular signals influence the selection of reach trajectories as we move among objects in the environment. We tested the hypothesis that vestibular signals influence the choice of reach trajectory around an obstacle and that this influence is mediated by distinct mechanisms during reach planning vs. execution. Human subjects made reaching movements while seated with their right arm supported in the horizontal plane by a robotic exoskeleton. Forward reaches were made in darkness to a remembered target located 20 cm away while avoiding collision with a remembered obstacle placed midway between the start position and the target. Subjects were free to choose whether to avoid the obstacle by reaching around it to the left or to the right, and we quantified how their choices varied with the obstacle's horizontal position. To address the influence of vestibular signals on choice behavior, in 20% of pseudo-randomly chosen trials galvanic vestibular stimulation (GVS) of unpredictable polarity (3 mA pulse) was applied to simulate body motion. In separate experiments GVS was applied either during the memory period prior to reach onset (2.5 s) or during reach execution (750 ms). We predicted that GVS applied *before* reach onset would increase the proportion of choices relative to no stimulation controls in the *same* direction as the simulated motion, consistent with vestibular influences on "spatial updating" mechanisms during planning. In contrast, we predicted that GVS applied *during* reaching would increase the proportion of choices in the *opposite* direction to the simulated motion, consistent with vestibular influences on online correction mechanisms that act to stabilize the hand's trajectory in space (e.g., Martin et al., 2021). In agreement with these predictions, average psychometric curves quantifying choice

behavior reflected significant shifts in the direction of simulated motion when GVS was applied before movement onset but shifted in the opposite direction when GVS was applied during reach execution. These results suggest that vestibular signals indeed contribute to trajectory selection and that their influence is mediated via distinct mechanisms during reach planning vs. execution.

Disclosures: **S. Haché:** None. **C.A. Canaveral:** None. **L. Vadnais-Cuddihy:** None. **P. Cisek:** None. **A.M. Green:** None.

Poster

473. Reaching Movement Selection and Strategy in Health and Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 473.02

Topic: E.04. Voluntary Movements

Support: The United States-Israel Binational Science Foundation (BSF) grant No. 2019222

Title: Higher cognitive load in three-dimensional virtual reality color trails test interferes with head-hand coordination

Authors: A. LUSTIG^{1,2}, *M. WILF^{1,2}, M. PLOTNIK^{1,2};

¹Sheba Med. Ctr., Ramat Gan, Israel; ²Tel Aviv Univ., Tel Aviv, Israel

Abstract: The Color Trails Test (CTT) is a well-validated version of the Trail Making Test which assesses executive function in a manually performed task using paper-and-pencil. The CTT consists of two parts: *Trails A*, which evaluates sustained visual attention (SVA) through target tracking and sequencing, and *Trails B*, which requires divided attention (DA), in which participants also alternate between target color groups while maintaining sequencing. The simplistic motor action required from the participant in the paper-and-pencil CTT, i.e., drawing a connecting line between targets, falls short in describing the complex demands of real-life cognitive-motor functioning. Accordingly, we recently translated the CTT task into a 3-dimensional virtual reality computerized platform (VR-CTT). The VR-CTT requires greater multi-directional hand movements and head rotations scanning the 3D environment, locating the virtual targets in space, and reaching them with an avatar hand representation. In this work, we introduce a robust method for quantifying the extent to which head rotations and manual movements are synchronized in time and space while performing the VR-CTT, and use it to investigate features associated with the type of cognitive load in the task (i.e., Trails A versus Trails B). Data was collected from 122 healthy participants (ages: 20 – 90 y; divided into 3 age groups: young, middle aged and older adults) who performed the VR-CTT in a fully immersive VR system (HTC-Vive; New Taipei City, Taiwan). The experimental set-up included a head mounted device and a controller by which the participant interacted with the virtual environment to hit a sequence of 25 circular numbered targets scattered in the virtual space. We employed a cross-correlation analysis on the hand horizontal trajectory kinematics and head rotation angles on the left-right axis and registered the best-fit coefficient, the phase shift (i.e., time lag) between

the signals and the total completion time of the task. We found that the level of spatial coherence of hand-head movements was high ($R \geq 0.76$) in both Trails A and B, in all age groups. However, assessing head-hand phase shifts revealed longer time lags (i.e., in which head leads hand) in Trails B versus Trails A, in all age groups (e.g., for the middle-aged group; 0.97 ± 0.57 s in Trails B vs. 0.55 ± 0.35 s in Trails A, $p < 0.0001$; with Trails A values differing between young and older adults). We conclude that allocating cognitive resources to DA task (i.e., Trails B) causes increased temporal disassociation of the hand-head coupled motions as compared to SVA conditions.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Program #/Poster #: 473.03

Topic: E.04. Voluntary Movements

Support: NIH-R01NS120579
NIH-R37HD087089

Title: Interaction of rhythmic and discrete control objectives in manipulation of complex objects

Authors: *M. SADEGHI, D. STERNAD;
Northeastern Univ., Northeastern Univ., Boston, MA

Abstract: Skilful actions often involve switching between different control objectives. For example, when swirling a glass of wine, the primary goal of control is to bring the wine (internal degree of freedom) into a rhythmic circular motion. However, when bringing the glass to the mouth, the goal is to translate the glass itself from one location to another. Such transition of control objectives, here from a rhythmic to a discrete goal, and from a focus on internal to external degrees of freedom, is an essential feature of dexterous object manipulation. This study designed a novel experimental paradigm where subjects manipulated a cup with a ball rolling inside (akin to a glass of wine) on a horizontal plane. The task was implemented in a virtual setting, where the cup-and-ball movements were displayed on a screen in top-down view and subjects controlled the cup via a robotic manipulandum, which provided haptic feedback. The task was to rhythmically "swirl" the ball close to the rim of the cup while keeping the cup within a "home" circular region. An auditory cue then signalled subjects to translate the cup in a reach-like manner towards a target and resume the ball swirling at the target. As such, the task involved two transitions, one from rhythmic ball motion to discrete cup translation at home position, and the other from discrete cup translation back to rhythmic ball motion when arrived at the target position. We examined how control mechanisms associated with rhythmic (ball) rotations and discrete (cup) translation interacted, and how such transitions affected planning and execution of the cup translation as a goal-directed reach-like movement. The results showed that subjects

initiated the transition from rhythmic to discrete movement by tuning to a certain phase of the ball rhythmic cycle, prior to and after the reach. More importantly, the duration of the reach was modulated by the ball rhythmic phase prior to reach initiation, showing a strong effect of ball rhythmic cycle on the reach execution. Simulations of the task dynamics revealed that such modulation facilitated the smoothness of transitions between rhythmic and discrete movements. These observations indicated that the control mechanisms responsible for rhythmic and discrete movements were not separated and the parameters of the discrete reaching movement were strongly affected by certain characteristics of the rhythmic movement.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 473.04

Topic: E.04. Voluntary Movements

Title: Evidence for dual processes in motor working memory

Authors: *H. HILLMAN, T. BOTTHOF, A. D. FORRENCE, S. D. MCDUGLE;
Yale Univ., New Haven, CT

Abstract: Working memory has been comprehensively studied in sensory domains such as vision, however, little attention has been given to motor working memory (MWM), that is the use and maintenance of movement information to serve an ongoing task. The limited research on this subject has focused on a bottom-up approach, examining effector-specific sensory representations. However, there is evidence for a concurrent top-down process in which movement information is represented in an abstract, effector-independent format, such as a goal or trajectory. While it is intuitive that top-down processing is present in MWM, it has only been studied indirectly. Our goal was to develop a more comprehensive framework of MWM that dissociates bottom-up and top-down processes.

To that end, we conducted two experiments in which participants encode a reaching movement with one hand (guided by a robotic arm), and then reproduce the movement with either the same hand or opposite hand. We reasoned that by transferring hands between encoding and recall, participants could no longer rely on the effector-specific sensory representations (bottom-up process), and thus would solely depend on abstract representations (top-down process) to recall the movement.

Experiment 1 (n = 21) examined MWM effects by manipulating the number of movements that had to be remembered (set size). As expected, subjects' recall errors reflected classic working memory effects (e.g., recency, primacy). Critically, in the high set size trials, there was a significant and substantial gap in performance between the hand switch and no-switch conditions, but only for the two most recently encoded movements. This key result suggested that two factors may contribute to MWM - an effector-specific representation that rapidly

decays, and a more robust, longer lasting effector-independent representation. Experiment 2 (n = 20) utilized the same design as Experiment 1, but participants maintained information for a single encoded movement (set size = 1) for 3, 6, or 12 seconds prior to executing recall. As predicted, hand switching between movement encoding and recall had a strong effect on performance on the short delay trials (3s), but not the longer delay trials (6 and 12s). Importantly, the temporal decay we observed was attenuated relative to the large set size effects seen in Experiment 1. This suggests that the putative effector-specific component of MWM is susceptible to both temporal decay and interference from other movements. Taken together, our results support a dual process theory of MWM. A better understanding of the MWM system may reveal important insights into the flexible nature of human motor skill.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Program #/Poster #: 473.05

Topic: E.04. Voluntary Movements

Support: Swiss National Science Foundation through the National Centre of Competence in Research (NCCR) Robotics
ERC under the European Union's Horizon 2020 research and innovation program (grant agreement no. 813713)
Bertarelli Foundation

Title: Assessment of a diaphragmatic respiration-based control strategy for extra robotic limbs

Authors: ***L. POLLINA**¹, **G. DOMINIJANNI**¹, **D. LEAL PINHEIRO**^{1,2}, **M. GINI**³, **G. GAMBERINI**³, **C. PIERELLA**⁴, **J. OLIVIER**⁵, **S. SHOKUR**¹, **S. MICERA**^{1,3};

¹Ctr. for Neuroprosthetics and Inst. of Bioengineering, École Polytechnique Fédérale de Lausanne (EPFL), Geneva, Switzerland; ²Depto de Neurologia e Neurocirurgia / Disciplina de Neurociência, Escola Paulista de Medicina, Univ. Federal de São Paulo, São Paulo, Brazil; ³The BioRobotics Inst. and the Dept. of Excellence in Robotics and Artificial Intelligence, Sant'Anna Sch. of Advanced Studies, Pisa, Italy; ⁴DINOEMI, Univ. of Genova, Genoa, Italy; ⁵Inst. of Sci. and Industrial Technologies, Haute École du Paysage, d'Ingénierie et d'Architecture (HEPIA), HES-SO Univ. of Applied Sci. and Arts Western Switzerland, Geneva, Switzerland

Abstract: Human enhancement is a new field at the frontier between engineering and neuroscience aiming at developing technologies to improve human capabilities. Extra robotic limbs (XRLs) represent an example of motor augmentation, where users control a robotic limb providing extra degrees of freedom. One of the challenges when designing XRLs is identifying the control strategy allowing for coordinated and independent movements with respect to the

biological limbs (Dominijanni et al., 2021). Here, we sought to investigate the control of a Third Arm (TA) via the modulation of the diaphragmatic respiration. To assess the level of independence and coordination of our control strategy, we designed two tasks in a virtual reality environment: a *Unimanual Reaching* (UR) task to evaluate the level of independence of each arm and a *Bimanual Clapping* (BC) task to measure the level of coordination. Ten participants performed three sessions (S1, S2, and S3) over 3 days for each task. The forward-backward movement of the TA was controlled by the modulation of the diaphragmatic respiration, while its orientation was controlled by eye-tracking. After 1 year, five participants came back for a follow-up analysis (S4) to assess the learning retained and the independence of our control strategy with respect to secondary tasks. For this, we designed two new UR tasks, one in which participants also had to gaze around to pop bubbles (S4-G), and one in which they had to count forward while moving the TA (S4-C). Finally, the same subjects and a size matched control group of naïve participants did the simple UR task with a physical robot (R). For both the UR and BC tasks, a significant learning effect was present across the three sessions, notably between the success rates in S3 and S1. For the UR task a plateau was reached, while this was not the case for the BC task. Regarding the follow-up analysis, no learning effect was retained, with the success rates in S4 being significantly lower than in S3 and not significantly different from S1. No significant difference was found between S4, S4-C and S4-G, suggesting that our control strategy still allowed to perform secondary tasks well, including speaking. Interestingly, the performance in R was significantly higher than in S4 and not significantly different from the control group. We present a first exhaustive assessment of a diaphragmatic respiration-based control strategy for XRLs. We showed that this strategy could be mastered efficiently both independently and in coordination with the biological limbs without significantly hindering concurrent tasks such as gazing and speaking. Moreover, participants were also able to use it to control a robotic prototype of the TA.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Topic: E.04. Voluntary Movements

Support: NIH Grant P20GM103430
URI Start-Up Funding

Title: Head motion detector as a universal joystick for disabled patients

Authors: *D. PETROU¹, A. CETERA¹, M. MORROW¹, M. SHAWA¹, J. DRISCOLL¹, R. ABIRI²;

²Dept. of Biomed. Engin., ¹Univ. of Rhode Island, Kingston, RI

Abstract: With 5.4 million individuals in the United States alone who suffer from paralysis, assistive devices are important to grant independence to these patients. Such devices may be invasive such as a recent implantable BCI designed for control of complex assistive devices or less invasive such as computer cursor control interfaces that have been developed using electroencephalography and head motion tracking with a camera. This study aims to develop a new and non-invasive head motion detector to serve as a universal joystick in controlling 2D and 3D assistive device applications. In our current work, the sensor and the 3D-printed, head-mounted case have been successfully integrated to comfortably control a computer cursor and virtual robotic arm. We used an Arduino MPU-6050 as our inertial measurement unit (IMU) and an Arduino Pro Micro as our microcontroller. With the ability to perform digital motion processing (DMP), our IMU can process head motion with six degrees of freedom. As a user performs head movements, the microcontroller receives data from the IMU and relays it to a Python script to be translated to cursor movement. In our Python script, a median filter with nine points in its filter window is applied to our raw data for processing, and the script has a sampling frequency of 100 Hz. If the cursor is maintained on a target for 2 seconds, it is able to perform a mouse click. In our pilot study development for a 2D cursor control task, the two degrees of freedom needed were pitch and yaw, and these motions were recorded using gravitational acceleration as a means to understand head orientation. We also developed a custom graphical user interface (GUI) that serves to test our 2D cursor control and familiarize users with the required head motion. When started, a timer begins and the user must accurately select and eliminate all targets on the screen using the cursor. In our current testing with two human subjects (IRB-approved), an average travel time of 0.9 seconds and a 100% accuracy rate was achieved. Additionally, we validated our cursor control platform to move a 3D virtual robotic arm. Our goal is to investigate the usability of our device in controlling semi-autonomous assistive devices such as a complex physical robotic arm with grasping capabilities, while addressing the control trade-off between a human and an intelligent robot.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Topic: E.04. Voluntary Movements

Support: Albert Einstein Society

Title: Target size influences arm choice and efficiency of reaching movements after stroke

Authors: J. JACOB¹, *S. KANTAK²;

¹Moss Rehabil. Res. Inst., Elkins Park, PA; ²Moss Rehabil. Res. Institute, Arcadia Univ., Philadelphia, PA

Abstract: Despite having the capacity to perform functional movements with the paretic arm, individuals with stroke often choose to use the nonparetic arm in their activities of daily life. This phenomenon, broadly categorized as nonuse, is a major barrier to participation after stroke. Determining factors that influence the choice of arm during functional actions may inform strategies for remediating arm nonuse. In this study, we sought to determine if dexterity requirements of the task influence arm choice in goal-directed reaching actions in individuals with stroke, compared to neurotypical individuals. We compared reaching performance to visual targets of two different sizes projected over a 2-D horizontal hemi-workspace in a spontaneous-use condition. Arm use was quantified as frequency of dominant/nonparetic and nondominant/paretic arm movements across the entire workspace. Arm choice inefficiency was quantified as the number of midline crosses with either hands. We hypothesized that stroke survivors will demonstrate greater use of the nonparetic arm and more midline crossings while reaching smaller targets compared to larger targets. Participants with stroke and neurotypical individuals performed reaching movements to targets with their movement times in accordance with the Fitt's law. Movement times were longer for smaller targets compared to larger targets. In neurotypical individuals, there was no significant difference in the frequency of reaches between the two arms for larger or smaller targets. Participants with stroke showed greater reaching frequency of the nonparetic arm compared to the paretic arm. The reaching frequency of the paretic arm was greater during the small target condition compared to the large target condition. In addition, midline crossings were greater for both arms in the small target condition compared to large target condition for both groups. Our results suggest that dexterity requirements of reaching actions bias the use of non-paretic arm in stroke survivors while increasing arm choice inefficiency in both neurotypical controls and stroke survivors.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 473.08

Topic: E.04. Voluntary Movements

Title: Individual cognitive representations of gravity

Authors: *A. DEVEMY¹, L. DA SILVA PONTES³, T. POZZO², P. HILT¹;

¹INSERM U1093, Dijon, France; ²INSERM U1093, DIJON, France; ³Univ. do Estado do Para, Belem, Brazil

Abstract: The existence of a neural representation of gravity benefiting anticipation and action planning has extensively been described (Pozzo et al. (2011) doi: 10.1152/jn.00081.2011). During pointing movements in the vertical plane, previous studies have reported direction-dependent kinematic differences: acceleration duration was greater in the downward than in the upward direction. According to recent studies (Gaveau et al. (2016) doi:10.7554/eLife.16394), these direction-dependent kinematics reflect a strategy of effort minimization implemented by the brain to take advantage of the gravity force field. Such a strategy maximizing the economy assumes universal, subject-independent internal models and that generic laws of action are guiding motor behavior. However, reducing the control of a task to its energy optimization confines the understanding of behavioral differences to economical or expensive subjects while other motivations may influence the movement. In the present study, we examine the potential role of subjective values in guiding human movements in the gravity field, by reducing as much as possible spatial and temporal task-related constraints. We expect to reveal interindividual differences linked to Individual Motor Signatures (Hilt et al. (2016) doi: 10.1038/srep23868). Forty right-handed voluntary subjects performed vertical upward and downward arm movements at three different speeds: Spontaneous (Spont), Fast (Fast) and Slow (Slow). One trial was carried out as follows: A first marker appeared, indicating the participant to point it with his right arm. Then it disappeared, and after a delay (0, 1 or 2 seconds) indicating movement speed, another marker briefly appeared, which was the signal to start the movement. Arm movements were recorded with a Vicon motion capture camera system. Several kinematic parameters were computed: Movement Duration (MD), Maximum Speed (Vmax), Movement Vigor (Vig) Time to Peak Velocity (TPV). Our results showed the existence of idiosyncratic kinematic features that were consistent across speeds, mechanical modalities (Upward vs Downward) and gravity contexts. This strong consistency suggests that individual components determine movement kinematics in the vertical plane. The observation of a continuum of behaviors ranging from fast to slow subjects is not compatible with the idea that the movements carried out in the vertical plane rely exclusively on a generic internal model of gravity minimizing energy expenditure. Rather, our data demonstrate that internal values specific to each individual (and the subjective rewards associated with each of these values) influence arm kinematic features.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Program #/Poster #: 473.09

Topic: E.04. Voluntary Movements

Title: Arm impedance in different movement directions

Authors: *C. ZHANG¹, J. HERMUS³, F. TESSARI³, N. HOGAN^{3,4}, A. B. SCHWARTZ^{2,1};
¹Dept. of Bioengineering, ²Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; ³Dept. of Mechanical Engin., ⁴Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Subjects performed a reaching task that encourages them to set their arm impedance before moving. In this ballistic-release task, subjects maintain a force on a stationary handle for a random duration before it is suddenly released. The handle is mounted on a WAM robot (Barrett Technology, Newton, MA) that opposes force exerted by the subject during the hold phase while allowing low-friction displacement upon release. After release, the handle needs to be smoothly and accurately arrested in a specified target zone. This is similar to picking an apple without knowing when the stem will break. A variety of forces and target distances are applied, so that pre-setting the arm's stiffness based on each force-distance combination, would be an optimal way to control the movement. Subjects perform the task in a block design, making repeated trials of the same force-target distance combination. Although the subjects cannot anticipate when the handle will be released, the block design allows the subject to develop a predictive strategy for controlling the arm's impedance to regulate the handle's motion upon release. Subjects performed this task by moving the handle in four orthogonal directions in a single plane. We modeled the endpoint movement as a second-order dynamic system consisting of a spring, damper, and mass. These parameters were estimated using the recorded force and displacement of the handle during the task. We found that in each direction of movement, subjects adjusted their impedance as if they intended to reach an equilibrium point that coincided with the target. This suggests that a control strategy is used to regulate component muscle activations flexibly for different behavioral criteria and for various direction-dependent arm configurations, to produce specific arm impedances. In the future, this experimental paradigm, in combination with neuronal recordings in monkeys, will be used to study neural correlates of impedance.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Topic: E.04. Voluntary Movements

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Title: Mental rotation incurs a cognitive cost during a visuomotor reaching task

Authors: *O. A. KIM, C. VELÁZQUEZ-VARGAS, J. A. TAYLOR;
Psychology, Princeton Univ., Princeton, NJ

Abstract: We often apply cognitive strategies to tailor our motor performance, such as when lining up a shot towards a sloped green during a round of golf. These kinds of strategies are also observed in the laboratory during visuomotor rotation tasks (VMR), in which participants reach to targets while visual feedback of the hand (cursor) is rotated by a fixed amount. Participants can strategically re-aim their movements to counteract a known rotation, causing the cursor to

land on the target by reaching in the direction opposite the rotation. These re-aiming strategies are supported by mental rotation (MR) of the reach trajectory and share a characteristic feature of classic MR: preparation time scales with the degree of MR necessary (Georgopoulos, 1995; McDougle & Taylor, 2019). Although most participants ought to be capable of the degree of MR required to counteract the rotations in VMR tasks, asymptotic performance during VMR training often falls short of countering the full rotation (Langsdorf et al., 2019; Weightman et al., 2022). This suggests that participants may be unwilling to perform the entire MR required to fully compensate for task errors. It is unclear whether any resistance to completing the MR is due to the additional cognitive effort or time required, both of which may compound with task success in estimating the return rate of reward. Here, we conducted a series of VMR studies to test whether MR for re-aiming movements incurs a cognitive cost independent from its potential temporal and performance costs. In all experiments, participants attempted to bring a visually-displayed cursor to a target in a center-out reaching task. The VMR task followed a dual-adaptation design in which participants learned to counteract both a small (25°) and large rotation (75°) on interleaved trials. Additionally, implicit adaptation was blunted by introducing a delay in cursor feedback, forcing participants to use an MR strategy to improve performance. After training, participants were given the choice of counteracting either the small or large rotation. Participants exhibited a strong preference for the smaller rotation. In multiple follow-up studies, this preference for the smaller rotation was observable even when differences in rotation amplitudes were as small as 10°. Furthermore, these preferences persisted when controlling for both the temporal costs of MR and the performance deficits that accompanied larger rotation amplitudes. Taken together, these studies indicate that MR strategies incur a distinct cognitive cost, independent of common factors that are known to influence decision making based on the return rate of reward.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Topic: E.04. Voluntary Movements

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CHRONOS Project
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Title: Auricular muscle control of extra degree of freedom for human enhancement

Authors: *D. LEAL PINHEIRO^{1,2}, J. FABER³, S. SHOKUR², S. MICERA^{2,4};

¹Depto de Neurologia e Neurocirurgia / Disciplina de Neurociência, Escola Paulista de Medicina,

Univ. Federal de São Paulo, São Paulo, Brazil; ²Ctr. for Neuroprosthetics and Inst. of Bioengineering, École Polytechnique Fédérale de Lausanne (EPFL), Genève, Switzerland; ³Depto de Neurologia e Neurocirurgia / Disciplina de Neurociência, Escola Paulista de Medicina, Univ. Federal de São Paulo, São Paulo, Brazil, São Paulo, Brazil; ⁴Scuola Superiore Sant'Anna, Pisa, Italy

Abstract: Human enhancement technologies aim to expand human sensory-motor capabilities through technological development. Finding a motor control strategy that is both intuitive and independent from the other biological limbs is a major challenge. In general, these approaches are based on motor, kinematic or neural redundancies (Dominijanni et al., 2021). However, for some users, these redundancies are not viable (e.g., high levels of spinal cord injury). Here, we propose the use of the auricular muscle (AM) to control extra degrees of freedom. As AM is a vestigial muscle with its neural connections preserved, it is possible to learn to activate it following training. Our approach is based on using different levels of contraction of both AMs to control a cursor in a two-dimensional paradigm with speed modulation. To allow the user to confidently stop the cursor in a given position, we have implemented a locker mechanism that gives the possibility to fix the current position of each axis independently. Five subjects (26.30 ± 3.70 years) followed a five-sessions training protocol (20-30 minutes per session) consisting of a 2D center-out task. Our preliminary study showed that all the participants significantly improved their success rate (Init: $37.78 \pm 11.16\%$ - End: $76.67 \pm 13.26\%$) and precision (as measured by the final distance from the target, Init: 22.15 ± 11.16 pts - End: 6.45 ± 2.70 pts) throughout the protocol. To evaluate the cognitive load, we performed a dual-task experiment consisting of adding the counting of random stimuli appearing on the screen to the before-mentioned approach. The performance results from this task were also compatible with those found in the single task, reaching a success rate of $74.44 \pm 16.94\%$ in the last day (Init: $36.67 \pm 25.64\%$) with concomitant improvement on the precision (Init: 30.59 ± 19.53 pts - End: 8.85 ± 3.49 pts). Finally, we found that in the last two sessions, the participants reported less mental demand and effort (using the Nasa Task Load Index questionnaire), which was aligned with a better performance. Comparing the questionnaires for both tasks, we see that they reported that the mental demand domain of the simple and dual tasks were significantly different during the first 3 days and then became similar, suggesting that through the learning process the mental load can be reduced. As such, we were able to show that the subjects can learn to control the movement of a cursor with two degrees of freedom using their AM. We also found that the cognitive load decreases over the sessions in a dual-task paradigm whilst improving the task performance. We believe AM control can be a viable solution for patients to recover some levels of independence.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 473.12

Topic: E.04. Voluntary Movements

Title: Do movement quality metrics improve our understanding of the restitution-compensation continuum after stroke?

Authors: C. MARTINEZ¹, A. CAIN¹, M. DEMERS¹, B. KIM², *C. J. WINSTEIN¹;
¹USC, Los Angeles, CA; ²SUNY Upstate Med. Univ., Syracuse, NY

Abstract: A common measure of paretic arm function is longitudinal movement time (MT) change using the Wolf Motor Function Test (WMFT) time score. While the 15-item WMFT average MT is used extensively as an efficacy endpoint in recovery trials, it lacks resolution about how functional improvement was achieved. Thus, it is unclear if patients achieve faster MT via restored movement patterns or improved efficiency of compensatory strategies. The WMFT-Reaching Performance Scale for Stroke (WMFT-RPSS) is a valid observational kinematic measure to assess performance quality for 2 WMFT tasks, 1 with object manipulation (Lift Can, max score = 19) and 1 with proximal control but no grasp (Hand to Box, max score = 16), where scores less than max indicate compensation (Martinez et al. 2020). Here, we sought to improve WMFT-RPSS rater reliability and explore longitudinal change in MT and performance quality. Two seasoned clinician researchers used WMFT-RPSS to rate WMFT videos from the ICARE database (Winstein et al. 2016) for 13 randomly selected participants (UE Fugl-Meyer range 29-56/66). Interrater agreement improved compared to our previous work (mean weighted Kappa: 0.63-0.66, mean Gwet's AC2 0.81-0.85). We assessed pre- (baseline) to post- (10 weeks) intervention MT change using the performance rate transformation (60 seconds/MT) (Hodics et al. 2012), and classified participants who achieved faster MT using a criterion threshold of 1.5x baseline rate. As expected, average task rate and total WMFT-RPSS scores improved ($p < .001$), indicating improved movement speed and quality. For Lift Can post-intervention, of 7 participants with 1.5x faster rate, none had max WMFT-RPSS score- evidence that all used compensatory movements to accomplish faster performance. For Hand to Box, 3 of the 10 participants (30%) with 1.5x faster rates had max WMFT-RPSS score-an indication that a restored movement strategy was used to achieve a faster MT. For both tasks, post-intervention compensation was reflected most commonly by deficits in "Movement Continuity" and "Task Completion" while post-intervention restitution (i.e., max score) was reflected most commonly by improved "Trunk Displacement" and "Elbow Movement." Together, task WMFT time score and WMFT-RPSS provide resolution about the specific movement elements associated with improved paretic limb MT. These findings provide motivation for analysis of a larger dataset, the ICARE video archive ($n > 300$) to: 1) characterize the nature of movement recovery, and 2) identify baseline movement predictors that may enhance predictor models which rely on standard clinical metrics (e.g. Fugl-Meyer, MT, CST integrity).

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Program #/Poster #: 473.13

Topic: E.04. Voluntary Movements

Title: Switching actions do not require an independent inhibitory mechanism to suppress initial motor plans

Authors: *S. ZHONG¹, V. N. CHRISTOPOULOS²;

¹Neurosci., Univ. of California Riverside, Riverside, CA; ²Bioengineering, Univ. of California, Riverside, Riverside, CA

Abstract: How actions are initiated and regulated based on changing demands of the environment is a fundamental neurobiological question. A key component of action regulation is action inhibition, which when abnormal contributes to various neuropsychiatric disorders. Recently, we studied the mechanism of action regulation that involves inhibition in tasks with competing or conflicting information (Zhong et al., bioRxiv 2022.04.20.488864). We presented for the first time an integrated mechanism of action regulation that includes cortical and subcortical regions and determines both action initiation and inhibition. In this study, we further explore the mechanism for switching-changing motor plans in response to environmental changes. We recruited healthy young adults (n=7, ages 21-40) to perform a series of behavioral experiments, in which they were trained to control a cursor on a computer screen using a joystick to reach towards targets presented at various locations. The experiment includes three tasks: decision-making task, in which subjects were instructed to reach towards a target or free to choose between two targets presented simultaneously; stop signal task, in which a stop signal was added in some trials of the decision-making task forcing the subjects to inhibit any action, and switch task, which required switching to another target in some trials of the decision-making task. We found that the reaction time (RT) for the one-target trials is always shorter than that of the two target trials, regardless of the task (two-sample t-test, $p < 0.001$). In the stop signal task, the probability of a successful stop is inversely correlated with the stop signal delay, and it's easier to completely suppress an action when there are two targets. Similarly, in the switch task, the probability of a successful switch is inversely correlated with the switch signal delay, and it's easier to switch when both the old and new targets are available prior to movement initiation. Interestingly, we found no difference in RT between two-target trials in which the subjects anticipated to switch their actions and control trials - i.e., no switch or stop signal was anticipated. On the other hand, subjects delayed their response when they anticipated a stop signal compared to the control trials or when they anticipated a switch signal. These findings provide strong evidence that unlike stopping, switching does not require an independent inhibitory mechanism to suppress ongoing/planned actions. Overall, our study showed that switching of motor plans could be achieved through a competition between the two actions associated with the old and the new targets, without engaging stopping circuitry.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Program #/Poster #: 473.14

Topic: E.04. Voluntary Movements

Support: NIH Res/0260/0271

Title: A primate model for the flexor synergy after stroke

Authors: *A. BAINES¹, S. N. BAKER²;

¹Biosci. Inst., ²Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: There are over a million stroke survivors in the UK. Some patients can become ‘stuck’ with unhelpful muscle co-contractions, whereby shoulder abductors obligatorily activate elbow flexors (flexor synergy). This makes reaching and grasping almost impossible. Despite this, no animal study has attempted to investigate the neural source of post-stroke synergies separate from weakness. The aim was to dissociate the contributions of different areas of the primary motor cortex (M1) to positive and negative motor signs after stroke. These areas were the ‘Old’ (anterior part) and ‘New’ M1. Two rhesus macaques were trained in a reach and grasp task. Reaches were recorded with both the arm free in space, and with an insert table which forced shoulder abductors to be more active. The comparison of these two conditions was critical to identify synergies from weakness. Wires for electromyography (EMG) recording were implanted into 12 upper limb muscles. Video footage allowed for kinematics analysis. After baseline recording, endothelin-1 infusions produced focal ischaemic lesions of upper limb representations in each cortical area. EMG and kinematics were analysed over 15 weeks post-lesion. The anterior Old M1 lesion did not produce muscle weakness. Post-lesion kinematics were near-normal, with significant slowing of maximum movement speed only for extension movements. There was slower extension where shoulder abductors were more active. In contrast, the New M1 lesion led to severe and immediate weakness in all muscles. This was irrespective of a requirement for increased shoulder abduction. Flexors recovered back to baseline within ~5 weeks. However, extensors remained persistently lower than baseline EMG, and kinematics of movements requiring extension never fully recovered. These results demonstrated that New M1 damage after stroke contributed to negative motor signs, whereas the anterior Old M1 lesion did not.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Program #/Poster #: 473.15

Topic: E.04. Voluntary Movements

Support: Vista
CFREF

Title: Influence of a visual landmark shift on memory-guided reaching in monkeys

Authors: *J. LIN, H. WANG, S. SUN, X. YAN, J. D. CRAWFORD;
York Univ., Toronto, ON, Canada

Abstract: The brain uses various sources of visual information, including both egocentric cues (e.g., object location relative to the eye) and allocentric (e.g., object location relative to other visual landmarks) to aim movements. It has been shown that humans optimally weigh egocentric and allocentric (landmark) cues when pointing (Bryne & Crawford 2010) but it is not known if monkeys do this. The main purpose of this study is to determine the influence of allocentric cue shifts on reaching responses in non-human primates. In order to do this, reach and gaze data were collected from one female *Macaca mulatta* monkey (ML) trained to perform a memory guided reaching task. The hand was initially placed at 1 of 3 varying locations of a waist level LED bar while gaze fixated centrally. A landmark (4 'dots' spaced 10° apart forming the corners of a virtual square) was then presented at 1 of 15 locations on a touch screen after a delay. A visual target then appeared transiently at a variable location within or outside this virtual square, followed by a visual mask. After the mask, the landmark either reappeared at the same location (stable landmark condition) or shifted by 8 degrees in one of 8 directions (landmark shift condition). The fixation light then extinguished, signaling a reach to the target. 'No-landmark' controls were the same, but without the landmark. Reaches correlated ($r = 0.82$) with target location relative to landmarks, showing animals did not simply reach to the landmark. Correlations of reach and gaze were poor ($r = 0.05$), suggesting gaze was not used to guide reach in this task. In the landmark shift condition, reaches shifted partially (mean=25%) with the landmark, with larger/smaller influence when the landmark shifted toward/away from initial gaze position. Overall, this data suggests that the monkey is influenced by visual landmarks when reaching to remembered target in a similar way as humans.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Program #/Poster #: 473.16

Topic: E.04. Voluntary Movements

Support: NIH NINDS K99/R00-NS101127
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Steve Palermo Foundation

Title: Evidence for corrective submovements in a precision center-out task with visual perturbations

Authors: *K. SCHWARTZE¹, A. G. ROUSE²;

¹Home, Univ. of Kansas Med. Ctr., Kansas City, KS; ²U of Kansas Med. Ctr., U of Kansas Med. Ctr., Kansas City, KS

Abstract: Whether in response to the environment spontaneously changing or due to motor error, it is necessary for individuals to be able to adjust behavior within a goal-directed movement. As a result, precise reaching movements are often multiphasic, with several sequential bell-shaped velocity profiles. Comprised of an initial and any required corrective submovements, these movement sequences give insight to how new sensory information is integrated at a neural level. Two macaques were trained to perform a precision center-out reaching task designed with small peripheral targets to necessitate movement correction. At a random time point during movement, a visual perturbation shifted the target location on some trials. The target perturbation was always 90° in relation to the straight-line path from the current hand position to the original target location. The perpendicular perturbation of the target required directional corrections but did not theoretically require a change in reach distance or hand speed. By probing hand speed during trials with the perpendicular target perturbation, one may gain insight into the timing of the updates of the sensorimotor plan. The macaques were required to adjust and reach the new perturbed target location to receive a liquid reward. The frequency of perturbations was adjusted so approximately 20% of the trials contained a perturbation. Our analysis classified trials into three groups based off perturbation time, trials with: 1) no perturbation, 2) an early perturbation 200-50ms before peak speed, 3) a late perturbation 50-200ms after peak speed. We observed similar speed peak-to-peak response times and speed profiles between the non-perturbed trials and trials with early perturbations. Interestingly the late perturbation group was split into two cohorts of fast response and slow response with a wider, bimodal distribution. The fast response trials showed a small, immediate correction followed by a second correction while the slow response late-perturbation trials had a longer delay before only one correction occurred. We hypothesize this bimodal distribution of fast and slow responses is evidence for corrective movements divided into submovements. For some perturbations, a fast response would already have been initiated and reached a “point of no return” as the perturbation occurred. Conversely, for other perturbations, the sensorimotor processing was interrupted and delayed as the perturbation information was integrated. We propose that further studies that include neural activity will identify phases dedicated to either movement execution or sensory integration and help explain these corrective submovements.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Topic: E.04. Voluntary Movements

Support: NSF OISE Grant 1854158

Title: Grip coordination patterns learned while manipulating rigid objects transfer incompletely to compliant objects but not vice versa

Authors: A. GRADY¹, O. LUTZ¹, M. CASADIO², *R. A. SCHEIDT¹;

¹Marquette Univ., Marquette Univ., Milwaukee, WI; ²Sensory Motor Performance Program, Univ. of Genoa, Genova, Italy

Abstract: The hand has more than 20 mechanical degrees of freedom; this complexity affords flexibility in how we interact with real-world objects. Some objects like piano keys are compliant (spring-like) such that finger forces induce correlated fingertip motions. Other objects like coffee mugs are rigid such that finger forces need not induce finger motion. To what extent can finger coordination patterns learned while manipulating rigid objects transfer to compliant objects and *vice versa*? 48 young adults consented to participate in this study. The dominant right arm and hand rested on a device that used plungers to position force sensors under each of the 4 fingers. Rigid rods or springs could be inserted in the plungers to make the interface rigid, stiff (1.65 lb/in) or compliant (0.83 lb/in). Forces from the 4 sensors were linearly mapped onto the 2-D position of a cursor on a display screen. The screen was a 5x5 grid of 25 target squares. Subjects were instructed to move the cursor into the cued target as quickly and accurately as possible. The mapping of finger forces onto cursor motion was formed from the first 2 principal components obtained during a calibration task where subjects made a sequence of single- and multi-finger presses against plungers with stiff inserted springs. Subjects were divided into 4 groups of 12 that differed in the sequence of mechanical interfaces experienced during 10 blocks of 25 target captures according to an “A-B-A” study design. Breaks were provided after blocks 5 and 8 to allow us to (potentially) swap the compliant springs for rigid rods or *vice versa*. One group (the RCR group) interacted with the rigid interface in blocks 1-5, the compliant interface in blocks 6-8, and the rigid interface in blocks 9-10. Another group experienced the complementary sequence (CRC - blocks 1-5: compliant; blocks 6-8: rigid; 9-10: compliant). Two control groups interacted with either the rigid or compliant interface in all 10 blocks. We used measures of kinematic- (path length) and temporal-efficiency (target capture time) to compare the extent of learning (from blocks 1 to 5) and transfer (between blocks 5 and 6) across groups. All 4 groups rapidly learned to coordinate forces generated by their 4 fingers to capture screen targets with efficiency; the groups did not differ in either efficiency measure in blocks 1 and 5. Only the RCR group showed decreased efficiency between blocks 5 and 6, although performance in block 6 was considerably better than in block 1 for all groups. These results thus reveal incomplete transfer of finger force coordination from rigid to compliant objects whereas transfer of coordination patterns from compliant to rigid objects appears more complete.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Topic: E.04. Voluntary Movements

Support: Stanford University Wu Tsai Neuroscience Institute
R01: NIH R01NS123517

Title: A freely moving behavioral and neural data set to explore neural correlates of locomotion

Authors: *A. S. LING¹, M. SILVERNAGEL¹, P. NUYUJUKIAN²;

¹Electrical Engin., ²Bioengineering, Neurosurgery, Wu Tsai Neurosciences Institute, Stanford Bio-X, Stanford Univ., Stanford, CA

Abstract: Motor systems neuroscience seeks to understand how the brain controls movement, and while we have some insight into simple, constrained behaviors, we have limited knowledge about complex, naturalistic ones. For example, how do we multitask while walking or performing different behavioral tasks? Advancements in markerless pose tracking using neural networks for smaller freely moving animals has helped systems neuroscience study this question, but these techniques still have limited impact on primate models due to the lack of availability of training data and the large size of the enclosure needed to be covered. To address this, we developed a novel primate pose tracking approach using point clouds instead of RGB, which contrasts currently established pose tracking methods. This helped us establish a multi-camera platform that synchronized wireless neural data and markerless 3D kinematics in freely moving macaques, allowing us to record a diverse range of neuroethologically relevant behaviors. In the observational enclosure, a macaque freely walked between two bowls placed on opposite walls for 20-30 minutes each run. Data collected from each run included point cloud data, full body and center of mass kinematics, and synchronized neural data from the arm regions of M1 and PMd. Three dimensional full body kinematics were extracted from the point cloud data using an articulated skeletal model and an optimization algorithm, and center of mass kinematics were extracted by localizing the macaque in the scene and taking the center of the point cloud. Across 3 months, we collected 1000s of laps of walking behavior that contain multiple behavioral event types synchronized to neural data which will facilitate answering basic motor systems neuroscience questions with sufficient statistical power. Additionally, neural state space analysis may be performed on the behavioral epochs of rhythmic walking, starting, stopping, turning, and sitting to standing to compare neural activity between rhythmic walking and abrupt movements. Exploring this question may help understand the mechanisms behind complex neural control of locomotion. Furthermore, these same analyses can be used to create ambulatory brain machine interfaces that have implications for improving general human mobility.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Topic: E.04. Voluntary Movements

Support: NSF GRFP DGE-1656518
Wu Tsai Neuroscience Institute
NIH R01NS123517

Title: Point clouds synchronized to M1, PMd electrophysiology in large animals

Authors: *M. P. SILVERNAGEL¹, A. S. LING¹, P. NUYUJUKIAN^{2,3,1,4,5};
¹Electrical Engin., ²Bioengineering, ³Neurosurg., ⁴Wu Tsai Neurosci. Inst., ⁵Bio-X Inst., Stanford Univ., Stanford, CA

Abstract: Motor systems neuroscience seeks to explain the neural mechanisms that guide movement from planning to execution. Historically, rhesus macaque studies in this field have limited free motion only to a particular region of interest. This limits the presence of confounding variables and permits the use of marker-based motion tracking technologies, providing a robust framework for repeatable tasks. Such studies have been the backbone of motor systems neuroscience for decades, providing numerous insights into how the mammalian brain controls movement. Yet, as these studies only allow for a subset of movement types, this data may lack the full expression of neural activity generated during naturalistic movement. This has led to repeated calls for studies that examine neuronal dynamics during complex, neuroethologically relevant behaviors (Gao et al., 2015; Bialek, 2022). To address this, our recent work has leveraged depth camera technology to capture full-body kinematics and wireless neural data as a macaque executes diverse, unconstrained movements (Silvernagel and Ling et al., 2021). Using an improved version of this experimental platform, we demonstrate the first synchronized recording of full-body point clouds to hundreds of electrophysiology channels across primary motor (M1) and dorsal premotor (PMd) cortex. Further, over a ten-week period we amassed thousands of trials of a rhesus macaque performing a stereotyped walking and reaching task. With ability to record from multiple brain regions and reliably capture repeated movements, free movement platforms can complement fixed-movement studies to provide a more comprehensive understanding of the motor cortex.



Figure 1: Synchronized neural and movement data during a walking and reaching task. **A.** Spike raster depicting 96 electrophysiology channels in M1. **B.** Spike raster depicting 96 electrophysiology channels in PMd. **C.** Point clouds of Monkey C. From left to right: Monkey C reaching for a treat reward; Monkey C walking to opposite side of enclosure; Monkey C reaching for a second treat reward. Vertical lines in **A**, **B** correspond to similarly colored point clouds in **C**.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Topic: E.04. Voluntary Movements

Support: MWK Lower Saxony ZB3422 "DeMoDiag"
German Research Foundation (DFG) Collaborative Research Consortium 1528
"Cognition of Interaction"

Title: The Exploration Room (ExR) - an experimental environment for systems neuroscience studies in freely moving macaques during naturalistic behaviors

Authors: *Z. AHMED^{1,2}, I. LACAL¹, R. VOGG³, M. NUSKE⁴, N. SHAHIDI^{1,5}, F. WÖRGÖTTER^{4,5}, A. ECKER^{3,5,6}, A. GAIL^{1,2,5,7};

¹Sensorimotor Group, German Primate Ctr., Göttingen, Germany; ²Fac. of Biol. and Psychology, ³Inst. of Computer Sci. and Campus Inst. Data Sci., Univ. of Göttingen, Göttingen, Germany; ⁴Dept. for Computat. Neurosci., Third Physics Inst., Göttingen, Germany; ⁵Leibniz ScienceCampus Primate Cognition, Göttingen, Germany; ⁶Max Planck Inst. for Dynamics and Self-Organization, Göttingen, Germany; ⁷Bernstein Ctr. for Computat. Neurosci., Göttingen, Germany

Abstract: Rhesus monkeys (*macaca mulatta*) are an important animal model in systems neuroscience. Widely used computer-based tasks and tethered neural recordings imply chair-seating and mostly also head movement constraints, preventing the study of more complex behaviors to improve our understanding of sensorimotor and decision making processes during ecologically more relevant behaviors like foraging or cooperation with others. Existing setups for freely moving macaques which include wireless neural recordings are of limited size and suited, for example, for short-distance walk-and-reach behaviors or treadmill walking. Larger environment feature markerless full-body motion capture, while neural recordings during complex, versatile full-body behaviors remain a challenge. We present a novel, highly modular, large-scale setup for neurophysiological recordings in freely moving macaques called the Exploration Room (ExR). The ExR is a ~4.3 x 2.6 x 2.5 m (W x D x H) setup suited for one or more animals simultaneously in the same physical space or separated in a transparent dyadic setting. For the latter, the room can be divided into two symmetrical halves using a motorized room divider. Walls and floor consist of panels, which can be exchanged individually against computer-controlled interactive feeders, or touchscreen kiosk systems (XBI). We demonstrate how the ExR's modularity and versatility can be used to elicit a variety of complex, yet experimentally induced behaviors within a single session without human interference (Playground Experiment, PE). PE consists of 36 potential food or fluid sources (patches) including flexible branches, XBIs, hollow toys, and litter piles. Using full-body markerless 3D pose estimation from an array of cameras, we identified "transition" behaviors during patch switching and patch-interaction behaviors. We demonstrate the ExR's suitability for systems neuroscience by presenting proof-of-concept wireless neural recordings obtained during PE. We recorded in the frontoparietal reach network of two rhesus macaques. We present neural data of complex behaviors, like reaching during a 2-leg-stand, which were not accessible so far, and 3D-tracked transition behaviors. As an example of patch interaction, we will compare reaches during different patch-specific postures and their neural correlates in cortical sensorimotor areas. We conclude that the ExR substantially extends the possibilities in systems neuroscience as it allows studying neural correlates of complex, ecologically relevant full-body behaviors beyond walking toward studying the cognition of interaction.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Topic: E.04. Voluntary Movements

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Simons Collaboration on the Global Brain
NIH grant DP2 NS120847

Title: A novel freely-moving paradigm for interrogating cortical-musculature and cortical-striatal dynamics during naturalistic behavior

Authors: *D. Y. XING¹, A. MIRI²;

¹Neurobio., Northwestern Univ., Chicago, IL; ²Neurobio., Northwestern Univ., Evanston, IL

Abstract: Our ability to generate a wide variety of movements reflects the profound flexibility of our motor system. However, how movements are controlled by neural populations across the full behavioral ethogram is still poorly understood. Specifically, it is unclear whether activity dynamics observed within motor cortex are preserved when expanding beyond artificially constrained and overtrained laboratory behaviors. Neural recordings from freely-behaving mice are a promising approach, but existing paradigms do not engage the remarkable dexterity exhibited by rodents when navigating complex terrains. Here, we present a novel paradigm where water-restricted mice traverse an arena in order to scavenge for water from ports positioned at multiple locations. By placing randomly spaced handholds throughout the arena, we are able to motivate mice to perform climbing and walking movements requiring precise, targeted placement of the limbs. Mice are able to proficiently walk across an irregularly spaced grid floor and climb up the walls, exhibiting a wide variety of movement trajectories and limb states. Mice also perform additional natural behaviors such as rearing, grooming, and eating. Eight intramuscular electrodes were chronically implanted to record muscle activity in flexor-extensor pairs across multiple joints in both forelimbs and one hind-limb. Analysis of muscle activity revealed rich and heterogeneous activity patterns across the space of motor behaviors expressed by the mice. Using chronically implanted neuropixels probes, we measured simultaneous activity from hundreds of motor cortical and striatal neurons and observed diverse neural responses and the emergence of distinct neural subspaces corresponding to separate motor behaviors. Using UMAP, an unsupervised dimensionality reduction technique, we found clusters corresponding to individual behaviors in both the muscle and neural activity. Therefore, our novel paradigm is able to reveal variation in neural activity structure across both dexterous and stereotyped motor behaviors.

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Poster

473. Reaching Movement Selection and Strategy in Health and Disease

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Topic: E.04. Voluntary Movements

Support: MWK Lower Saxony ZN3422 “DeMoDiag”
DFG CRC-1528 “Cognition of Interaction”

Title: Inferring commitment to choice from full-body trajectories during go-before-you-know decision making in freely moving rhesus monkeys

Authors: *I. LACAL¹, Z. AHMED^{1,2}, N. SHAHIDI^{1,3}, A. GAIL^{1,2,3,4},

¹Sensorimotor Group, German Primate Ctr., Göttingen, Germany; ²Fac. for Biol. and Psychology, Univ. of Göttingen, Göttingen, Germany; ³Leibniz ScienceCampus Primate Cognition, Göttingen, Germany; ⁴Bernstein Ctr. for Computat. Neurosci., Göttingen, Germany

Abstract: Neurophysiological recordings in chair-seated animals have extensively demonstrated that action goals are dynamically represented in primate sensorimotor cortex during movement planning and execution. To which extent established concepts gained from lab studies generalize to more complex, ecologically-relevant contexts remains unknown. We address this question by studying goal-directed, full-body movements in a behavioral paradigm that requires the experimental animal to decide while acting and that can allow us to investigate the neural basis of decision making in freely moving monkeys. Two rhesus macaques were trained to perform a walk-and-reach task in a 4.6 m (W) x 2.5 m (D) x 2.6 m (H) enclosure (Exploration Room) with two synchronized touchscreen-based kiosk systems (XBIs) serving as potential targets on a short side of the room. To start a trial, the monkey had to take a centered position at the opposite side of the room. Once the touchscreens turned white, the animal was allowed to walk towards the offered targets within a pre-defined time window. During this movement epoch, a change in color of the two screens instructed the reward associated to each target (blue = low, red = high) at varying stimulus-onset asynchrony (SOA). The monkeys' full-body postures were tracked offline in 2D and reconstructed in the 3D space via six cameras. The animals showed motivation to engage in the task and performed the walk-and-reach movement with a success rate above 90%. Moreover, within the first 30 sessions, both monkeys transitioned from a directionally-biased guessing behavior to a predominant reward-based choice strategy. The analysis of their velocity profiles showed that longer SOAs correlate with shorter peak velocity latency and longer movement time. To identify the point of commitment to the chosen option, we applied an adapted version of the Cone method (Ulbrich & Gail, 2020), a tool for single trial readout of target commitment from 3D trajectories. A strong correlation between the SOA and the time of commitment to the chosen option was found. Our results support three conclusions: (i) monkeys can learn to engage in experimentally controlled, goal-directed and unconstrained walk-and-reach movements in a trial-wise fashion within a large environment; (ii) full-body movement kinematics depend on the timing of environmental salient information and can be extracted from video-based markerless motion capture; (iii) the moment of commitment to a target can be quantified in single walking trajectories. These conclusions support the feasibility of the adopted

paradigm for trial-wise neurophysiological testing of choice behavior in freely moving macaques.

Disclosures: I. Lacial: None. Z. Ahmed: None. N. Shahidi: None. A. Gail: None.

Poster

473. Reaching Movement Selection and Strategy in Health and Disease

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 473.23

Topic: E.04. Voluntary Movements

Support: GE-2-2-023A (REXO)
IT-2-2-023 (VAFES)

Title: Three-dimensional center-out human upper-limb transportation motion measured by a single IMU

Authors: T. SZIBURIS^{1,2}, S. BLEX¹, *I. IOSSIFIDIS¹;

¹Dept. of Computer Sci., Ruhr West Univ. of Applied Sci., Muelheim an der Ruhr, Germany;

²Inst. for Neural Computation, Ruhr Univ. Bochum, Bochum, Germany

Abstract: This work presents a novel systematic dataset of 3D center-out transportation movements as the foundation for analysis and understanding of human upper-limb movements. The majority of former studies focused on 2D motion in rather restricted settings. The recording and analysis of 3D movements in a natural task setting, though more challenging, is essential for comprehending actual human motion.

Among the variety of applicable sensors and systems, we decided to utilize a single Inertial Measurement Unit (IMU). In general, motion trajectory recordings with infrared or optical sensors (3D or multiple 2D cameras) provide a higher precision. However, they are not well suited for integration in embedded systems due to constraints regarding size, power consumption, etc. Also, networks of multiple IMUs are not suitable when solely focusing on hand transportation trajectories. In the scope of the VAFES project (Virtual-Reality-based Machine Learning for Arm-Hand Function Evaluation and Support System), we develop a glove which serves as an easy-to-use diagnostic tool for movement disorders. This may be applied under clinical as well as ambulant conditions requiring portability without sophisticated set-ups. In particular, for recording the introduced dataset, an IMU sensor of the Xsens Awinda motion capture system is utilized and mounted on a cylindrical object to be transported. The measurement procedure is automatized; for each trial, one of nine targets aligned on a semicircle is randomly chosen. To avoid rhythmic movement patterns and specific time dependencies, random delays are introduced between a visual target cue and an acoustic start signal. The data is gathered from both hands of young adults without known movement disorders or impairments.

From the accelerations measured by the IMU, velocities and positions are integrated after initial filtering and drift compensation. Typically, the velocity profile of transportation movements

exposes one minimum and one maximum. Corresponding to the extrema, we decompose the trajectories into three movement phases: initiation, travel, and approach phase.

Besides a general analysis of transportation trajectories, the experiments will provide insights regarding inter-trial and inter-subject deviations by comparing variability measures. Together with the evaluation of the Edinburgh Handedness Inventory, the collected data allows for the study of handedness effects, too.

In future work, we will also capture data from persons experiencing movement disorders like Parkinson's disease. By methodical comparison, we aim at finding deviations which may allow pathological analysis and diagnosis.

Disclosures: T. Sziburi: None. S. Blex: None. I. Iossifidis: None.

Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.01

Topic: E.04. Voluntary Movements

Support: NIH R01 NS105697
Whitehall Foundation 2017-12-94

Title: Optical resting state reveals network architecture of sensorimotor cortex in monkeys

Authors: *N. S. CARD¹, O. A. GHARBAWIE²;
¹Bioengineering, ²Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Skilled movements require the coordination of neural activity throughout sensorimotor cortex. The most direct route of connectivity to achieve this coordination is corticocortical connections within and between the somatotopic representations of sensorimotor cortical areas including premotor cortex (PM), primary motor cortex (M1), and primary somatosensory cortex (S1). Although many such connections have been identified in previous work, the architecture of the functional network that they form is not clear due to limitations in spatial resolution or the number of sites studied per animal. Consequently, how neural activity in sensorimotor cortex is coordinated in service of skilled movements is not known. To address this knowledge gap, we investigated the connectivity of thousands of sites spanning sensorimotor cortex in 3 squirrel monkeys to reveal network organization. In longitudinal experiments (~1.5 years), we partitioned sensorimotor cortex using dense microelectrode mapping (>400 sites/hemisphere). PM and M1 were mapped with intracortical microstimulation and S1 was mapped with multi-unit recording. We used resting state intrinsic signal optical imaging (RS-ISOI, 630 nm illumination) to measure spontaneous hemodynamic fluctuations under isoflurane anesthesia. High resolution optical images (1312 x 1082 pixels, 19 $\mu\text{m}/\text{pixel}$, ~26 x 22 mm field of view) were acquired in >12 sessions/animal (15 min/session, 10 frames/s) over 14-16 months. Optical images were processed, temporally filtered (0.01-0.1 Hz), then used in cross-correlation

analyses between pixels to generate functional connectivity maps for thousands of sites spanning sensorimotor cortex. RS-ISOI revealed the functional connectivity of thousands of cortical sensorimotor sites at high spatial resolution, which was central to the investigation of cortical network architecture. We used an unsupervised community detection approach and a supervised network connectivity strength approach to identify organizational features of cortical sensorimotor network architecture. Cortical sensorimotor networks preferentially bound somatotopic representations across cortical areas. Connectivity between non-matched functional zones was dependent on cortical area. Connections between motor and somatosensory areas mostly targeted proprioceptive somatosensory zones. These organizational features converge to grant insight into how the sensorimotor network contributes to the generation of skilled movements.

Disclosures: N.S. Card: None. O.A. Gharbawie: None.

Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.02

Topic: E.04. Voluntary Movements

Title: Causal links between cortical circuits and motor expertise

Authors: *Q. NGUYEN, T. LEE;
Univ. of Michigan, Ann Arbor, MI

Abstract: Despite the importance of motor skill expertise in human behavior, the neural mechanisms underlying long-term motor learning remain poorly understood. While there is growing interest in mapping the neural reorganization due to motor development, commonly used neuroimaging techniques such as functional magnetic resonance imaging limit the strength of inferences that can be made about the causal role of commonly implicated brain regions, namely the dorsolateral prefrontal (DLPFC) and the primary motor (M1) cortices. The present study aims to address such shortcomings by combining non-invasive brain stimulation with a multivariate analysis of cross-expertise patterns of behavioral performance after six weeks of training on a discrete sequence production task. Our results show that over the course of training, participants markedly speed up sequence execution to achieve different yet concurrent levels of expertise on different sequences. While disruption of both the DLPFC and M1 produce deficits at all levels of expertise, we find evidence of a double dissociation as a function of expertise. We also validate the utility of Pattern Component Modeling in detecting the most likely behavioral pattern that is associated with the disruption of either the DLPFC or M1. This allows us to test hypotheses about how the strength of sequence encoding varies across the brain over the development of skilled motor performance.

Disclosures: Q. Nguyen: None. T. Lee: None.

Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.03

Topic: E.04. Voluntary Movements

Support: National Science Foundation

Title: Stability of motor cortex projection neuron population dynamics with long-term prehension task training

Authors: *F. APARICIO¹, S. BARATI², C. KIRST³, K. GANGULY², H. GHUMAN⁴;
¹Univ. of California San Francisco Neurosci. Grad. Program, San Francisco, CA; ²Neurol., UCSF, San Francisco, CA; ³Ctr. For Theoretical Studies and Kavli Neural Systems Inst., The Rockefeller Univ., New York, NY; ⁴Univ. of California, San Francisco, Alameda, CA

Abstract: Skilled movement control relies on a highly distributed network of cortical, subcortical, brainstem and spinal areas. Recent work, done in rodents, have suggested that with extended skill training, the skilled movement control becomes independent of primary motor cortex (M1); instead, it becomes reliant on subcortical structures. While initially this was shown for gross proximal arm movement control, more recently, this has also been suggested to be the case for fine motor control, i.e., prehension. Consistent with this is the finding of apparent task ‘disengagement’ of upper cortical layers in M1. Together, this suggests that rodents are fundamentally distinct from primates, given that cortical lesions in both non-human primates and humans results in loss of dexterity even for well-rehearsed tasks. Alternatively, it is possible that more precise characterization of behavior and delineation of activity patterns linked to behavior can reconcile differences. Here we aimed to measure dexterity more precisely in a single pellet prehension task along with long-term monitoring of layer 5 M1 projection neurons, i.e., pathways we know are more likely to reflect control signals from M1. Using retrograde labeling of brainstem nuclei red nucleus (RN) and epifluorescence imaging in M1, the current study investigated the timing of when M1 projection neurons are recruited during motor learning. After the mice reached a performance plateau within 2 weeks, we continued to train them for additional 8-10 weeks to examine the stability of the projection neurons during long term motor execution. Results show that M1 projection neurons continued to show strong modulation during skilled movements for at least 10 weeks after reaching plateau performance. To further explore the role of cortical projections to and from layer 5 neurons in M1, we removed the preparatory input signals to M1 neurons in the same mice by inducing a permanent stroke into the secondary motor cortex (M2). By eliminating the reliable preparatory input to M1 after a M2 stroke, we hypothesized that it would lead to severely diminished activity in projection neurons resulting in forelimb deficits and decreased motor performance. During early post-stroke rehabilitation, the motor performance of mice was severely impaired. During 8 weeks of poststroke rehabilitation, task-modulated projection neurons again emerged that strongly correlated to the functional recovery of the forelimb. These results indicate that M1 projection neuron activity remain

engaged even with long term task practice. Overall, our results indicate that control of prehension in mice requires M1 even with long-term task practice.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.04

Topic: E.04. Voluntary Movements

Support: DFG Walter-Benjamin Fellowship
ERC-2012-SyG_318987

Title: Cortical neuron types differentially encode mouse motor behavior during the progression of Huntington's Disease

Authors: *S. BLUMENSTOCK^{1,3,4}, K. VÖLKL^{3,4}, E. GJONI¹, N. DEL GROSSO⁴, R. KLEIN³, T. KOMIYAMA^{1,2}, I. DUDANOVA^{4,5};

¹Dept. of Neurobiology, Ctr. for Neural Circuits and Behavior, ²Halicioğlu Data Sci. Inst., UCSD, La Jolla, CA; ³Dept. of Molecules – Signaling – Develop., Max-Planck-Institute for Biol. Intelligence, Martinsried, Germany; ⁴Mol. Neurodegeneration Group, Max Planck Inst. for Biol. Intelligence, Martinsried, Germany; ⁵Ctr. for Anatomy, Fac. of Med. and Univ. Hosp. Cologne, Univ. of Cologne, Cologne, Germany

Abstract: The dysfunction of the corticostriatal circuit is central to the development and progression of neurodegenerative movement disorders such as Huntington's disease (HD). Previous electrophysiological and functional imaging studies have shown HD-related changes in cortical excitatory neuron activity, possibly related to a loss of inhibitory control through interneurons. Despite its relevance, the involvement of specific cortical excitatory and inhibitory neuronal populations in HD remains largely unexplored. To dissect the contributions of major cortical neuron subtypes, we performed chronic *in vivo* 2-photon calcium imaging of excitatory corticostriatal projection neurons (CPN) as well as inhibitory parvalbumin (PV), somatostatin (SST) and vasoactive intestinal peptide (VIP) populations in the R6/2 HD mice and wildtype littermate controls. Neuronal activity and mouse behavior were monitored over the presymptomatic and symptomatic phase of disease progression. Using three-dimensional pose estimation, we show abnormalities in gait and behavioral state dynamics in R6/2 mice compared to healthy controls. Furthermore, specific inhibitory neuron subtypes were differentially activated during motor behavior in control mice, with VIP neuron activity positively and SST neuron activity negatively correlating with locomotion, whereas grooming was accompanied by strong SST activity. The behavioral abnormalities of R6/2 mice were mirrored in aberrant activity of inhibitory neurons, with an overall reduction of PV and VIP activity and an increase

of SST activity. These neural changes were observed before the onset of overt behavioral symptoms and became more pronounced with disease progression. Our results indicate that HD affects cortical circuits in a cell-type specific manner, opening new possibilities for targeted therapeutic intervention.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.05

Topic: E.04. Voluntary Movements

Support: RO1EY002686
McDonnell Foundation 220020516
5T32EY015387-15

Title: Movement representations in the primary somatosensory cortex (S1) of the greater galago *Otolemur garnetti* elicited by intracortical microstimulation

Authors: ***A. HALLEY**¹, **I. STEPNIIEWSKA**², **Q. WANG**², **J. L. REED**⁵, **H. X. QI**³, **J. H. KAAS**⁴, **L. A. KRUBITZER**⁶;

¹Univ. of California Davis, Davis, CA; ³Dept Psychol, ⁴Psychology Dept., ²Vanderbilt Univ., Nashville, TN; ⁵Dept. of Radiology and Radiological Sci., Vanderbilt Univ. Med. Ctr., Nashville, TN; ⁶UC Davis, Davis, CA

Abstract: What role does primary somatosensory cortex (S1) play in generating movements that are unique to primates compared to other mammals? The role of S1 in generating movement has been demonstrated by the application of long-train intracortical microstimulation (LT-ICMS) in a range of mammals, including primates, rodents, and bats. Recent work has shown that distinct movement types - e.g. forelimb extension vs. retraction - can be elicited from stimulation of S1, primary motor cortex (M1), premotor cortex (PMC), and posterior parietal cortex (PPC). Stimulation studies have shown that in New and Old World monkeys, S1 plays a central role in motor control, but no study has yet applied these methods to a species of prosimian primate - the extant lineage thought to most closely resemble the earliest primate ancestors. Here we used LT-ICMS in order to characterize movement representations in the primary somatosensory cortex (S1/3b), frontal motor (e.g. M1, PMC), and posterior parietal (PPC) cortex of the greater galago (*Otolemur garnetti*). Within galago S1, we found motor representations of most body parts, including hindlimb, torso, forelimb, jaw, lips, tongue, ears, and eyelids. Stimulation of M1, PMC, S1, and PPC elicited distinct movement types of the forelimb, hindlimb, and tongue, and each region included a large representation of the forelimb. While we could evoke movements of individual digits, the representation of the digits alone was relatively small in the cortical areas

that we examined, unlike other primates. We also observed a large region of tongue representation across cortical fields. This study shows that, as in other primate lineages, S1 in the galago plays a central role in motor control of forelimb and other parts of the body, but with motor specializations that are adapted to their unique locomotor behaviors.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 474.06

Topic: E.04. Voluntary Movements

Support: JSPS

Title: Modulation effect of transcranial direct current stimulation over the right inferior frontal cortex on response inhibitory function depends on the stimulus intensity and polarity.

Authors: *K. YAMANAKA;
Showa Women's Univ., Tokyo, Japan

Abstract: The right inferior frontal cortex (IFC) is considered to be critical for response inhibition, which is essential for adaptive behavior in human daily life. Previous studies suggest that transcranial direct current stimulation (tDCS) over the right IFC can modulate response inhibitory function. However, it remains unclear how the modulation effect of tDCS over the right IFC on response inhibitory function depends on the stimulus intensity and polarity. Here, we compared the performance of Go/Stop task before, during, and after the tDCS with 2 stimulus intensities (2 mA/1 mA) and 2 polarities (Anodal/Cathodal). A total of 38 female volunteers participated in this study and assigned to 4 tDCS conditions; 2 mA-Anodal (10 participants), 1 mA-Anodal (9 participants), 2 mA-Cathodal (10 participants), and 1 mA-Cathodal (9 participants). All participants performed 3 sessions (before, during, and after tDCS) of Go/Stop task, each consisting of randomly ordered 60 Go and 60 Stop trials. During the second Go/Stop task session, a direct current of 2 mA or 1 mA was delivered with battery-driven stimulator (DC-STIMULATOR Plus, neuroConn GmbH). In the Anodal tDCS conditions, the anode electrode was placed over the right IFG, and the cathode electrode was placed over the left IFC. In the Cathodal tDCS conditions, the cathode electrode was placed over the right IFG, and the anode electrode was placed over the left IFC. In the Go/Stop task, participants were instructed to click a computer mouse by a right index finger when an indicator moving with a constant velocity reached a target (Go trial) or to avoid the click when the indicator randomly stopped 250-100 ms before it reached the target (Stop trial). From the %correct in Stop trials and mean response time in Go trials, stop-signal reaction time (SSRT), which is a standard index for response inhibition level, was calculated for each participant and session. As a result, SSRTs were modulated

differently in the 4 conditions. In the 2 mA-Anodal condition, SSRTs tended to decrease during and after tDCS sessions (before: 217 ± 16 ms, during: 214 ± 9 ms, after: 207 ± 6 ms), while, in the 1 mA-Anodal condition, SSRTs tended to increase during and after tDCS sessions (before: 217 ± 12 ms, during: 228 ± 6 ms, after: 225 ± 11 ms). In contrast, in the two Cathodal conditions, SSRTs did not modulated. These results indicate that the response inhibitory function is modulated by the anodal tDCS over the right IFC but the direction of the modulation might change depending on the stimulus intensity.

Disclosures: **K. Yamanaka:** None.

Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.07

Topic: E.04. Voluntary Movements

Support: NSERC Discovery Grant

Title: The effect of transcranial magnetic stimulation pulse width on the sensorimotor integration temporal window: A short-latency afferent inhibition study

Authors: ***K. R. GRAHAM**, M. N. SHAHEN, N. E. BARCLAY, K. D. HAYES, S. K. MEEHAN;

Dept. of Kinesiology and Hlth. Sci., Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Short-latency afferent inhibition (SAI) is a method of probing sensorimotor integration. The different interneuron populations recruited by anterior-posterior (AP) and posterior-anterior (PA) current directions during SAI provide convergent afferent input to the motor cortex. Past work established that a peripheral electrical and transcranial magnetic stimulation (TMS) interval of 20 ms produced maximal inhibition regardless of TMS-induced current direction. However, past work employed a fixed TMS pulse width that may still recruit a mix of sensorimotor circuits. Controllable pulse parameter TMS provides control over pulse width, potentially better isolating sensorimotor circuits. However, whether the temporal windows of sensorimotor integration are similar across all circuits recruited by varying pulse widths is unclear. Therefore, the present study examined the effect of interstimulus interval (ISI) on SAI elicited by different current directions and pulse widths. Participants completed two similar sessions. During each session, motor evoked potentials (MEP) were measured from surface electromyographic electrodes over the first dorsal interosseus muscle (FDI). MEPs were elicited using both AP and PA current and 120, 70 and 30 μ s pulse widths. SAI was quantified for each combination of current direction and pulse width by comparing the MEPs elicited by a single pulse of TMS alone to when the sample TMS stimulus was preceded by electrical stimulation of the median nerve. Twelve different ISIs (10-36 ms) were employed for each current direction and pulse width. To establish the effect of tonic contraction on the various combinations, SAI

was assessed at rest (Session 1) or while participants maintained a minimal voluntary contraction of the FDI (Session 2). Preliminary results (n=7) demonstrate that PA SAI was weaker when maintaining a voluntary contraction, whereas AP SAI was unaffected by muscle state. PA SAI peaked across ISIs of 20-22 ms regardless of pulse width and muscle state. Inhibition persisted but progressively weakened across the 22-30 ms ISI. For AP SAI, inhibition was maximal at 22-24 ms regardless of pulse width and muscle state. However, while inhibition decreased progressively from 24 to 28 ms for AP₇₀ and AP₁₂₀, inhibition persisted to 32 ms for AP₃₀. The current results expand past work using a fixed pulse width (70 μ s) and demonstrate multiple PA sensorimotor loops are sensitive to muscle state, whereas AP loops are not. Further, the broader inhibition window for AP₃₀-induced current suggests the involvement of a shorter, direct sensorimotor loop and a second circuitous loop.

Disclosures: **K.R. Graham:** None. **M.N. Shahan:** None. **N.E. Barclay:** None. **K.D. Hayes:** None. **S.K. Meehan:** None.

Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 474.08

Topic: E.04. Voluntary Movements

Support: NSERC Discovery Grant

Title: The contribution of oscillatory activity to the modulation of different sensorimotor circuits under varying working memory load

Authors: N. E. BARCLAY, K. R. GRAHAM, K. D. HAYES, *S. K. MEEHAN;
Kinesiology and Hlth. Sci., Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Sensorimotor integration is a complex process that involves multiple sensorimotor loops converging to shape motor output. Potential targets of these convergent loops have been identified in the motor cortex using posterior-anterior (PA) and anterior-posterior (AP) induced current during short-latency afferent inhibition (SAI). However, we recently reported that increasing verbal working memory demands similarly reduced both PA-SAI and AP-SAI with a fixed pulse width (\sim 70 μ s). However, a fixed pulse width of \sim 70 μ s may recruit a mix of distinct AP interneuron circuits. Whether the effect of working memory is consistent across unique AP-SAI interneuron circuits recruited by different pulse widths is unknown. Further, the origin of the afferent projections influencing the different sensorimotor circuits in the motor cortex is also unclear. The current study combined controllable pulse parameter transcranial magnetic stimulation (cTMS) and electroencephalography (EEG) to probe the sensitivity of different sensorimotor circuits to verbal working memory demand and identify unique contributions of oscillatory frequencies to their response. Participants completed a single session during which EEG was simultaneously recorded while PA induced current lasting 120 μ s (PA₁₂₀), AP induced

current lasting 120 μs (AP_{120}), or 30 μs (AP_{30}) were used to elicit MEPs in the presence (conditioned trials) or absence (unconditioned trials) of a peripheral electrical stimulus over the contralateral wrist. cTMS was timed to occur during the maintenance period of a modified Sternberg memory task. EEG oscillatory activity was quantified using event-related spectral perturbation (ERSP) amplitude across five different frequency bins (Theta: 4-8 Hz, Alpha: 8-12 Hz, Low Beta: 12-18 Hz, High Beta: 18-30 Hz and Gamma: 30-45 Hz) just before the cTMS stimulus artifact. Linear mixed models were used to quantify the fixed effects of memory load and ERSP amplitude on SAI. Preliminary results ($n=14$) demonstrate that PA_{120} , AP_{120} and AP_{30} SAI were all reduced by increasing working memory load, although the magnitude of the reduction in SAI qualitatively decreased from PA_{120} to AP_{120} to AP_{30} . Alpha ERSP amplitude at Cp3 and low beta ERSP amplitude at FCz significantly shaped the load response. The current results suggest overlapping projections to different sensorimotor circuits converging on the motor cortex with differential responses across the circuits potentially dependent on additional, unique modulatory projections.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.09

Topic: E.04. Voluntary Movements

Support: NSERC Discovery Grant RGPIN-2020-04255

Title: The response of distinct sensorimotor circuits in motor cortex to valid and invalid cues during a cued response task

Authors: *K. D. HAYES, M. E. R. KHAN, K. R. GRAHAM, N. E. BARCLAY, S. K. MEEHAN;

Dept. of Kinesiology and Hlth. Sci., Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Skilled motor action requires the integration of sensory afference across multiple sensorimotor loops converging on motor corticospinal neurons. Short-latency afferent inhibition (SAI) can be used to investigate functionally distinct sensorimotor circuits in the motor cortex by manipulating the properties of the transcranial magnetic stimulus. However, the specific functional contribution of each circuit to different sensorimotor tasks is not clear. Therefore, the present study quantified SAI using controllable pulse parameter transcranial magnetic stimulation (cTMS) during a cued finger response task. Participants completed a single session during which they used their index, middle, ring and little finger to respond to a target stimulus. On 70% of trials the target stimulus was preceded by a cue that correctly predicted the target that would appear after a delay. On 30% of the trials the target stimulus was preceded by an invalid

cue. Motor evoked potentials (MEP) were elicited in the first dorsal interosseous (FDI) muscle 200-275 milliseconds after the target presentation. MEPs were elicited using either posterior-anterior induced current lasting 120 μ s (PA₁₂₀) or anterior-posterior induced current lasting 30 μ s (AP₃₀) while participants maintained a slight tonic contraction. To quantify SAI, MEPs for both current types were elicited in the presence (conditioned trials) or absence (unconditioned trials) of a peripheral electrical stimulus over the contralateral wrist. Preliminary findings ($n_{PA}=13$, $n_{AP}=9$) demonstrate that the magnitude of PA₁₂₀ SAI was less compared to AP₃₀ SAI, consistent with an increased sensitivity of PA₁₂₀ SAI to movement onset. Preliminary results also demonstrate that PA₁₂₀ SAI decreased across valid to invalid trials whereas AP₃₀ SAI increased across valid to invalid trials. The opposite direction of change in SAI amplitude in these PA₁₂₀ and AP₃₀ sensorimotor circuits indicates that the circuits use sensory afference for different purposes during movement consistent with previous work linking the PA₁₂₀ circuit to movement planning and the AP₃₀ circuit to movement modulation by perceptual load and cerebellar input. The current results highlight the importance of the different functions of convergent sensorimotor loops to motor learning and performance.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.10

Topic: E.04. Voluntary Movements

Support: DOD W81XWH-19-1-0810
NIH P01 NS083514

Title: Decrease in movement-related gamma amplitude modulation after extended practice

Authors: ***E. TATTI**¹, **A. CACCIOLA**², **A. QUARTARONE**², **M. GHILARDI**³;
¹CUNY Sch. of Med., New York, NY; ²Univ. of Messina, Messina, Italy; ³Dept. of Molecular, Cell. & Biomed. Sci., CUNY, Sch. of Med., New York City, NY

Abstract: In previous work, we showed that the magnitude of beta event-related synchronization (ERS) (13-25 Hz) is not related to kinematic features but increases with extended practice; these increases are local, depend on the type of practiced activity, and return to baseline after two hours of rest either with or without sleep. Differently from beta, movement-related gamma (30-80 Hz) ERS is strongly associated with the motor output, in that the greater the gamma ERS, the higher the peak velocity. While the prokinetic role of gamma is known, the effect of practice on gamma ERS is largely unexplored. High-density EEG activity was thus recorded to verify whether gamma ERS and its relationship with the task parameters would be affected by motor practice. 51 healthy subjects were allocated to two practice groups: 28 participants (mean \pm SD:

24.38±3.96 years, 16 women) and 23 subjects (23.3±4.6 years, 12 women) completed the ROT and VSEQ task sessions, respectively. Both groups underwent a baseline assessment (*mov*) before and after completing either three one-hour blocks of ROT, an implicit motor learning task, or VSEQ, a visual sequence learning task. The *mov* test consisted of 96 planar reaching movements towards targets located at three distances. In line with our previous study, during *mov*, gamma ERS occurred before movement onset over the centro-parietal regions and over the parieto-occipital regions during movement execution (Cluster 1 $t=14,964$, $p<0.001$; Cluster 2 $t=2169$, $p=0.012$), and was appropriately scaled to target distance in both *mov* tests and groups (planning: $F=38.28$, $p<0.001$, $n^2p = 0.44$; movement: $F=91.10$, $p<0.001$, $n^2p =0.66$). Importantly, and differently from beta, gamma ERS decreased with practice (planning: $F=20.84$, $p<0.001$, $n^2p=0.30$; movement: $F=45.81$, $p<0.001$, $n^2p=0.48$); such a decrease occurred independently of the type of interposed learning and was not related to significant changes in the behavioral performance. Altogether, our findings confirm that cortical gamma oscillations have a crucial role in the selection, execution, and control of the proper kinematic parameters of goal-directed reaching movements. They also suggest that practice affects gamma amplitude without compromising its prokinetic role. Thus, its amplitude reduction might either represent reduced neural recruitment to perform the motor act or, as an alternative, a marker of fatigue induction. This evidence is relevant in light of what was previously observed with beta. In that case, the beta amplitude increase was greater after ROT than VSEQ, suggesting that practice-related changes in beta and gamma amplitude might reflect different mechanisms for motor control and learning.

Disclosures: E. Tatti: None. A. Cacciola: None. A. Quartarone: None. M. Ghilardi: None.

Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.11

Topic: E.04. Voluntary Movements

Title: Rediscovering the Human Motor Homunculus in 3-Dimensions with Implanted Electrodes

Authors: *M. JENSEN, K. MILLER;
Mayo Clin., Mayo Clin., Rochester, MN

Abstract: Introduction: Most have seen the hand-made drawing of the body's motor representation on the convexity of the brain surface, the Penfield homunculus. Since Dr. Penfield's work, we have seen the development of implantable electrodes for treatment of neurologic disease such as refractory epilepsy. Electrophysiological mapping has been primarily performed with 2-dimensional electrocorticography (ECoG) grids that sit atop the convexity of the brain. Recently, we have seen a shift from brain surface measurement with ECoG to widespread depth electrode measurement with implanted stereoelectroencephalography (SEEG) which has allowed us to begin to study the 3-dimensional electrophysiological properties of the

brain. **Methods:** Twelve adult and pediatric patients with intractable epilepsy were implanted with SEEG to localize the onset of their seizures. While being monitored, we performed a simple task consisting of visually-cued movement blocks of their hand, tongue, or foot, interleaved with blocks of rest. We segmented electrophysiological data into epochs of each movement modality and rest using parallel recordings of EMG, and computed r^2 correlation values for each movement modality vs rest using normalized broadband power shifts. Results are plotted as functions of r^2 values. **Results:** Representations of move-rest r^2 cross correlation were plotted and showed the relationship of electrophysiological changes to precise brain anatomy. We found volumetric regions of specific representation for foot, hand, and tongue representation, respectively, along a medial-to-lateral gradient. The surface convexity confirms the classic pattern described by Penfield with new electrophysiological evidence of extension into sulcal depths. **Conclusion:** In our population of twelve patients, we demonstrate electrophysiologically, for the first time, the existence of a volumetric homunculus using electrophysiological data.

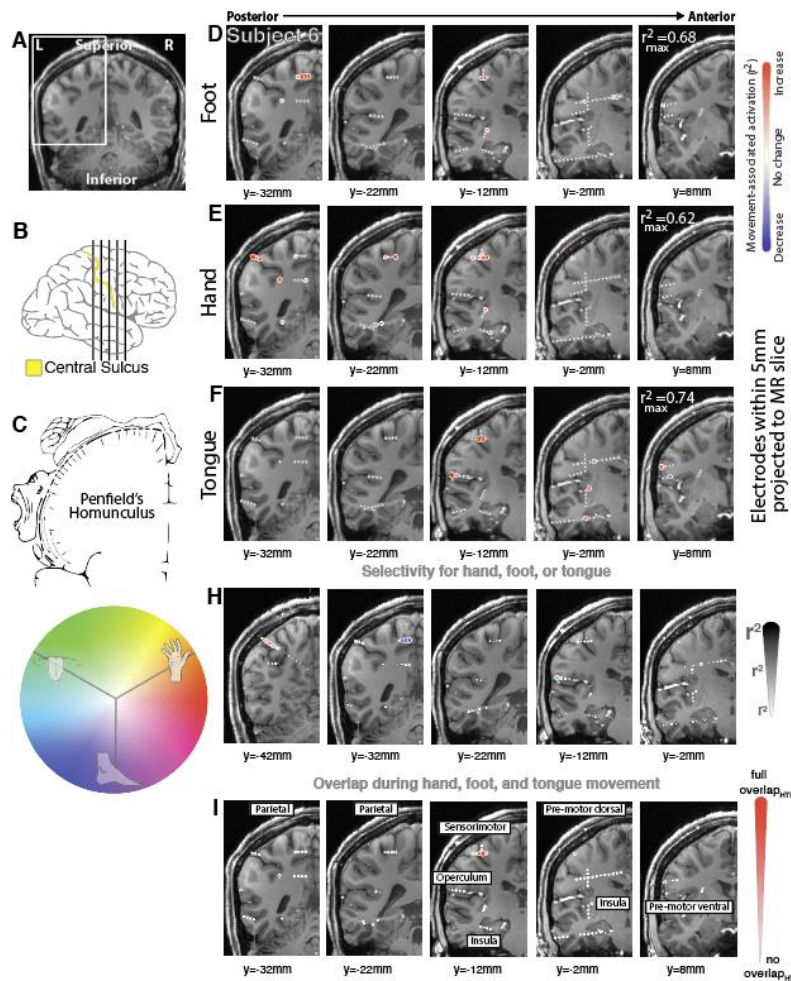


Figure 1. The three-dimensional Penfield homunculus parsed into selective and common regions. A. Orientation of slices shown in D-F. B. Slices displayed are peri-central as shown with black vertical lines. C. Classic Penfield Motor Homunculus shown as reference. D. Electrode pairs with significant increase in power from 65-115Hz during foot movement. E. Electrode pairs with significant increase in power from 65-115Hz during hand movement. F. Electrode pairs with significant increase in power from 65-115Hz during tongue movement. G. Colormap in the complex plane utilizing the unique complex number assigned to each electrode pair based on each pair's selectivity for a modality (hand, tongue or foot). H. Peri-central slices with colored electrode pairs based on selectivity of an electrode pair for hand, tongue, or foot movement. I. Primary motor and pre-motor coronal slices with electrode pairs plotted based on overlap between hand, tongue, and foot movement with corresponding brain regions labeled.

Disclosures: M. Jensen: None. K. Miller: None.

Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 474.12

Topic: E.04. Voluntary Movements

Support: Innovation Fund Denmark (IFD) for the Grand Solution project “PRECISION-BCT” (Grant number: 9068-00025A)
Hartwig R. Siebner holds a 5-year professorship in precision medicine at the Faculty of Health Sciences and Medicine, University of Copenhagen which is sponsored by the Lundbeck Foundation (Grant Nr. R186-2015-2138).

Title: Early transcranially evoked potentials of sensorimotor cortex are modulated by intensity and motor state

Authors: *M. MALLING BECK¹, L. CHRISTIANSEN¹, M. HEYL¹, A. MASTROPASQUA¹, L. TOMASEVIC¹, H. SIEBNER^{1,2,3};

¹Danish Res. Ctr. for Magnetic Resonance, Copenhagen Univ. Hosp., Hvidovre, Denmark;

²Dept. of Neurol., Copenhagen Univ. Hosp. Bispebjerg and Frederiksberg, Copenhagen, Denmark; ³Dept. of Clin. Med., Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Transcranial magnetic stimulation (TMS) targeting the hand area of the primary motor cortex (M1-HAND) can lead to motor evoked potentials (MEPs) in the contralateral hand reflecting the evoked activation of the corticomotor pathway. It is well known that amplitudes of the MEPs are modulated by the intensity of TMS and the physiological state of the corticospinal system at the time of stimulation. A single TMS pulse also evokes a cortical response that can be recorded using electroencephalography (EEG). This response is characterized by a distinct sequence of positive and negative peaks known as a TMS-evoked EEG potential (TEP). Little is known about how TEPs change with stimulation intensity or changes in motor state. To advance our current understanding of how TMS engages the targeted pericentral cortex, we recorded EEG responses to single-pulse TMS of the left M1-HAND in 28 healthy adults. TMS was delivered at six different stimulation intensities, including both sub- and suprathreshold stimuli, while participants rested or performed a voluntary isometric contraction of the right first dorsal interosseous (FDI) muscle. EEG data of sufficient quality and with a clearly visible, early negative peak was obtained and analyzed in 16 individuals. TEPs were elicited at all stimulation intensities, including both sub- and suprathreshold stimuli, during both rest and voluntary contraction. The earliest TEP responses (N15, P30, N45 and P60) were topographically localized close to the site of stimulation. Furthermore, amplitudes of the earliest responses generally increased with stimulation intensity regardless of corticomotor state. Comparisons between motor states revealed that the amplitude of the N15 component, but not the remaining, was consistently smaller when participants performed a voluntary contraction compared to rest. These results show that early TEP peaks may be used as a neurophysiological signature of functional engagement of the targeted M1-HAND. Furthermore, specific peaks of the TEP,

namely the earliest of the locally elicited peaks (N15), may be particularly sensitive to changes in the physiological state of the corticomotor system. These preliminary findings contribute to an increased understanding of how TMS at different stimulation intensities evokes cortical activity. They also provide a greater understanding of what the different TEP peaks represent physiologically. This information can be used to inform targeting and dosing of TMS in research and clinical settings.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.13

Topic: E.04. Voluntary Movements

Support: NIH Grant F32HD105458
AHA Grant 909059

Title: State-dependent interhemispheric inhibition reveals individual differences in motor behavior in chronic stroke

Authors: **J. L. MIRDAMADI**¹, **J. XU**², **N. A. BAUNE**¹, ***M. R. BORICH**¹;
¹Emory Univ., Atlanta, GA; ²Kinesiology, Univ. of Georgia, Athens, GA

Abstract: The objective of this study was to investigate interhemispheric inhibition (IHI) in chronic stroke survivors compared to neurotypical older adults (NOA) during different motor states, and to determine if state-dependent IHI was associated with upper extremity motor behavior. We employed a dual-coil transcranial magnetic stimulation (TMS) approach to elicit IHI bi-directionally, between non-lesioned and lesioned motor cortex (M1) in two motor states: 1) at rest and 2) during contralateral isometric hand contraction. IHI was assessed by delivering a conditioning stimulus 8-msec or 50-msec prior to a test stimulus over contralateral M1. We evaluated paretic motor behavior with clinical measures of impairment, strength, and dexterity, and quantified mirroring activity in the non-paretic hand during the active motor state condition. Associations between IHI modulation from rest to active states with behavioral measures were evaluated with bivariate correlations. Results showed that individuals with stroke demonstrated reduced IHI at rest, and less IHI modulation (active – rest), bi-directionally, compared to NOA. Greater IHI modulation was associated with greater paretic upper limb motor impairment and more mirroring activity. Findings suggest that abnormal state-dependent interhemispheric circuit activity may be more sensitive to post-stroke motor deficits than when activity is assessed in a single motor state. Characterizing state-dependent changes in neural circuitry may contribute to

biologically-informed models of stroke recovery to inform development of more effective rehabilitation interventions.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.14

Topic: E.04. Voluntary Movements

Support: NIH Grant 1R01HD095975-01A1
NIH Diversity Supplement 3R01HD046922-13S1

Title: Aging-related decline in descending cortical modulation of spinal sensorimotor circuits and its relation to dynamic balance performance

Authors: *A. J. LOPEZ¹, C. F. MASON¹, L. H. TING², M. R. BORICH¹, T. M. KESAR¹;
¹Rehabil. Med., ²Biomed. Engin., Emory Univ., Atlanta, GA

Abstract: Aging-related decline in sensorimotor connectivity can impair balance, resulting in increased fall risk. Significant knowledge gaps exist in our understanding of aging effects on cortical and spinal sensorimotor neural circuit connectivity. Here, we utilized paired cortical and peripheral nerve stimulation (PNS) to evaluate aging effects on descending modulation of spinal reflexes during resting and active conditions. When a subthreshold transcranial magnetic stimulation (TMS) conditioning pulse is delivered before or after PNS, the resulting modulation of Hoffman's (H-) reflexes probes the influence of direct, faster and indirect, slower descending volleys on the spinal motoneuron pool. We hypothesized that: 1) task-dependent descending modulation of spinal reflexes will be reduced in older versus young adults, and 2) descending modulation of spinal reflexes will be related to dynamic balance performance. Healthy young (YA, n=19, age=28±4 years) and older (OA, n=17, age=62±10 years) adults have been evaluated. PNS was delivered to the posterior tibial nerve to generate a soleus H-reflex recruitment curve during seated rest (SR), seated active (SA), and quiet stance (QS) conditions. Subthreshold TMS to the soleus motor cortex (M1) hotspot was paired with PNS (intensity: 50% Hmax) at three inter-stimulus intervals (ISIs): -1.5ms, +10ms, +40ms. TMS-conditioned H-reflex amplitudes were used to calculate Conditioned H-reflex % (conditioned H-reflex / unconditioned H-reflex * 100%). The narrowing beam walking test (NBWT) assessed balance performance. Both groups showed significantly increased direct pathway modulation of spinal reflexes during activation at the -1.5ms ISI (SR vs. SA: p=0.01; SR vs. QS: p<0.0001). Alternatively, both groups showed significantly decreased indirect pathway modulation during activation at the +10ms ISI (SR vs. SA: p=0.002; SR vs. QS: p=0.02). Older adults showed a significant reduction in late indirect pathway modulation at the +40ms ISI compared to young adults during both SA (p<0.0001) and QS (p=0.002). Young adults performed better in the

NBWT compared to older adults ($p=0.0003$); however, neither group showed a relationship between dynamic balance performance and descending modulation of spinal reflexes. Task-related activation increased direct pathway modulation and decreased indirect pathway modulation. Interestingly, older adults showed decreased late indirect pathway modulation suggesting age-related changes in descending inhibitory control of reflex gain during task-related activation. Aging impacted dynamic balance performance, and no relationship to descending modulation was observed.

Disclosures: **A.J. Lopez:** None. **C.F. Mason:** None. **L.H. Ting:** None. **M.R. Borich:** None. **T.M. Kesar:** None.

Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.15

Topic: E.04. Voluntary Movements

Support: DOD Grant 81XWH-18-1-0707

Title: Neuro-correlate between knee proprioception error and force control brain activity at six weeks post anterior cruciate ligament reconstruction: A preliminary report

Authors: ***H. KIM**, A. J. SCHNITTJER, B. T. FARRAYE, M. CHAPUT, J. E. SIMON, D. R. GROOMS;
Ohio Univ., Athens, OH

Abstract: Anterior cruciate ligament (ACL) injury induces proprioceptive deficits due to loss of somatosensory input from the ACL, which is not completely restored with reconstructive surgery (ACLR). In addition to proprioceptive deficits, individuals with a history of ACLR have altered brain activation patterns, that may be initiated by deafferentation, to perform lower extremity tasks. Disrupted proprioception and altered brain activity may associate with reduced functional performance and increase risk of re-injury following ACLR. However, no study has investigated the relationship between brain activity and proprioceptive deficits in an ACLR cohort. The purpose of this study was to determine the relationship between brain activity during force control task and proprioception error at 6-weeks post ACLR. Active joint position sense (AJPS) and a force control task were used to measure proprioception error and unique neural strategy for force regulation, respectively. Seven participants with a history of unilateral ACLR were enrolled (4 male, 4 left ACLR, 18.86 ± 1.77 yrs, 57 ± 15.96 days post-ACLR). Participants performed a unilateral quadriceps force control task during functional magnetic resonance imaging, matching a sinusoidal wave (range 0-5 N) with visual biofeedback during movements blocks (4 movement and 5 resting blocks, 30 seconds per block). In a separate session, individuals performed an AJPS task in a concentric manner to 45° knee flexion as a target angle. AJPS error was calculated by subtracting the reproduction angle from the target angle. FSL (FMRIB,

Oxford, UK) was used to analyze the data. Individual brain activation was generated by contrasting between rest and movement blocks after preprocessing to improve signal to noise ratio. A general linear model group-level mixed effect correlation analysis with an explanatory variable (AJPS error) and three contrasts (group mean and brain activity positively and negatively correlated with AJPS error) was performed with an *a priori* threshold at $z=3.1$ and $p<.05$ with cluster correction for multiple comparisons. Brain activity in the bilateral intracalcarine cortex, cingulate gyrus, precuneus, lingual gyrus, left lateral occipital cortex, occipital fusiform gyrus, and crus I of cerebellum was positively correlated with AJPS error. Also, brain activity in left frontal pole was negatively correlated with AJPS error. Individuals with AJPS deficits may have a compensatory neural activation pattern to perform a force control task. This compensation may indicate early sensory reweighting following ACLR, increasing reliance on cross-modal and visual regions to compensate for disrupted proprioception.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.16

Topic: E.04. Voluntary Movements

Support: P2CHD086844
NSF Award # 1650536

Title: Altered Cortico-muscular Functional Coupling for Balance Control in Chronic Stroke Survivor.

Authors: *K. KUKKAR¹, N. RAO⁵, S. SHAH², D. HUYNH³, J. CONTRERAS-VIDAL⁴, P. PARIKH¹;

¹Hlth. & Human Performance, ²Psychology, ³Biol., ⁴Electrical & Computer Engin., Univ. of Houston, Houston, TX; ⁵Haskins Lab., New Haven, CT

Abstract: Nearly 50% of stroke survivors experience a fall within 6-12 months after discharge from hospital, which leads to significant complications and financial burden on the society. Balance control is a key indicator of mobility and independence in ADLs, and its impairment is an important factor contributing to falls in stroke patients. Therefore, it is important to understand the mechanisms underlying impaired balance control following stroke. Remarkably, the contribution of cortical reorganization following stroke to impaired balance control remains unknown. Our recent work in healthy individuals identified a network of fronto-parietal brain (i.e. cortex) regions that adjusts its activity based on the difficulty of the balance control task. In this study, we are investigating the changes in the functional coupling between the brain and the muscles during a challenging balance task and how these changes in the cortico-muscular

coherence influence balance control in chronic stroke survivors. We recruited 7 stroke patients with mild-to-moderate severity and 4 age-gender matched healthy adults. Clinical assessment was performed using Berg Balance Scale and Time Up and Go tests. Participants were instructed to maintain balance in response to balance perturbations with varying difficulty levels (low, medium, and high) on a computerized balance platform with simultaneous neuroimaging using electroencephalography (EEG). On the clinical tests and the laboratory-based balance task, stroke patients showed poor performance when compared with healthy controls. We observed a shift in the peak fronto-parietal cortico-muscular (tibialis anterior) coherence to higher frequency bands in stroke patients when compared with healthy controls. That is, the peak coherence in healthy controls was usually observed in theta and alpha frequency bands while that in patients was observed in the low gamma frequency band. Furthermore, healthy controls showed modulation in the cortico-muscular coherence, mainly in the delta band, with changes in the difficulty level of the balance task. In contrast, the modulation in the cortico-muscular coherence with the balance task difficulty was smaller in stroke patients. The alterations in functional cortico-muscular coherence may contribute to poor balance control in stroke patients. Findings from this study are expected to inform the design of interventions aimed at reducing falls among patients.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.17

Topic: E.04. Voluntary Movements

Title: Functional Connectivity as a Method for Identifying Eloquent Cortex in a Cohort of Epilepsy Patients

Authors: ***K. TYNER**¹, **D. ZHOU**², **O. TARASCHENKO**², **V. GUMENYUK**², **S. GLISKE**¹;
¹Neurosurg., ²Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Approximately 50 million people worldwide suffer from epilepsy. Currently, intracranial EEG monitoring and functional mapping using EEG electrodes is the most common method to identify eloquent cortex in patients who undergo resective surgery. As these methods are invasive, costly, and time consuming, noninvasive methods to identify eloquent cortex could serve as an appropriate alternative. In this study, we aim to identify whether functional connectivity could accurately localize the primary motor cortex in a cohort of epilepsy patients. Patients (N = 8) received a magnetoencephalography (MEG) scan while performing a motor task with their left hand. MEG data were processed with MaxFilter software and analyzed in MNE-Python. Data were filtered 1 - 40 Hz, and heart and ocular artifacts were removed using independent component analysis. One second epochs were made (500 ms before and after trigger

onset) and averaged to generate evoked waveforms. An inverse operator was applied using standardized low resolution brain electromagnetic tomography (sLORETA) to the entire duration of the evoked data. Source time courses were extracted based on the Destrieux Atlas (75 regions per hemisphere) and loaded into MATLAB for dynamic functional connectivity analysis. Data were broken into 50 ms sliding windows starting at the beginning of the recording, with a 5 ms overlap. Principal component analysis was performed on each window, and individual components that explained $\geq 95\%$ of the variance were identified. Varimax rotation was applied, and node locations with an absolute value of 0.20 or higher were identified as major nodes. Functional connectivity from each node pair in the window was calculated using Granger Causality, and all node pairs with a p-value ≤ 0.05 were identified as significant. We then determined if the precentral gyrus was identified as an important node in each window across all regions. Our results show that Granger Causality correctly identified the right precentral gyrus in 6 of the 8 patients who performed the left-handed motor task with spatial and temporal precision. These results suggest that functional connectivity could be used as a non-invasive method to identify the motor cortex in epilepsy patients who receive a MEG scan. This approach could be extended to other task-based MEG recordings to identify other regions of eloquent cortex.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.18

Topic: E.04. Voluntary Movements

Support: Burroughs Wellcome Foundation CA-0142133

Title: Cortico-subcortical dynamics predict motor sequence features during multiday motor sequence performance in patients with Parkinson's Disease

Authors: ***K. PRESBREY**¹, K. LOUIE¹, P. A. STARR², D. D. WANG³;
¹Neurolog. Surgery, Univ. of California, San Francisco, San Francisco, CA; ²Univ. of California, San Francisco, Mill Valley, CA; ³Neurolog. Surgery, Univ. of California, San Francisco, SAN FRANCISCO, CA

Abstract: The neural dynamics of explicit motor sequence learning in Parkinson's Disease (PD) are poorly understood, and there are currently no effective treatments for impairments in motor learning in PD patients. Either off or on basal ganglia deep brain stimulation (DBS), PD patients practiced known sequences for multiple days in a typing task that required discrete sequence production upon presentation of visual cues. Clinical motor assessments were used to verify treatment state and score acute motor impairment each day of the experiment. Chronic wireless brain implants located in the motor cortex and basal ganglia recorded local field potential during

motor planning and execution, and a custom behavioral apparatus determined finger kinematics. In patients exhibiting learning, both cortical and subcortical neural activity predicted sequence-specific features and cortico-subcortical interactions correlated with behavior.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Topic: E.04. Voluntary Movements

Support: NIH Grant AG 072262
University of Michigan Rackham Graduate Travel Award
University of Michigan School of Kinesiology Travel Award

Title: Additive effects of multiple, spaced cortical paired associative stimulation sessions on cortical excitability in the human motor system

Authors: *E. GOLDENKOFF, T. DAVIS, A. RETTMANN, J. RASHI, B. HAMPSTEAD, M. VESIA;

Univ. of Michigan, Univ. of Michigan, Ann Arbor, MI

Abstract: Cortical paired associative stimulation (cPAS) of interconnected parietal and motor areas can induce changes in cortical excitability in the human motor system that last beyond stimulation. Novel accelerated repetitive transcranial magnetic stimulation (TMS) protocols, which consist of multiple theta burst stimulation (TBS) sessions, have been shown to have an enhanced effect on neural plasticity induction when delivered at optimally spaced intervals. However, it remains to be shown whether the TBS-induced aftereffects for the spaced application protocol also hold for cPAS. We investigated whether cortical plasticity induced by cPAS to a parieto-motor circuit involved in skill grasp control can be modulated by multiple, spaced stimulation sessions. We hypothesized that multiple, spaced cPAS sessions increase motor excitability wherein one cPAS session influences the next cPAS session. We used a crossover within-subjects design to test the effects of a standard cPAS (single-cPAS session) and accelerated cPAS (three repeated cPAS sessions) on motor excitability. To induce motor associative plasticity, we repeatedly applied paired TMS pulses over the posterior parietal cortex (PPC) and primary motor cortex (M1) using two coils. Two interventional protocols were compared in the experiment. The accelerated-cPAS protocol tested the effects of three serial sessions of a Hebbian-like cPAS protocol where PPC stimulation preceded M1 stimulation by 5ms (cPAS5ms). In the other single-cPAS protocol, participants underwent one session of

cPAS5ms and two sessions of a non-Hebbian (sham) cPAS protocol where PPC was stimulated 500 ms prior to M1 (cPAS500ms). Each cPAS session lasted 8.3 min followed by a 50 min intersession interval, as per the principles of spaced learning theory. We investigated the responses to cPAS by measuring the amplitude of motor evoked potentials (MEPs) elicited by single-pulse TMS over M1 before and at 20 min and 40 min after each cPAS session. We observed a significant increase in MEP amplitudes after the 3-session cPAS5ms protocol (accelerated-cPAS protocol) compared to one cPAS5ms session followed by two sham cPAS500ms sessions (single-cPAS protocol). Our results indicate an additive increase in MEP amplitudes only after the third cPAS5ms session. These findings reveal that multiple, spaced cPAS sessions summate to increase motor excitability induced by single cPAS. This knowledge might inform clinical applications of cPAS, when lasting effects of stimulation on parietal-frontal grasping circuits are needed to produce meaningful clinical responses in stroke patients with neuromotor impairments.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.20

Topic: E.04. Voluntary Movements

Support: onsciousness and Free Will: A Joint Neuroscientific-Philosophical Investigation
 (John Templeton Foundation #61283
 Fetzer Institute, Fetzer Memorial Trust #4189

Title: Baseline correction can lead to spurious results in response-locked potential research

Authors: *L. JEAY-BIZOT, U. MAOZ, A. SCHURGER;
Chapman Univ., Irvine, CA

Abstract: In electroencephalography (EEG) research, event related potential analysis (ERP) is a ubiquitous method. It refers to averaging EEG signals across trials over a short window of time surrounding an event of interest. Often, the activity of interest is the brain's response to a stimulus. However, in response-locked potentials, the activity of interest can also be the signal that precedes the response. One very well-known response-locked potential is the readiness potential (RP). The RP extends back one second or more before the onset of a spontaneous voluntary movement. To reveal the RP, multiple data epochs encompassing spontaneous voluntary movements are time locked to movement onset and then averaged together. While the RP is a very reliable feature of the movement-locked average, where to place the onset of the RP remains open to debate. Some recent interpretations argue that the RP onset reflects a decision process that originates as early as the start of the trial, which may be several seconds prior to the

movement. When processing EEG data, it is standard practice to subtract a baseline from the signal as a data preprocessing step, in order to correct for slow drifts and offsets in the signal. In RP research, the baseline is frequently placed at the start of the epoch. This places the baseline period anywhere from a few hundreds of milliseconds to multiple seconds before movement. However, baseline correction relies on the assumption that the activity during the baseline period is independent from the task. Using a statistical argument we demonstrate that this fundamental assumption of baseline correction is violated for event-preceding potentials. Given that the response-locked potentials may start in this early time frame, baseline correction could, arbitrarily, determine a study's results. We further show, using a meta-analysis, that when comparing the RP across conditions, baseline correction can generate false positives and even invert the interpretation of the results. Finally, we consider and propose alternative processing steps and advocate for a shift away from time-locked analysis in response-locked potential research.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.21

Topic: E.04. Voluntary Movements

Support: DARPA Award HR001120C0120

Title: Motor cortical control of sustained isometric contractions

Authors: A. CASAMENTO-MORAN¹, *A. S. PAWAR², T. GEORGE¹, M. SINGHALA¹, R. W. NICKL², L. OSBORN³, V. S. CHIB¹, J. D. BROWN¹, M. S. FIFER³, F. V. TENORE³, G. L. CANTARERO², P. A. CELNIK²;

¹Biomed. Engin., ²Physical Med. & Rehabil., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; ³Res. and Exploratory Develop., Johns Hopkins Applied Physics Lab., Laurel, MD

Abstract: It is well-known that motor cortical activity relates to different aspects of force, such as the magnitude and direction of the generated force output. However, most studies investigating how primary motor cortex (M1) encodes force used ballistics tasks of a duration shorter than 2 sec or techniques that measure cortical activity indirectly such as functional magnetic resonance. This has led to a limited understanding of how the M1 encodes longer and sustained isometric contractions. In this study, we used a 5 sec sustained isometric contraction task, where a spinal cord injury participant implanted with intracortical microelectrode arrays was required to maintain 60% of his maximum voluntary contraction (MVC). He achieved this by pressing against a force transducer using wrist extension. We concurrently measured exerted force, neuromuscular activity via electromyography (EMG), multi-unit firing activity (MUA), local-field potentials (LFP) from M1. To examine how M1 encodes sustained isometric

contractions, we divided the contraction into three phases: initiation (from EMG onset until the end of the force increase), maintenance (1 sec window in the steady state), and termination (from the beginning of force descent to EMG offset). As expected, we found that M1 firing rate increased during the initiation window. Surprisingly, the firing rate decreased for the rest of isometric contraction (i.e., maintenance and termination phases), even though the exerted force and EMG were maintained at a high amplitude for the duration of the task. Similarly, we found that while the EMG power in the alpha, beta, and gamma bands was maintained, LFP power in the same bands decreased in the maintenance and termination phases, relative to initiation. Altogether, we observed different cortical patterns during initiation, maintenance, and termination of long isometric contractions. This suggests that M1 appears to play a key role during force generation/modulation, while additional sources (subcortical or spinal) contribute to maintenance of force. These results have important implications for brain-computer interfaces techniques aiming to use cortical activity to decode behavior, as well as to basic and clinical neuroscience by demonstrating that intricate system interactions are responsible for exerting a simple motor task that requires maintaining a constant force output.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.22

Topic: E.04. Voluntary Movements

Support: MRC Grant MR/W004798/1

Title: Sensorimotor deficits in post-COVID fatigue

Authors: *N. J. MAFFITT¹, A. M. E. BAKER¹, A. DEL VECCHIO², K. M. MCKEATING¹, M. R. BAKER¹, S. N. BAKER¹, D. S. SOTEROPOULOS¹;

¹Fac. of Med. Sci., Newcastle Univ., Newcastle Upon Tyne, United Kingdom; ²Dept. Artificial Intelligence in Biomed. Engin., Friedrich-Alexander Univ., Erlangen-Nurnberg, Germany

Abstract: Following infection from SARS-CoV-2, a substantial minority of people develop lingering after-effects known as ‘long COVID’. Fatigue is a common complaint with substantial impact on daily life, but the neural mechanisms behind post-COVID fatigue remain unclear. We recruited 37 volunteers with self-reported fatigue 6-26 weeks after a mild COVID infection and carried out a battery of 35 behavioural and neurophysiological tests (33 relating directly to the state of the nervous system, plus blood oxygen saturation and tympanic temperature) to assess the state of the central and peripheral nervous systems. In comparison to age and sex matched volunteers without fatigue (n=52), we show underactivity in specific cortical circuits and

myopathic change in skeletal muscle. Cluster analysis revealed no sub-groupings, suggesting post-COVID fatigue is a single entity with individual variation, rather than a small number of distinct syndromes. Based on our analysis we were also able to exclude dysregulation in sensory feedback circuits and descending neuromodulatory control. These abnormalities on objective tests may indicate novel avenues for principled therapeutic intervention, and could act as fast and reliable biomarkers for diagnosing and monitoring the progression of fatigue over time.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.23

Topic: E.04. Voluntary Movements

Support: KAKENHI grant 20H04594

Title: Neural substrates of performance failure under pressure - A 7T-fMRI study

Authors: *K. OGASAWARA, T. KOIKE, M. FUKUNAGA, A. YOSHIOKA, N. SADATO; Natl. Inst. for Physiological Sci., Okazaki, Japan

Abstract: The decrease in performance under psychological pressure is known as ‘choking’. While it is thought that an excessive arousal level due to psychological pressure causes choking, its underlying mechanisms and neural basis are still unknown. We conducted a fMRI study with a 7-Tesla machine using high-stakes scenarios. 23 right-handed volunteers underwent visuomotor tasks, in which they knew in advance the amount of the reward to receive upon success. There were four conditions with different rewards: None (N), Small (S), Large (L), and Jackpot (J). The reward under the J-condition, an infrequent event, was more than ten times higher value than the L-condition. Therefore, we expected the J-condition to provide high psychological pressure. Behavioral data showed that, compared to other three conditions, the J-condition was characterized by significantly lower success rate, higher arousal level defined by pupil diameter, and higher psychological pressure in subjects report. The neuroimaging data revealed the J-condition irrespective of the result of success or failure contrasted with other conditions showed greater activation in the bilateral anterior insular cortex (AIC), the anterior cingulate cortex (ACC), basal forebrain, midbrain, cerebellum, and the parietal cortices. The saliency network (ACC, AIC) plays a crucial role in controlling arousal level and bridging cortico-subcortical networks. Given this, the greater activation in J-condition in the salience network indicates that the elevated arousal levels, that is represented on mid brain areas, may affect the cortical networks for task execution via the salience network. In failure compared with success, the J-condition contrasted with other conditions showed the activation in the anterior rostral medial prefrontal cortex (arMPFC). Because the arMPFC is reported as a core of self-

monitoring that is one factor in decrease of performance, the arMPFC activation in failure specific to J-condition represents the relation between self-monitoring and choking. On the other hands, within the failure-related areas defined by the N, S, and L-conditions, the failure during the J-condition specifically activated the bilateral caudate nucleus, which associate with the fine motor control. Taken these series of findings together, it is suggested that the mechanisms of choking as follows: the psychological pressure is accompanied by the enhanced salience network. As the salience network mediates the effect of arousal level to cortical areas, it may induce self-monitoring processes represented by arMPFC that in turn modulates the subcortical motor control system, resulting in failure motor control.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.24

Topic: E.04. Voluntary Movements

Support: NSERC Grant 418589

Title: Eeg theta dynamics for error processing during online movement control in a manual tracking task

Authors: ***S. KESSOURI**^{1,2}, F. DANION³, J.-F. LEPAGE², P.-M. BERNIER¹;

¹Dept. de kinanthropologie, Faculté des sciences de l'activité physique, Univ. de Sherbrooke, Sherbrooke, QC, Canada; ²Dept. de pédiatrie, Faculté de médecine et des sciences de la santé, Ctr. de Recherche du Ctr. Hospitalier Universitaire de Sherbrooke., Sherbrooke, QC, Canada; ³Ctr. de Recherches sur la Cognition et l'Apprentissage, CNRS, Univ. de Poitiers, Poitiers, France

Abstract: The neural mechanisms underlying the online control of visuomotor tracking in humans remain poorly understood. Frontoparietal modulations in the EEG theta band [4-8 Hz] have been shown to correlate with error processing (Perfetti et al., 2011), especially in contexts of cognitive control (Pellegrino et al., 2018; van Driel et al., 2012). However the contribution of these oscillations to error processing during motor control is still unclear. The objective of this study was to test the hypothesis that online control of movement is reflected in the oscillatory activity of the theta band. Electroencephalography (EEG) was recorded in 29 healthy adult participants while they performed a manual tracking task with their right hand. Two conditions were used to control the demands for error processing during movement tracking: in the first condition, the target moved along a repeating (i.e., predictable) pattern, generating low tracking errors; in the second condition, the target moved pseudo-randomly, inducing large tracking errors. Behavioral analyses confirmed a significant difference in mean errors across conditions,

but importantly they revealed similar mean hand velocities across conditions. EEG results showed that theta power was significantly different in the context of Low vs High tracking error; specifically, theta power was significantly greater in the High error context, with a peak difference occurring at electrodes overlaying the left central regions. Interestingly, although beta desynchronization was observed over these same electrodes during movement, it was not significantly different across the two error conditions. Overall, this study extends current knowledge of the role of theta oscillations for error processing to the context of motor control. These modulations are likely to reflect cortical activity mediating the communication and integration of information within sensorimotor circuits including the motor cortex, the dorsal premotor cortex, and the parietal cortex, all of which are known to mediate online movement control (Takei et al., 2021; Archambault et al., 2015).

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 474.25

Topic: E.04. Voluntary Movements

Title: Validating the threshold of conscious sensory perception

Authors: *A. SCHURGER, C. URIBE;

Dept. of Psychology and Brain Inst., Chapman Univ., Orange, CA

Abstract: Experimental manipulations of conscious sensory perception ordinarily rely on behavioral metrics that can be used to infer the presence or absence of consciousness. Such metrics imply that there is a threshold intensity above which subjects are conscious of stimuli and below which they are not. Wherever the “true” threshold of conscious perception might lie, any behaviorally-based metric of conscious perception makes a commitment to where to place that threshold. However, no metric has become universally agreed upon. Importantly, placement of this threshold determines how to group the data in experiments aimed at identifying neural correlates of consciousness. Here we argue that if a given purported threshold is a threshold of subjective experience, then there is one test that, at a minimum, this threshold must pass: If identical stimuli are presented at two different levels of intensity that straddle the threshold, then subjects should tend to report that the two stimuli are subjectively different. Furthermore, the probability that both stimuli are reported as different should be lower if both stimuli are below or both are above the putative threshold. Any threshold that does not pass this test, though it might be a threshold of something, is not a threshold of subjective experience. We estimated thresholds using this approach and compared them to thresholds obtained using d-prime, accuracy, meta d-prime, and subjective report. Unexpectedly, the measure that is most closely aligned with this test is the classical psychophysical threshold of 75% correct on a binary discrimination task. Our

data offer a means to test the subjective validity of perceptual thresholds and also provide evidence for an abrupt non-linear threshold of conscious perception.

Disclosures: A. Schurger: None. C. Uribe: None.

Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.26

Topic: E.04. Voluntary Movements

Support: TWCF0608

Title: Empirically studying neuroscientific theories of consciousness: an update on The ConTraSt database

Authors: *L. MUDRIK, K. JOSPE, I. YARON;
Tel Aviv Univ., Tel Aviv, Israel

Abstract: How does the brain give rise to conscious experience? In the last decades, several neuroscientific theories of consciousness have provided different answers to this long-lasting question, and the field has yet to converge on an agreed upon account. The ConTraSt database (<https://ContrastDB.tau.ac.il>) is aimed at exploring how four leading theories have been tested experimentally: Global Neuronal Workspace, Higher Order Thought, Integrated Information Theory and Recurrent processing Theory. Incorporating data from 412 experiments, we recently unraveled potential biases in the neuroscientific study of consciousness: we found a strong confirmation bias, unpredicted heterogeneity of findings and little scientific cross talk between the theories. We were further able to predict which of the four theories will be supported solely from methodological choices, irrespective of findings. Here, we provide updated analyses that now spans over 471 experiments, with 59 new studies that were published since November 2019. The updated database thus provides a current overview of the field and an analysis of major trends over time; it further strengthens our conclusions about the way neuroscientific theories of consciousness have been studied and calls for a-priori experiments aimed at testing opposing predictions of more than one theory in the same experiment.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

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Program #/Poster #: 474.27

Topic: E.04. Voluntary Movements

Title: Prestimulus alpha oscillatory activity interacts with evoked recurrent processing to facilitate conscious visual perception

Authors: *K. KRASICH¹, C. SIMMONS¹, C. M. GIATTINO¹, W. SINNOTT-ARMSTRONG¹, F. DE BRIGARD³, L. MUDRIK⁴, M. G. WOLDORFF²;

²Ctr. Cognitive Neurosci, ¹Duke Univ., Durham, NC; ³Psychology, Harvard Univ. Press, Durham, NC; ⁴Sagol Sch. of Neurosci. & Sch. of Psychological Sci., Tel Aviv Univ., Tel Aviv, Israel

Abstract: Prominent theories have argued that stimulus-elicited recurrent processing in the visual neural hierarchy is critical for conscious visual perception. More recent accounts have also implicated prestimulus alpha (8-12 Hz) oscillatory activity as important for conscious perception. The current work investigated whether prestimulus alpha activity and subsequent stimulus-elicited recurrent processing interact to facilitate conscious perception. Participants attempted to identify the location of a visual cue that was perceptually masked by object substitution masking (OSM). Specifically, on each trial, a transient cue appeared with a surrounding, nonspatially-overlapping four-dot mask. The mask then lingered after the cue offset to produce the OSM effect. It has been previously argued that in OSM, the lingering mask elicits additional feedforward processing of the mask-only stimulus, which disrupts recurrent processing of the cue+mask stimulus. We show that in trials when prestimulus alpha power was low, there was a more negative-polarity cue-evoked ERP response resembling the visual awareness negativity (VAN), which is thought to reflect recurrent processing related to conscious perception. Distribution considerations, including the cruciform model of early visual processing, suggested that this effect originated from dorsal parietal visual cortex. These findings thus suggest that attenuated prestimulus alpha power was linked to a greater innervation needed for stimulus-elicited recurrent processing within the dorsal visual pathway. However, this prestimulus-engendered effect did *not* correspond with better conscious cue-perception. Rather, conscious cue-perception was best in trials when prestimulus alpha power was elevated *and* the cue occurred at a preferred alpha phase *and* there was a robust cue-evoked VAN-like ERP response. In contrast, perception was worse when prestimulus alpha power was elevated but the stimulus appeared at the opposite (i.e., nonoptimal) phase and there was less stimulus-evoked VAN-like negativity. We propose that in OSM, when the cue+mask stimulus appears at an optimal, excitatory phase of the alpha oscillation, recurrent processing for the cue+mask stimulus will be more resilient to suppression from the feedforward processing of the lingering mask-only stimulus. In this way, our findings suggest that prestimulus alpha phase facilitates *temporally selective* recurrent processing. These findings thus help to delineate key parts of the cascading neurocognitive pathways that lead from prestimulus oscillatory activity to evoked recurrent processing and on to eventual conscious perception.

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Poster

474. Sensorimotor and Perceptual Mechanisms in Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 474.28

Topic: E.04. Voluntary Movements

Support: John Templeton Foundation #61283
Fetzer Institute, Fetzer Memorial Trust #4189

Title: Non-conscious reactions and reactions to non-conscious stimuli

Authors: *T. DOMINIK¹, M. SHAW¹, T. BAYNE², L. MUDRIK³, A. SCHURGER¹, U. MAOZ¹;

¹Brain Inst., Chapman Univ., Orange, CA; ²Monash Univ., Melbourne, Australia; ³Tel Aviv Univ., Tel Aviv, Israel

Abstract: We spend much of our awake lives monitoring our environment and reacting to stimuli. When reacting to stimuli, we typically experience ourselves as conscious of both the stimulus triggering our action (stimulus awareness) and the action itself (action awareness). It might thus seem that stimulus awareness and action awareness necessarily accompany (or at least follow) any stimulus-reaction event. But is this always the case, for any stimulus and any reaction? Here, we aim to shed light on the divide between behaviors that can and cannot manifest without stimulus and action awareness by testing whether certain simple reactions can take place with neither stimulus nor action awareness. We tested 16 university students (8 females, 8 males, aged 18-23). We used a go/no-go paradigm, with both supraliminal stimuli (Task 1) and subliminal stimuli (Task 2), while recording subjects' muscle activity using electromyography (EMG). After each trial in Task 1, we asked the subjects whether they were aware of their own reaction; and in Task 2, we additionally asked whether they were aware of the stimulus. We found that no-go stimuli in Task 1 sometimes invoked measurable muscle activation—clearly in (erroneous) response to the stimulus—while the subjects reported being unaware of any response. Specifically (after removing two subjects who exhibited signs of misunderstanding the task), we compared the pre- and post-stimulus EMG amplitude in the no-go trials, where subjects reported not being aware of any motor activity, and found the latter to be reliably larger (one-tailed t-test $t(13) = 2.958$, $p = 0.006$, $d = 0.791$). We also found that subliminal go stimuli in Task 2 significantly increased muscle activity, as well as the probability of muscle activation in general, when subjects reported not being aware of any stimulus. Specifically, after removing one subject due to potentially misunderstood instruction, we examined the EMG amplitude in the trials where subjects reported not being aware of stimulus and found that EMG amplitude was much higher when the subliminal go stimulus was present (one-tailed t-test $t(14) = 3.563$, $p = 0.002$, $d = 0.920$). Our results suggest that neither action awareness nor stimulus awareness is necessary for simple muscle contractions in response to sensory stimuli. Our results provide evidence that some simple behavioral reactions can take place without any conscious processing. Future research should test whether more complex behavioral patterns—such as cued choices—can manifest without both stimulus and action awareness as well.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

Location: SDCC Halls B-H

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Topic: E.05. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D service, Department of Veterans Affairs (N2864C, A2295R)
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NINDS UO1-NS098968, U01 DC1943, UH3 NS107709

Title: Leveraging Language Structure for Unsupervised Recalibration of Communication BCIs

Authors: *C. FAN¹, F. R. WILLETT⁷, D. T. AVANSINO⁷, F. KAMDAR⁷, L. R. HOCHBERG^{8,10,9,11}, J. M. HENDERSON^{2,3,4}, K. V. SHENOY^{5,7,3,4,6};

¹Dept. of Computer Sci., ²Dept. of Neurosurg., ³Wu Tsai Neurosciences Inst., ⁴Bio-X Inst., ⁵Dept. of Electrical Engin., ⁶Dept. of Bioengineering, Stanford Univ., Stanford, CA; ⁷Howard Hughes Med. Inst. at Stanford Univ., Stanford, CA; ⁸Sch. of Engin., ⁹Carney Inst. for Brain Sci., Brown Univ., Providence, RI; ¹⁰VA RR&D Ctr. for Neurorestoration and Neurotechnology, Rehabil. R&D Service, Providence VA Med. Ctr., Providence, RI; ¹¹Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: Intracortical brain-machine interfaces (BCIs) have shown promise for restoring communication to people with paralysis. However, to maintain high performance over time, intracortical BCIs typically need frequent recalibration to combat changes in the neural recordings that accrue over days. This requires BCI users to stop using the BCI and engage in supervised data collection, making the BCI system hard to use. Here, we propose a method to leverage the structure in language to enable unsupervised recalibration of communication BCIs without interrupting the user. We use a language model (LM) to automatically correct communication BCI outputs and generate pseudo-labels for recalibration. Language models (LMs) capture statistical structure in language and can be used to infer the user's intended message given both the BCI output and the typical construction of words and sentences, yielding output that has fewer errors than the BCI alone. With this method, BCI users can continue to engage with the system while the unsupervised LM recalibration runs in the background. As a proof of principle, we performed offline analysis of data collected from participant T5 who was enrolled in the BrainGate 2 pilot clinical trial. T5 has a C4 spinal cord injury and is

implanted with two microelectrode arrays in the “hand knob” area of the dorsal motor cortex. Using offline data spanning almost two months, we show that unsupervised LM recalibration on average has only 0.8% more character error rate (CER) compared to models recalibrated with ground truth labels. In contrast, unsupervised recalibration without an LM or no recalibration has 12.9% and 39.8% more CER, respectively. Therefore, unsupervised LM recalibration is able to fix most of the errors caused by changes in neural signals that accrue over time, and the results are stable across two months. These findings indicate that unsupervised LM recalibration is a promising tool to make communication BCI more practically usable.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 475.02

Topic: E.05. Brain-Machine Interface

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CAUTION: Investigational Device. Limited by Federal Law to Investigational Use.

Title: Ten finger decoding from left precentral gyrus of a person with tetraplegia using an intracortical BCI

Authors: ***T. HOSMAN**^{1,2,4}, C. E. VARGAS-IRWIN^{3,2,4}, D. J. THENGONE^{1,2,5,4}, A. KAPITONAVA⁵, L. R. HOCHBERG^{4,1,5,6,2}, J. D. SIMERAL^{4,1,2};

¹Sch. of Engin., ²Carney Inst. for Brain Sci., ³Dept. of Neurosci., Brown Univ., Providence, RI; ⁴VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of VA Med. Ctr., Providence, RI; ⁵Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; ⁶Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: Intracortical brain computer interface (iBCI) systems record neural activity with high spatial and temporal resolution providing information-rich command signals that people with

limited mobility can use to control assistive devices in efficient and intuitive ways. Here, we evaluate the performance of an iBCI system for classification of individual finger movements across 10 digits using signals from the left precentral gyrus. BrainGate trial participant T11, a 37-year-old man with tetraplegia (C4 AIS-B) with two 96-channel microelectrode arrays implanted in the left precentral gyrus, attempted finger movements to play a simulated piano. Ten piano keys were shown with images of left and right hands directly below the keys. Keys were randomly presented in a cue-response paradigm. Each trial began with the instructed finger and accompanied key turning yellow. If the cued movement was correctly decoded the instructed finger and key turned green and the key's note was played. If the instructed key was not decoded after two seconds, the trial would end. After an intertrial period of 0.75 seconds during which no key was cued or decoded the next trial would begin. Sessions began with an open-loop block of the task where the correct feedback was always displayed after 1.25 seconds. Subsequent closed-loop (CL) decoders incorporated data from all previous blocks. The iBCI decoder used 20ms binned threshold crossings (3.5 RMS) and local field potential (LFP) spike power (250-5000 Hz) that were significantly selective between fingers (Kruskal-Wallis, $p < 0.001$). A regularized linear discriminant analysis (LDA) classifier with a hidden Markov model (HMM) was used to decode the ten digits and null class. Over seven research session days, T11 achieved an average accuracy of 90% across all ten fingers after the first CL block. The average per-digit right (left) percent accuracy for: thumb 94.2% (92.0%), index finger 92.6% (90.5%), middle finger 85.2% (83.1%), ring finger 92.2% (83.5%), pinky finger 95.1%, (92.0%). Errors were most often associated with adjacent digits (3.9% of all trials), the same digit from the opposing hand (1.5%), and trial timeout (2.1%) averaged over all digits. On average, the time from cued instruction to decode was 1.07 seconds. While this study was performed with a single participant with tetraplegia, these results suggest the ability for single hemisphere iBCI to decode discrete contralateral and ipsilateral digits with high accuracy. In conclusion, this approach shows promise of intracortical BCI decoding toward accurate prosthetic digit control and the ability to interact with computer interfaces and modern touch-based technology.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

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Topic: E.05. Brain-Machine Interface

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CAUTION: Investigational Device. Limited by Federal Law to Investigational Use

Title: Tracking Nonstationarity in Multi-Day Intracortical Neural Recordings During iBCI Cursor Control by a Person with Tetraplegia

Authors: ***T. PUN**^{1,2}, **T. HOSMAN**^{1,2,5}, **A. KAPITONAVA**⁶, **C. E. VARGAS-IRWIN**^{2,3,5}, **J. D. SIMERAL**^{1,2,5}, **M. T. HARRISON**⁴, **L. R. HOCHBERG**^{1,2,5,6,7};

¹Sch. of Engin., ²Carney Inst. for Brain Sci., ³Dept. of Neurosci., ⁴Div. of Applied Math, Brown Univ., Providence, RI; ⁵Dept. of VA Med. Ctr., VA RR&D Ctr. for Neurorestoration and Neurotechnology, Providence, RI; ⁶Dept. of Neurol., Ctr. for Neurotechnology and Neurorecovery, MGH, Boston, MA; ⁷Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: Intracortical brain-computer interfaces (iBCIs) have enabled individuals with tetraplegia to control external devices via decoding movement intentions from neural recordings. However, neural activity underlying consistent motor intentions varies over time due to changes in recording conditions, individuals' cognitive states, etc.. There is within- and across-day nonstationarity in the relationship between neural activity and intended movements that can lead to a drop in performance if the decoder is not robust against such changes. To study nonstationarity, a fixed robust online long-short term memory (LSTM) decoder was used for human iBCI cursor control for 142 days. We propose a statistical method to detect when the decoder should be updated solely based on neural activities and decoder outputs, agnostic to the decoder performance.

Neural activity was recorded with two Blackrock Utah microelectrode arrays from the hand-arm area of the precentral gyrus of a participant, T11, who is a 37-year-old male with C4 AIS-B spinal cord injury (enrolled in BrainGate2 pilot clinical trial). Raw neural data were processed in real time into threshold crossing events and power in the spike band (250-5kHz). We trained a LSTM model with recordings from 20 prior sessions of T11 completing point-and-select tasks (trial days 576-646). We analyzed 1832 trials in a closed-loop radial-8 task in 15 sessions (trial days 658-800).

The same decoder achieved a mean 93.8% success rate in the first 11 sessions without parameter updates, but subsequently declined to 33.1% in later sessions. We use Kullback-Leibler divergence to measure changes in the distribution of sampled neural features and decoder outputs. This metric highly correlated with the online cursor angular error ($r=0.94$, Pearson's, $p<0.01$). The same approach also tracked with offline performance in random target Fitts task (794 trials in 4 out of 15 sessions; $r=0.94$, Pearson's, $p<0.01$). This suggests that KL divergence metric is sensitive to nonstationarities that affect decoder performance. Towards translating iBCIs for practical everyday use, this metric may be effective to track nonstationarity online and be useful for triggering either a user-engaged or background update of the decoder as it begins to degrade.

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Paradromics, and Synchron, for which LRH provides consultative input., These entities did not support this work..

Poster

475. Neurophysiology: Decoding and Neural Processing III

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 475.04

Topic: E.05. Brain-Machine Interface

Support: Emory Neuromodulation and Technology Innovation Center (ENTICE)
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DARPA PA-18-02-04-INI-FP-021
NIH Eunice Kennedy Shriver NICHD K12HD073945
NIH-NINDS/OD DP2NS127291
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Burroughs Wellcome Fund

Title: Stabilizing brain-computer interfaces through alignment of latent dynamics

Authors: ***B. M. KARPOWICZ**¹, Y. H. ALI¹, L. N. WIMALASENA¹, A. R. SEDLER^{1,2}, M. KESHTKARAN¹, K. BODKIN³, X. MA³, L. E. MILLER^{3,4,5,6}, C. PANDARINATH^{1,7};

¹Biomed. Engin., Georgia Inst. of Technol. and Emory Univ., Atlanta, GA; ²Ctr. for Machine Learning, Georgia Inst. of Technol., Atlanta, GA; ³Neurosci., Northwestern Univ., Chicago, IL; ⁴Biomed. Engin., Northwestern Univ., Evanston, IL; ⁵Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; ⁶Shirley Ryan AbilityLab, Chicago, IL; ⁷Neurosurg., Emory Univ., Atlanta, GA

Abstract: Intracortical brain-computer interfaces (iBCIs) restore motor function to people with paralysis by translating brain activity into control signals for external devices. In current iBCIs, instabilities at the neural interface degrade decoding performance, which necessitates frequent supervised recalibration using new labeled data. One potential solution is to use the latent manifold structure that underlies neural population activity to facilitate a stable mapping between brain activity and behavior. Such recent efforts have used unsupervised approaches to improve iBCI stability; however, existing methods treat each time step as an independent sample and do not account for latent dynamics. Dynamics have been used to enable high performance prediction of movement intention, and may also help improve stabilization. Here, we present a platform for Nonlinear Manifold Alignment with Dynamics (NoMAD), which stabilizes iBCI decoding using recurrent neural network models of dynamics. As instabilities cause changes in the recorded neural population, NoMAD uses unsupervised distribution alignment to update the mapping of this nonstationary neural data to fixed neural dynamics without knowledge of the subject's behavior, thereby providing stable input to the iBCI decoder.

We applied NoMAD to recordings from monkey primary motor cortex (M1) collected during motor tasks in sessions that span multiple weeks. We compared our results to two previous state-

of-the-art stabilization approaches that use latent manifolds (ADAN - Farshchian 2019 and Aligned FA - Degenhart 2020). To evaluate on as much data as possible, we tested each method by aligning every day within a dataset to every other day. When applied to data from a two-dimensional isometric wrist force task, NoMAD achieved strikingly higher decoding performance (median R^2 of all pairs = 0.91, 0.65, 0.59 for NoMAD, ADAN, and Aligned FA respectively) and improved stability (slope = -0.033, -0.087, -0.13 R^2 /month) compared to those previous approaches over 3 months. Further, when applied to recordings spanning five weeks from a center-out reaching task with very different output dynamics, NoMAD again achieved substantially higher decoding performance (median R^2 = 0.77, 0.29, 0.17 for NoMAD, ADAN, and Aligned FA respectively) and stability (slope = -0.13, -0.48, -0.64 R^2 /month) than previous approaches. These results demonstrate that by incorporating the temporal structure of the neural activity into the manifold-alignment process, NoMAD enables more stable neural decoding that would presumably require less frequent iBCI recalibration procedures and provide a pathway to more practical iBCIs.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

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Topic: E.05. Brain-Machine Interface

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NIH Eunice Kennedy Shriver NICHD K12HD073945
NIH-NINDS/OD DP2NS127291
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Burroughs Wellcome Fund

Title: Realtime asynchronous decoding for closed-loop neuroprosthetics

Authors: ***Y. H. ALI**¹, **K. BODKIN**², **M. RIGOTTI-THOMPSON**¹, **L. E. MILLER**^{2,3,4,5}, **C. PANDARINATH**¹, **D. M. BRANDMAN**⁶;

¹Wallace H. Coulter Dept. of Biomed. Engin., Emory Univ. and Georgia Inst. of Technol., Atlanta, GA; ²Neurosci., Northwestern Univ., Chicago, IL; ³Biomed. Engin., Northwestern Univ., Evanston, IL; ⁴Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; ⁵Shirley Ryan AbilityLab, Chicago, IL; ⁶Neurolog. Surgery, Univ. of California, Davis, CA

Abstract: Artificial neural networks (ANNs) have advanced our ability to model and decode neural activity, but deploying them in closed-loop experiments has been challenging due to their limited support in existing real-time frameworks. Researchers need a flexible framework that provides full support for interpreted languages for running ANNs (e.g., Python) while maintaining support for other languages that are critical for low-latency data acquisition (e.g., C and C++) and providing seamless data flow between them. To address these needs, we introduce the BRAND Realtime Asynchronous Neural Decoding system (BRAND). BRAND consists of a set of Linux processes, termed “nodes”, that each receive inputs and/or produce outputs during an experiment. Three main features differentiate BRAND from other real-time data processing systems: 1) it runs nodes asynchronously, 2) it supports a wide variety of programming languages, and 3) it can store and replay the data sent between any of the nodes. Its asynchronous design allows for acquisition, control, and analysis to be executed in parallel processes on streams of data that may operate at different timescales. By using a Redis database to send data between nodes, BRAND takes advantage of Redis’s existing support for 54 different programming languages, allowing developers to select the language that is most suitable for the problem at hand. Furthermore, storing inter-process communication in a database allows developers to replay the data flow from an experiment and prototype modifications to the experiment’s design. In our testing, BRAND has shown an inter-process communication latency of less than 350 microseconds when sending 640 channels of 30 kHz 16-bit neural data in 1-millisecond blocks. In a monkey experiment, we demonstrate that BRAND can acquire 30 kHz microelectrode array recordings and compute threshold crossings with a typical latency of 360 microseconds. We also demonstrate that BRAND can perform LFADS inference on replayed spiking data in less than 9.2 milliseconds per 10-millisecond bin (Pandarinath et al., 2018). Lastly, as an example of the system’s use in more general applications, we built a BRAND-based simulator which mimics standard neural data acquisition hardware by generating 30 kHz continuous neural data from user movement and sending it out via network packets at a consistent 1kHz rate (1.000 ms mean inter-sample period, <20 μ s std. deviation). As shown by these applications, BRAND provides a flexible framework for building closed-loop experiments while supporting the latest tools in neuroscience and machine learning.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 475.06

Topic: E.05. Brain-Machine Interface

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Wu Tsai Neurosciences Institute
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Larry and Pamela Garlick
Simons Foundation Collaboration on the Global Brain 543045

Title: Leveraging task structure for unsupervised recalibration of cursor BCIs

Authors: *G. WILSON¹, F. WILLET^{2,3,4}, D. AVANSINO^{2,3,4,5}, F. KAMDAR², L. R. HOCHBERG^{9,10,11,12,13}, J. M. HENDERSON^{2,5,6}, S. DRUCKMANN^{5,7}, K. V. SHENOY^{3,8,7,5,4},
¹Neurosciences Program, ²Neurosurg., ³Electrical Engin., ⁴Hhmi, ⁵Wu Tsai Neurosciences Inst.,
⁶Bio-X Program, ⁷Neurobio., ⁸Bioengineering, Stanford Univ., Stanford, CA; ⁹Carney Inst. for
Brain Sci., ¹⁰Sch. of Engin., Brown Univ., Providence, RI; ¹¹Dept. of VA, Med. Ctr., RR&D Ctr.
for Neurorestoration and Neurotechnology, Providence, RI; ¹²Neurol., Harvard Med. Sch.,
Boston, MA; ¹³Neurol., Massachusetts Gen. Hosp., Ctr. for Neurotechnology and
Neurorecovery, Boston, MA

Abstract: Intracortical brain-computer interfaces (iBCIs) require repeated recalibration to maintain robust performance across days due to nonstationary neural features. An ideal solution to this problem would enable high performance across time without the need for supervised recalibration periods, where users cannot engage in free use of their device. Here we introduce a probabilistic model (“PRI-T”, Probabilistic Retrospective Inference of Targets) for unsupervised recalibration of 2D cursor velocity decoders. PRI-T infers likely target positions from a noisy decoder’s outputs and uses this information to then recalibrate the decoder.

To develop the approach, we recorded from two microelectrode arrays placed in “hand knob” area of dorsal motor cortex in a participant with C4 spinal cord injury (“T5”) while he engaged in a series of cursor control tasks. In a closed-loop simulation environment matched to T5’s SNR and neural drift characteristics, we found that PRI-T enabled better control than two prior recalibration methods - subspace stabilization (Degenhart, Bishop et al., 2020), and RTI (Jarosiewicz et al., 2015) - with no detectable performance difference compared to supervised recalibration after 60 days of cumulative recalibration. In contrast, subspace stabilization accumulated error over time and performance eventually diverged. We also found that PRI-T was far more robust to smaller dataset sizes than RTI while maintaining similar performance to supervised recalibration. With offline analyses of pairs of days spanning four years, we show that integrating PRI-T and subspace stabilization achieves higher performance than either alone on nearly all tested evaluations (> 91% of the time for both; 116/127 versus PRI-T, 118/127 versus stabilizer).

The latter finding suggests that combining both neural structure and task structure can optimize recalibration beyond what is achievable with either source alone. More broadly, our results demonstrate how task structure can be used to bootstrap a noisy decoder into a high-performant one and highlight the utility of adaptive algorithms for BCI robustness.

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Druckmann: F. Consulting Fees (e.g., advisory boards); consults for CTRL-Labs (acquired by Facebook Reality Labs in Fall 2019, and is now a part of Meta Platform's Reality Labs). **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); serves on the Scientific Advisory Boards (SABs) of MIND-X Inc. (acquired by Blackrock Neurotech, Spring 2022), Inscopix Inc. and Heal Inc., he also serves as a consultant / advisor (and was on founding SAB) for CTRL-Labs (acquired by Facebook Reality Labs in Fall 2019, and is now a part of Meta Platform's Reality Labs), and serves as a consultant / advisor (and is a co-founder, 2016) for Neuralink.

Poster

475. Neurophysiology: Decoding and Neural Processing III

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Program #/Poster #: 475.07

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Title: How many people with upper extremity paralysis or communication deficits might benefit from an intracortical brain BCI?

Authors: *A. M. DUFFY^{1,4,2}, D. B. RUBIN^{1,2}, R. M. MARUJO^{1,2}, D. A. SCHOENFELD³, L. CHIBNIK², R. A. AHMED², M. K. C. DOROTAN⁵, L. K. OETJEN⁵, P. SRIKANTH², A. TANG⁵, L. R. HOCHBERG^{1,4,6,2,7};

¹Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., ²Dept. of Neurol., ³Dept. of Biostatistics, Massachusetts Gen. Hosp., Boston, MA; ⁴VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of Veterans Affairs Med. Ctr., Providence, RI; ⁵Dept. of Neurol., Brigham and Women's Hosp., Boston, MA; ⁶Sch. of Engin., ⁷Carney Inst. for Brain Sci., Brown Univ., Providence, RI

Abstract: Motivation: For individuals with paralysis, intracortical brain-computer interfaces (iBCIs) have the potential to restore motor function and communication. The prospective applications of iBCIs are wide, however the number of people who may benefit from an iBCI has not been quantified. We sought to develop a methodology that quantifies an estimate of this population size.

Methods/Results: To quantify the number of people within the Mass General Brigham (MGB) healthcare system who may benefit from an iBCI, we queried the MGB Research Patient Data Registry (RPDR) - a database of all MGB clinical data, which includes 7 million patients since

1975 (3 billion corresponding datapoints [e.g. diagnoses, procedures etc.]). To determine the number of people who might benefit from an iBCI, we sought to review clinical records, which provide sufficient detail to make this determination. We first queried the diagnosis codes for “locked-in state” (LIS) (ICD10:G83.5 and ICD9:344.81) and developed a strategy to characterize iBCI benefit of the 132 individuals who had been assigned these ICD codes since 1975. We are working with a group of 9 clinicians/neuroscientists to assess inter-rater reliability. We also queried 733 diagnosis codes relevant to upper extremity and/or communication deficits, yielding >240,000 patients. We plan to sample a subset of these cases to determine the likelihood of iBCI benefit by first clustering diagnoses by similar etiologies and clinical phenotypes. We then estimate the proportion of people who would benefit from an iBCI for each cluster. These estimated proportions and the number of people in each cluster can then be used to extrapolate incidence and prevalence that can be applied to the larger populations.

Conclusion: This study will quantify the number of patients seen within a large, multi-hospital academic medical center with specific diagnosis codes who may benefit from an iBCI, based upon retrospective, efficient, and statistically rigorous chart review of patients with upper extremity and/or communication deficits. Extrapolating these results to larger national databases (e.g., private insurance, Medicare) will help to clarify the size of the US population who may benefit from these initial indications for novel iBCI technologies.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

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Topic: E.05. Brain-Machine Interface

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Office of the Vice President for Research - Brown University
Robert J and Nancy D Carney Institute for Brain Science - Brown University
Brown University School of Engineering

Title: Engaging individuals with tetraplegia in the user-centered design of a home intracortical BCI.

Authors: ***R. GROSS-LEWIS**¹, T. SINGER-CLARK³, T. HOSMAN^{1,2,4}, R. CRAWFORD³, A. KAPITONAVA³, J. D. SIMERAL^{4,1,2}, L. R. HOCHBERG^{4,1,3,5,2},

¹Sch. of Engin., ²Carney Inst. for Brain Sci., Brown Univ., Providence, RI; ³Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; ⁴VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of VA Med. Ctr., Providence, RI; ⁵Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: Objective. Intracortical brain-computer interfaces (iBCIs) have been shown to provide high-throughput point-and-click communication and access to computers and the internet. Successful long-term adoption of iBCI assistive technologies will require these complex systems to be easily configured, operated, and customized by end-users and their caregivers without expert assistance. To this end, we aimed to create a user interface (UI) that could facilitate access to iBCI functions and an intuitive and satisfying user experience. To meet these goals, we applied a user-centered design (UCD) framework for UI development that engaged iBCI end users early and often in the design process with the goal of enhancing the usability of the iBCI system. Methods. We engaged study participant T11, a 37-year-old man with tetraplegia (C4 AIS-B), in an iterative UCD process to create new software features intended to enhance the usability of the BrainGate iBCI. T11 used the wireless BrainGate iBCI in his home using neurally-decoded imagined hand movements and gestures to generate mouse and touch-like computer interactions. Over the course of a year, a series of novel interface features were deployed on T11's Windows desktop computer and wheelchair-mounted iPad for his personal use and evaluation of UI behavior, utility, and on-screen aesthetics. Through research sessions utilizing qualitative open-ended questions and a UCD process, T11 provided feedback on his experience, suggested design changes, and requested specific new software features. Assessments measuring effectiveness, efficiency, and satisfaction were also developed to quantitatively evaluate system usability. Results. Over the course of 100+ personal use days, and 20+ usability sessions, a user-facing app (BG Home) was developed and refined with input from the trial participant to improve his experience with the BrainGate iBCI. Some features included the ability to adjust the iBCI cursor speed, a novel multi-gesture sequence for switching neural control between devices such as a desktop computer or wheelchair-mounted iPad, and the ability to customize imagined hand gestures to priority screen functions such as recentering the cursor or right-click. Input from T11 also refined the on-screen presentation and technical implementation of the usability surveys. Conclusion. Elements of UCD have previously been applied to EEG BCIs. Here, UCD robustly engaged a BrainGate trial participant in long-term, iterative UI/UX development that informed the functionality, design, and metrics of an intracortical BCI. This process identified foundational design elements for an iBCI UI for individuals with paralysis.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

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Topic: E.05. Brain-Machine Interface

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NINDS U01-NS098968, U01 DC1943, UH3 NS107709

Title: Neural ensemble encoding of future speech production in oral facial motor cortex

Authors: ***E. KUNZ**^{1,2}, **F. WILLETT**^{3,2}, **D. AVANSINO**³, **F. KAMDAR**⁴, **L. R. HOCHBERG**⁵, **K. V. SHENOY**^{3,2,6,1,7}, **J. M. HENDERSON**^{4,6,2};

¹Dept. of Electrical Engineering, Stanford Univ., Stanford, CA; ²Wu Tsai Neurosciences Institute, Stanford Univ., Stanford, CA; ³Howard Hughes Med. Inst. at Stanford Univ., Stanford, CA; ⁴Dept. of Neurosurgery, Stanford Univ., Stanford, CA; ⁵Providence VA Med. Ctr., VA RR&D Ctr. for Neurorestoration and Neurotechnology, Rehabil. R&D Service, Providence, RI; ⁶Bio-X Institute, Stanford Univ., Stanford, CA; ⁷Dept. of Bioengineering, Stanford Univ., Stanford, CA

Abstract: Language and rapid, efficient vocal communication are uniquely human attributes. While functional MRI (fMRI) and electrocorticography (ECoG) have paved the way in studying the neural basis of speech production in people by recording from broad regions of the brain, revealing the precise mechanisms of human speech production requires single-neuron level recordings. Here, we present evidence from intracortical microelectrode array recordings in ventral Precentral Gyrus (vPCG) that neural ensemble activity encodes information related to preparing to speak, not just at the level of upcoming articulatory gestures, or individual sound units, or phonemes, but at the mesoscale level of words and potentially further into the future. We also examine preparatory activity for non-words and non-speech motor sequences. Simultaneous single-neuron resolution population activity was recorded from four intracortical microelectrode arrays (64 channels each, two in oral-facial vPCG and two in the posterior

portion of inferior frontal gyrus (IFG; encompassing Broca's Area), from a human subject ("T12") with dysarthria as a result of bulbar-onset ALS. The subject was instructed to attempt to speak sets of words and phrases or perform motor sequences. Each trial was cued with displayed text during a 'delay' period followed by a 'go' period, during which the participant attempted to speak or perform the sequence. Trial conditions included sets of words, short phrases, sentences and motor sequences with variation of single items at each ordinal position. We did not find information in IFG related to the speaking conditions during either the delay or go periods, supporting recent literature that Broca's area may be further divided into functional subgroups that are not always language or phonology-specific. In contrast, decoding analyses from vPCG during the preparatory period showed high decode-ability between words regardless of the ordinal position for which single phonemes were varied, revealing that vPCG encodes future phonemes for the duration of entire words and possibly beyond. These preliminary findings indicate that area 6V of PCG may have more complex motor sequence planning functionality than previously known.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

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Topic: E.05. Brain-Machine Interface

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Title: Representation of the whole body in orofacial versus arm/hand motor cortex in people

Authors: ***D. R. DEO**^{1,2,3}, **F. WILLETT**^{1,3,4}, **F. KAMDAR**¹, **L. R. HOCHBERG**^{8,10,9,11,12}, **K. V. SHENOY**^{3,4,2,5,6}, **J. M. HENDERSON**^{1,2,7};

¹Neurosurg., ²Wu Tsai Neurosciences Inst., ³Electrical Engin., ⁴Howard Hughes Med. Inst.,

⁵Dept. of Bioengineering, ⁶Dept. of Neurobio., ⁷Bio-X Program, Stanford Univ., Stanford, CA;

⁸Carney Inst. for Brain Sci., ⁹Sch. of Engin., Brown Univ., Providence, RI; ¹⁰Dept. of VA Med.

Ctr., VA RR&D Ctr. for Neurorestoration and Neurotechnology, Providence, RI; ¹¹Dept. of Neurol., Harvard Med. Sch., Boston, MA; ¹²Dept. of Neurology, Massachusetts Gen. Hosp., Ctr. for Neurotechnology and Neurorecovery, Boston, MA

Abstract: Traditionally, it was thought that face, arm, and leg movements are represented in distinct areas of human precentral gyrus. We recently discovered that the whole body is represented in a 4 mm x 4 mm patch of the ‘arm/hand’ premotor cortex component of the precentral gyrus. In addition, we found a compositional neural code that links matching movements from all four limbs as well as a departure from this compositionality during bimanual movements. Here, we investigate the arm/hand premotor cortex movement representation in orofacial motor cortex to understand how the whole body is represented along the precentral gyrus.

Our study participant (T12) has bulbar-onset ALS and is enrolled in the BrainGate2 clinical trial. Four 64-channel microelectrode arrays were surgically placed in March 2022, two of which were placed in ventral precentral gyrus which is believed to be responsible for orofacial movement. T12 performed a series of attempted individual and bimanual movements spanning the entire body while we recorded spiking activity at single-neuron resolution. Similar to our findings in arm/hand premotor cortex (Willett*, Deo*, et al. Cell 2020), we found that orofacial motor cortex is tuned to the entire body with the strongest tuning to orofacial movements—congruent with the arm/hand area which was most strongly tuned to arm movements. A cross-validated naive Bayes classifier was able to decode 45 discrete movements across the body from neural activity with 83.7% accuracy. Also consistent, we found a compositional code in orofacial motor cortex with correlations between pairs of homologous movements (e.g., hand grasp and toe curl) and neural dimensions that code for movement direction, limb, and side of the body that the limb resides. Lastly, and also consistent with arm/hand premotor cortex, we found tuning to simultaneous multi-limb movements in orofacial motor cortex. Future work will further characterize how tuning changes during simultaneous movements.

In sum, we found that orofacial motor cortex is similar to arm/hand premotor cortex in that the "preferred" body part (face and mouth) is represented most strongly, but the rest of the body is also represented there in a compositional way. This suggests that compositional whole-body representation may be an organizing principle for understanding human motor cortex.

Disclosures: **D.R. Deo:** None. **F. Willett:** None. **F. Kamdar:** None. **L.R. Hochberg:** Other; MGH Translational Research Cntr. has a clinical research support agreement with Neuralink, Paradromics, and Synchron, for which LRH provides consultative input. These entities did not support the work. **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); Prof. Shenoy serves on the Scientific Advisory Boards (SABs) of MIND-X Inc. (acquired by Blackrock Neurotech, Spring 2022), Inscopix Inc. and Heal Inc., he also serves as a consultant / advisor (and was on founding SAB) for CTRL-Labs (acquired by Facebook Reality Labs in Fall 2019, and is now a part of Meta Platform's Reality Labs), and serves as a consultant / advisor (and is a co-founder, 2016) for Neuralink. **J.M. Henderson:** F. Consulting Fees (e.g., advisory boards); Serves on the Scientific Advisory Board of Enspire DBS and serves as a consultant for Neuralink. Other; Dr. Henderson has sponsored research from Boston Scientific.

Poster

475. Neurophysiology: Decoding and Neural Processing III

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 475.11

Topic: E.05. Brain-Machine Interface

Support: Pilot Award - Simons Collaboration for the Global Brain
Career Award to S. D. Stavisky - Scientific Interface from BWF

Title: Decoding intracortical neural activity from motor cortex to synthesise speech

Authors: *M. WAIRAGKAR¹, L. R. HOCHBERG², D. M. BRANDMAN¹, S. D. STAVISKY¹;

¹Neurolog. Surgery, Univ. of California, Davis, Davis, CA; ²Neurol. / Engin., Massachusetts Gen. Hospital/Brown University/Providence VAMC, Boston, MA

Abstract: The ability to speak is a key determinant of quality of life, but is disrupted in people with brain injury and neurodegenerative diseases such as ALS. Brain-Computer Interfaces (BCIs) can potentially restore speech in individuals who have lost the ability to speak by interpreting their speech-related neuronal activity. Current intracortical BCIs can enable users to write with high accuracy, but these modes of communication are slow and do not capture the full expressive range of speech. Recent studies have identified speech features from ECoG and sEEG recordings and even demonstrated closed-loop word classification and attempts at voice synthesis; however intelligible speech synthesis using these mesoscale recordings has not yet been demonstrated. Our previous work has shown that intracortical recordings from dorsal motor cortex show distinct speech-related patterns, motivating exploring an intracortical (microscale) approach for voice synthesis. Here, we present a neural decoder to synthesise speech by directly translating neural activity recorded from human motor cortex using intracortical multi-electrode arrays. We have designed a deep learning neural network decoder framework with signal processing and feature extraction modules to decode neural firing rates from intracortical electrodes in a BrainGate clinical trial participant. First, we processed raw neural firing rates from short sliding windows to extract features by normalisation, dimensionality reduction, and quantisation. We used these features to train a neural network to predict spectral and pitch features associated with speech corresponding to the neural activity. To improve the robustness of the decoder, we also trained the neural network with augmented neural data by introducing noise and artefacts simulating non-stationarities observed in intracortical recordings over time. Finally, we used LPCNet, a deep neural network vocoder to synthesise speech frame-by-frame from the predicted low dimensional spectral speech features. We predicted speech features from short windows of neural data every 10 ms which is essential for real-time speech synthesis. Our decoder was able to reconstruct the input speech spectrum which was processed through LPCNet to synthesise speech directly from the corresponding intracortical neural activity in motor cortex. Offline reconstruction of Penn Treebank passages read out loud (87 min) from dorsal motor cortex activity yielded $r=0.71\pm 0.02$ correlation with true speech (averaged across 40 Mel frequencies). Our approach is suitable for real-time applications in BCI to restore lost speech, which is the focus of our ongoing work.

Disclosures: **M. Wairagkar:** None. **L.R. Hochberg:** F. Consulting Fees (e.g., advisory boards); Neuralink, Paradromics, Synchron. **D.M. Brandman:** None. **S.D. Stavisky:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Wispr.ai, Blackrock Neurotech.

Poster

475. Neurophysiology: Decoding and Neural Processing III

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 475.12

Topic: E.05. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D service, Department of Veterans Affairs (N2864C, A2295R)
Wu Tsai Neurosciences Institute
Howard Hughes Medical Institute
Larry and Pamela Garlick
Simons Foundation Collaboration on the Global Brain 543045
NIDCD R01-DC01434, NIDCD U01-DC017844, NINDS UH2-NS095548,
NINDS U01-NS098968, U01 DC1943, UH3 NS107709

Title: An intracortical speech BCI for high-performance brain-to-text communication

Authors: ***F. WILLETT**¹, **C. FAN**², **E. KUNZ**^{3,4}, **D. AVANSINO**¹, **F. KAMDAR**¹, **L. R. HOCHBERG**^{8,9,10,11}, **K. V. SHENOY**^{1,4,3,5,6}, **J. M. HENDERSON**^{7,4,5};

¹HHMI at Stanford Univ., Stanford, CA; ²Computer Sci., ³Electrical Engin., ⁴Wu Tsai Neurosciences Inst., ⁵Bio-X Inst., ⁶Bioengineering and Neurobio., ⁷Neurosurg., Stanford Univ., Stanford, CA; ⁸Sch. of Engin., ⁹Carney Inst. for Brain Sci., Brown Univ., Providence, RI; ¹⁰Ctr. for Neurotechnology and Neurorecovery, Massachusetts Gen. Hosp., Boston, MA; ¹¹VA RR&D Ctr. for Neurorestoration and Neurotechnology, Providence VA Med. Ctr., Providence, RI

Abstract: Speech-to-text brain-computer interfaces (BCIs) have the potential to restore rapid communication to people with paralysis by neurally decoding attempted speaking movements into text. However, early demonstrations have not yet achieved sufficient decoding accuracy for real-world use, especially on large vocabularies. We hypothesized that recording neural ensemble activity with microelectrode arrays would improve speech decoding accuracy relative to prior work with larger electrode contacts (e.g., ECoG grids). In this study, we placed four microelectrode arrays into speech-related areas of cortex in a participant with bulbar ALS who could no longer speak intelligibly, as part of the BrainGate2 pilot clinical trial. We found that although neural tuning to attempted speech was weak in Brodmann area 44 (Broca's area), ventral precentral gyrus (area 6v) contained rich neural representations of attempted orofacial movements and speech. Tuning to speech articulators in 6v was intermixed at the single electrode level, and all speech articulators and phonemes were clearly represented even within a single array (3.2 x 3.2 mm).

Using neural population activity from area 6v, we achieved decoding accuracies that, to our knowledge, set a new standard for speech BCIs. We used a recurrent neural network to translate neural activity into decoded phonemes and a 3-gram language model to translate decoded phonemes into words. Offline, we achieved phoneme error rates as low as 24% on held-out sentences of general English spoken at a rate of 66 words per minute (WPM). Using a large vocabulary language model (130k words) to translate these phonemes into text yielded a word error rate (WER) of 20%. Word error rates were much lower on more restricted vocabularies - for example, 5-7% on a 50-word vocabulary. We are currently translating these results online with a real-time system. Early online demonstrations have achieved a WER of 7.9% at 61 WPM using a 50-word vocabulary (compare to 25% WER and 15 WPM for the prior state of the art, Moses et al. 2021), and a 30% WER using a large vocabulary (130k words). These results show the potential of microelectrode recordings for high-performance speech decoding, although more electrodes may be needed to achieve low WERs on large vocabularies.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 475.13

Topic: E.05. Brain-Machine Interface

Support: AHA (19CSLOI34780000)
Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (N2864C, A2295R)
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CAUTION: Investigational Device. Limited by Federal Law to Investigational Use.

Title: Response of human motor cortex to observed effector movement is modulated by anthropomorphicity

Authors: ***J. T. GUSMAN**^{1,2,4}, **Z. BECKMAN**¹, **T. SINGER-CLARK**⁵, **A. KAPITONAVA**⁵, **J. P. DONOGHUE**^{6,1,2,7}, **C. E. VARGAS-IRWIN**^{3,2,4}, **L. R. HOCHBERG**^{4,1,5,8,2};
¹Sch. of Engin., ²Carney Inst. for Brain Sci., ³Dept. of Neurosci., Brown Univ., Providence, RI;
⁴VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of VA Med. Ctr., Providence, RI; ⁵Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; ⁶Dept. of Neurosci., BROWN UNIVERSITY, Providence, RI; ⁷Wyss Ctr., Geneva, Switzerland; ⁸Dept. of Neurosci., Harvard Med. Sch., Boston, MA

Abstract: As researchers of intracortical Brain-Computer Interface (iBCI) technology develop ever more sophisticated methods for extracting user intent from motor areas of the human brain, understanding the influences of sensory and other non-motor signals in these areas has become increasingly important. It is well documented that neurons involved in motor execution can also be activated by observation of movement performed by other humans or monkeys. However, little is known about the behavior of these neurons when observing other anthropomorphic agents like prosthetic limbs and assistive robots. In this study, we used virtual robotic hands and random dot configurations to evaluate neural activity in the human motor cortex as an iBCI clinical trial participant observed effectors of differing degrees of *anthropomorphicity*. Neural data were recorded from participant T11, a 37-year-old man with spinal cord injury (C4 AIS-B), who had two 96-channel electrode arrays (Blackrock Microsystems) implanted in his left precentral gyrus as part of the BrainGate Clinical Trial. In the first task, T11 attempted “power” and “pinch” grips while watching a screen displaying movements of an animated human hand, an anthropomorphic robot hand, a three-fingered robot claw, a cube, and a fixation cross. He also passively observed the virtual effectors without attempting grasps. In the second task, T11 passively observed the human hand, the fixation cross, and a series of random dot stimuli that were systematically altered to look more or less like a human hand. After each trial, T11 rated on a scale of 1 to 4, how human-like the effector appeared. We compared population and single unit activities across conditions. Whereas visual feedback did not significantly affect neural activity when T11 was attempting grasps, passive observation of the most anthropomorphic effectors (human hand, anthropomorphic robot) yielded significantly greater ensemble firing rates and grasp classification accuracies over the other conditions (Rank Sum, $p < 0.01$). The random dot stimuli demonstrated that a monotonic relationship exists between effector anthropomorphicity and neural modulation magnitude; ensemble activity and grasp type classification accuracies gradually increased as random dot motion became more hand-like. This relationship was also evident among individual neurons, as neurons were nearly six times more likely than chance to have anthropomorphically ordered mean firing rates. These results suggest that visual activation of the human motor cortex is highly dependent on the relative human-likeness of the observed movement.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 475.14

Topic: E.05. Brain-Machine Interface

Support: NSF Grant 2011595
NSF Grant 1902395
BMBF 01GQ1602

Title: Comparative Analysis of Speech Activity Detection from Overt, Mouthed, and Imagined Speech using Stereotactic EEG

Authors: *P. ZANGANEH SOROUSH¹, C. HERFF², S. K. RIES³, J. SHIH⁴, T. SCHULTZ⁵, D. J. KRUSIENSKI¹;

¹Biomed. Engin., Virginia Commonwealth Univ., richmond, VA; ²Maastricht Univ., Maastricht, Netherlands; ³San Diego State Univ., San Diego, CA; ⁴UCSD Hlth., San Diego, CA; ⁵Univ. of Bremen, Bremen, Germany

Abstract: Recent studies have shown promise toward the development of speech neuroprosthetics using intracranial signals. For those who have completely lost the ability to speak, the objective is to synthesize acoustic speech directly from brain activity during imagined or attempted speech. Due to the lack of auditory and behavioral output during imagined speech, current approaches commonly rely on surrogate acoustic speech for training imagined speech decoding models. However, performance of such models remains far inferior to the better-established overt speech decoding counterparts. With the objective of elucidating and comparing the distinct neural feature contributions to overt and imagined speech models, the present study uses stereotactic EEG (sEEG) recordings collected during a speech task designed to produce three graded degrees of behavioral output. sEEG data were collected from 4 native English-speaking participants being monitored as part of treatment for intractable epilepsy at UCSD Health. For the experiment, participants were presented with a sentence displayed on a computer monitor and simultaneously narrated via computer speakers. Immediately following this presentation, participants were prompted in sequence to respectively: (1) overtly speak, (2) mouth (articulate without speaking), and (3) imagine speaking the sentence. Data were collected for 50 sentences per participant. The 4-second overt speech trials were extracted and labeled with 'speaking' and 'non-speaking' intervals using automatic speech transcription software. Due to unavailability of labels for the mouthed and imagined speech tasks, the corresponding overt task labels were used to derive surrogate labels for these tasks. Speech activity detection decoding models were developed and applied within and across the overt, mouthed, and imagined speech tasks to reveal the similarities and differences in the spatial, temporal, and spectral features among the models. The results indicate a hierarchy in which the relevant features for the lower behavioral output modalities form nested subsets of the relevant features from the higher behavioral output modalities. The feature subset relevant to imagined speech was found to reside

in bilateral frontal and temporal regions at various depths, whereas the features unique to overt speech predominantly resided in more superficial temporal regions, despite prescreening features for auditory feedback. These findings provide important insights toward the development of more effective imagined speech decoding models.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 475.15

Topic: E.05. Brain-Machine Interface

Support: C.H. acknowledges funding by the Dutch Research Council (NWO) through the research project 'Decoding Speech In SEEG (DESI)' with project number VI.Veni.194.021
M.V., P.L.K. and C.H. acknowledge funding for the project 'NWO TTW INTENSE'

Title: User training for real-time speech neuroprostheses

Authors: *M. VERWOERT¹, M. C. OTTENHOFF¹, J. AMIGÓ-VEGA^{1,2}, S. GOULIS¹, A. J. COLON³, L. WAGNER³, S. TOUSSEYN³, J. P. VAN DIJK^{3,4,5}, P. L. KUBBEN¹, C. HERFF¹; ¹Neurosurg., Maastricht Univ., Maastricht, Netherlands; ²Computer Sci., Gran Sasso Sci. Inst., L'Aquila, Italy; ³Academic Ctr. for Epileptology, Kempenhaeghe/Maastricht Univ. Med. Ctr., Heeze, Netherlands; ⁴Orthodontics, Ulm Univ., Ulm, Germany; ⁵Electrical Engin., Eindhoven Univ. of Technol., Eindhoven, Netherlands

Abstract: Speech brain-computer interfaces (BCIs) aim to restore intuitive communication in individuals who are unable to vocalize their thoughts. The most severe use-case for such a system is for individuals with locked-in syndrome (LIS), who have no means of communicating at all due to complete loss of motor control. A speech BCI system for this target population ideally adheres to the following conditions to be practical: 1) provides real-time feedback, 2) utilizes imagined/attempted speech and 3) the output is intelligible. Though significant progress has been made in recent years, thus far no speech synthesis system has achieved all conditions at once. Here we focus on increasing the intelligibility of a closed-loop speech synthesis system through user training. BCI control is a skill whereby the user needs to learn to produce stable neural features. It is important that the user learns to modulate their own signal, before an adapting system will be effective. We evaluate whether real-time auditory feedback is sufficient to help the user adapt and thereby increase the intelligibility of the output.

Participants were asked to generate the training data by speaking 20 words, relevant to LIS patients, out-loud 5 times in random order. Stereo-electroencephalography (sEEG) and acoustic

signals were recorded simultaneously during the speech production. A closed-loop unit-selection speech synthesizer was initialized using the training data. Participants were subsequently asked to practice imagined speaking while receiving the synthesized speech as auditory feedback. Participants were instructed to imagine speaking at their own pace, try out different techniques and repeat the word presented on a screen as often as they like before proceeding to the next word or a previous word. This design introduces autonomy which may facilitate the learning process.

Preliminary results indicate that the difference between the proportion of speech detected during speech compared to rest trials increased after 30 minutes of practice with the closed-loop system. Importantly, the training data remained exactly the same between the evaluations and the participant perceived an improvement in control of the system. This indicates that users are able to adapt and improve the output of the system based on auditory feedback.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 475.16

Topic: E.05. Brain-Machine Interface

Support: National Science Foundation (NSF) 2011595/1902395
BMBF 01GQ1602

Title: Characterization of Traveling Waves in the Human Brain During a Speaking Task using Stereotactic EEG

Authors: P. ZANGANEH SOROUSH¹, C. HERFF², S. RIES³, J. SHIH⁴, T. SCHULTZ⁵, *D. J. KRUSIENSKI¹;

¹Biomed. Engin., Virginia Commonwealth Univ., Richmond, VA; ²Maastricht Univ., Maastricht, Netherlands; ³San Diego State Univ., San Diego, CA; ⁴UC San Diego, San Diego, CA; ⁵Univ. of Bremen, Bremen, Germany

Abstract: Recent electrophysiological studies in humans and animals have provided evidence that certain oscillatory activity appears to propagate across the cortex and within the brain. This phenomenon, known as traveling waves, has been observed during performance of visual and memory tasks, for example, and is hypothesized to be associated with information transfer in the brain. While prior studies have primarily identified traveling waves on the cortex of humans and animals via intracranial electrocorticography (ECoG), the increasing clinical prevalence of stereotactic electroencephalography (sEEG) allows for the exploration of traveling waves propagating to deeper and broader areas beyond localized cortex. The present exploratory study

aims to identify and characterize traveling waves in sEEG recordings during a speaking task. sEEG data were collected from 4 native English-speaking participants being monitored as part of treatment for intractable epilepsy at UCSD Health. For the experiment, participants were presented with a sentence displayed on a computer monitor and simultaneously narrated via computer speakers. Immediately following this presentation, participants were prompted to vocalize the sentence. Data were collected for 50 sentences per participant. The 4-second speaking trials were extracted and labeled with 'speaking' and 'non-speaking' intervals using automatic speech transcription software. The trials were analyzed for consistent traveling waves present below 30 Hz along individual sEEG shafts. The detected traveling waves were comprehensively characterized using measures such as location, frequency of occurrence, and propagation speed. The results show distinctive, significant differences in each measure within and across the speaking and non-speaking intervals. In particular, traveling waves were most prominently observed in the beta band and there was a marked difference in the number of traveling waves detected between the speaking and non-speaking intervals. It is envisioned that this work will advance the understanding of information transfer in the brain during speech production and inform the design of future speech neuroprosthetics.

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Poster

475. Neurophysiology: Decoding and Neural Processing III

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 475.17

Topic: E.05. Brain-Machine Interface

Support: UTAP grant 30973783N

Title: Executed and imagined grasping movements can be decoded from lower-dimensional representation of distributed non-motor brain areas.

Authors: *M. C. OTTENHOFF¹, M. VERWOERT¹, S. GOULIS¹, A. J. COLON², L. WAGNER², S. TOUSSEYN², J. P. VAN DIJK², P. L. KUBBEN¹, C. HERFF¹;

¹Univ. Maastricht, Maastricht, Netherlands; ²Kempenhaghe, Heeze, Netherlands

Abstract: Using brain-activity directly as input for assistive tool control can circumvent muscular dysfunction and increase functional independence for physically impaired people. Most invasive motor decoding studies focus on decoding neural signals from the primary motor cortex, which provides a rich but superficial and spatially local signal. Initial non-primary motor cortex decoding endeavors have used distributed recordings to demonstrate decoding of motor activity by grouping electrodes in mesoscale brain regions. While these studies show that there is relevant and decodable movement related information outside the primary motor cortex, these methods are still exclusionary to other mesoscale areas, and do not capture the full informational

content of the sampled parts of the motor system. In this work, we recorded intracranial EEG of 8 epilepsy patients, including all electrode contacts except those contacts adjacent to the central sulcus. We show that executed and imagined movements can be decoded from non-motor areas; combining all non-motor contacts into a lower-dimensional representation provides enough information for a Riemannian decoder to reach an area under the curve of 0.83 ± 0.11 . Additionally, by training our decoder on executed and testing on imagined movements, we demonstrate that there exists shared distributed information in the beta frequency range. By combining relevant information from all areas, the decoder was able to achieve high decoding results without information from the motor cortex. Additionally, the lower dimensional representation also makes the decoder more robust to perturbations, signal non-stationarities and neural tissue degradation. Our results indicate to look beyond the motor cortex and open up the way towards more robust and more versatile brain-computer interfaces.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 476.01

Topic: E.06. Posture and Gait

Support: Clinical and Translational Science Award (CTSA) program, through the NIH National Center for Advancing Translational Sciences (NCATS), grant UL1TR002373
Clinical and Translational Science Award (CTSA) program, through the NIH National Center for Advancing Translational Sciences (NCATS), grant TL1TR002375

Title: Vision and proprioceptive effects on force intersection point in quiet human standing.

Authors: *K. GRUBEN¹, C. GROVE², J. BARTLOFF¹;

¹Univ. of Wisconsin-Madison, Madison, WI; ²Johns Hopkins Univ., Baltimore, MD

Abstract: This study evaluated effects of reduced and disruptive visual stimuli and altered proprioception on sagittal-plane coordination during quiet human standing. Ten human female volunteers (aged 23 to 62 years) 5 with motion sickness and 5 age-matched controls, stood quietly for 30s in various conditions, repeated on four separate days within a 6-week period. The volunteer participants provided informed consent to a protocol approved by the local IRB. Coordination was assessed with the frequency-dependent intersection point analysis which characterizes the emergent property of the ground-on-feet force (F) to exhibit an intersection point (IP) within narrow frequency bands. The height of the IP (zIP) reflects the ratios of muscle

torque at the primary joints and summarizes the complex interaction between the multi-segmental inverted pendulum dynamics and neuromuscular coordination strategies. Three representative frequencies (1, 2.2, 3.4 Hz) were chosen to characterize the zIP dependence on frequency. Previous work showed that sloped surfaces and volitional co-activation alter the zIP in a systematic frequency-dependent manner. Visual and proprioceptive inputs typically contribute to feedback during quiet standing, though the contributions vary across individuals. This study manipulated visual input via reduced complexity and disruptive character. The reduced complexity stimulus displayed a single world-fixed vertical bar of white light directly in front of the individual. The disruptive stimulus was an annulus of dots that filled most of the visual field and spun counterclockwise (30 deg/s) about the visual axis. The stimuli were delivered in a visual void with a virtual reality headset. This study also altered proprioceptive feedback with a foam pad under the feet. F was measured with custom 6-axis force plates. Student's t-tests ($\alpha = 0.05$) with Holm-Bonferroni adjustment assessed the comparisons. Results show that on a firm surface viewing the light bar in the VR headset as compared to viewing the laboratory raised the zIP at the higher two frequencies. Standing on foam, compared to firm, increased zIP at the higher two frequencies when viewing the laboratory. However, with VR, standing on foam decreased zIP at 1 Hz, regardless of visual stimulus. These results provide evidence that the zIP provides a means of discriminating among the effects of visual and proprioceptive influences on the frequency components of coordination that ensure upright posture during quiet standing. Future work is needed to elucidate the mechanisms by which visual and proprioceptive feedback are integrated into this metric of coordination.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 476.02

Topic: E.06. Posture and Gait

Support: NIGMS 5T32GM132498

Title: Associative learning in serial motor decisions

Authors: ***H. E. PARK**¹, P. DIZIO³, J. D. HOWARD²;

¹24 Adams st., 3R, ²Brandeis Univ., Brandeis Univ., Waltham, MA; ³Brandeis University, MS033, Brandeis University, MS033, Bedford, MA

Abstract: Falling is a leading cause of mortality in older adults. Fall prevention research indicates a combination of physiological (e.g., exercise) and higher-level interventions will be most successful for lowering falls (Martins et al., 2021). Higher-level control mechanisms can be studied with a visual inverted pendulum (VIP) balancing tasks. It is not known whether aversive associative learning may attenuate falling in VIP tasks. Learning to balance a VIP requires

corrective manual joystick commands within a critical decision-making window (when the pendulum is moving toward the nearest fall boundary), and falling is aversive because it represents a balancing failure. In an analogous manual self-balancing paradigm, training which attuned subjects to imminent falls improved joystick control (Vimal et al., 2019). Positive feedback is a more effective valence than punishment in retaining learned behavior, while punishment is more effective for faster motor learning (Galea et al., 2015). Therefore, this study aimed to augment the learning of the position-velocity state which defines the danger zone for imminent falls (an outcome with intrinsically negative valence) with an aversive odor (punishment that has negative valence). Healthy young adults (18 to 30) were recruited and divided into two groups (n=3 per group, data collection ongoing). The Experimental Group (EG) received an aversive odor when they failed to maintain the VIP balancing task. The Control Group (CG) received the same odor but uncoupled to the VIP balance state, thus controlling for arousal level related to odor stimulation while preventing the possibility of an association. Preliminary analysis revealed an association between the number of VIP falls and pleasantness ratings of the aversive odor. Although CG had a significantly smaller number of falls than EG ($t=4.44$, $p<.001$), EG had a significantly lower rating score for the pleasantness of the odor than CG ($t=-3.01$, $p=0.004$). Further, EG had a high negative correlation between odor pleasantness and falls ($r=-0.99$, $p=0.017$), whereas CG did not show a significant correlation ($r=-0.34$, $p=0.78$). The effect of learning over trials was marginal ($p=.09$). These preliminary findings suggest that the EG underwent learning from punishment, such imminent falls in the VIP task become associated with the aversive stimuli. Constraints on the feasible rate and amount of odor could have been responsible for the marginal learning in EG. More rapidly deployable aversive stimuli could enhance learning and transfer to different balancing tasks, such as bipedal self-balancing, and interventions to prevent falls in vulnerable populations.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 476.03

Topic: E.06. Posture and Gait

Title: Effects of hot and cold cognitive loads on the control of locomotion with a neuromuscular impairment

Authors: *J. MANCZUROWSKY¹, T. L. CLINE², C. H. HILLMAN², C. J. HASSON¹;
¹Dept. of Physical Therapy, Movement and Rehabil. Sci., ²Dept. of Psychology, Northeastern Univ., Boston, MA

Abstract: Many locomotor adaptation studies are performed under cognitively sterile conditions, yet rehabilitation may be accompanied by significant mental and emotional challenges. Real world locomotion requires executive function (EF) to navigate environments and fluidly respond

to changing circumstances. EF can be organized on a cold-hot axis according to the logical (cold) and emotional (hot) content. Despite the importance of EF for real-world ambulation, it is unclear how locomotor control is affected by cold and hot cognitive loads, and vice versa. To gain an understanding, we asked participants to walk on a motorized treadmill while experiencing an artificial impairment. This impairment was elicited with dysfunctional electrical stimulation (DFES), which produced an uncomfortable (nociceptive) disruption in muscular coordination (discoordination) by involuntarily activating the right hamstrings during the early swing phase of walking. We hypothesized that 1) DFES-induced discoordination and nociception impair locomotor control, and 2) discoordination and nociception exert a differential cognitive loading effect, with discoordination taxing the cold end of the EF spectrum and nociception taxing the hot end. DFES cognitive loads were amplified with added flanker tasks during impaired locomotion. A cold flanker required correct identification with button presses of a centrally presented letter flanked by distractor letters, and a hot flanker replaced the letters with affective faces. No stimulation and SHAM (only a nociceptive stimulus) conditions were also included. Locomotor control was quantified by the step length standard deviation (SL variability; an increase is operationalized as worse locomotor control), and EF was quantified by reaction time (RT; flanker onset to button press). Here, we present early results with one male and one female as exemplary cases. As hypothesized, DFES and SHAM increased SL variability (0.15-0.57 cm), but SL variability did not differ between DFES and SHAM. Changes in flanker RT from the neuromotor perturbations and added cognitive load (cold/hot) depended on participant sex. The female had longer RTs with DFES (5-15 ms) but shorter RTs during SHAM with hot flankers (20-45 ms). Conversely, the male had a very large increase in RT, but only for hot EFs (DFES = 200-400 ms; SHAM = 230-275 ms). These early results support DFES as an approach for temporarily disrupting locomotor control. It may be prudent to consider participant sex as a factor mediating the degree to which emotionally laden stimuli affect executive function during locomotion, although caution is warranted given the preliminary nature of these findings.

Disclosures: J. Manczurowsky: None. T.L. Cline: None. C.H. Hillman: None. C.J. Hasson: None.

Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 476.04

Topic: E.06. Posture and Gait

Support: CP-PPRAI1/2-02

Title: Early decoding of walking tasks with minimal set of EMG channels

Authors: F. BARBERI, F. IBERITE, *E. ANSELMINO, A. MAZZONI, S. MICERA;
Scuola Superiore Sant'Anna, Scuola Superiore Sant'Anna, Pisa, Italy

Abstract: Even with the state-of-the-art lower-limb prostheses, the majority of lower-limb amputees present a less energy-efficient and stable gait compared to able-bodied individuals. These deficits emerge in particular during the execution of challenging tasks. Most prosthetic legs are not able to provide net-positive mechanical energy, which contrasts with the capabilities of a non-damaged human neuromuscular system and can lead to the modification of the gait pattern. Powered lower-limb prostheses, instead, can actively provide mechanical energy, improving the quality of life of amputee subjects. Powered prostheses usually rely on decoding motor intentions from non-invasive sensors, like electromyographic (EMG) signals, to adapt the prosthesis conformation to the desired gait pattern. The decoding procedure benefits from an increase in the EMG sensors set, but this might be discomfortable for the patients: the optimal combination of high decoding performance and minimal set-up burden is yet to be determined. Here we propose an efficient decoding approach obtaining top-level decoding performance by observing only a fraction of the gait duration with a limited number of recording sites. Eleven transfemoral amputee subjects performed five motor tasks (ground level walking, stairs ascending and descending, ramp ascending and descending) while recording EMG signals from four muscles (Biceps Femoris, Tensor Fasciae Latae, Rectus Femoris and Adductor) and inertial signals from the prosthesis. A Support-Vector-Machine-based algorithm was trained to decode motor intention over subwindows of the gait cycle. We investigated the trade-off between the robustness of the classifier's performances and the minimization of i) the duration of the observation window, ii) the number of EMG recording sites, and iii) the computational load of the procedure. Our results demonstrate how including pre-foot-strike data into the decoding leads to classification accuracies above 94% at the 20% of the gait cycle, using a combination of three EMG recording sites and the inertial signals, which showed to be the best trade-off between the invasiveness of the setup and performances of the classifier. The complexity of the algorithm proved to be significantly higher when applying a polynomial kernel compared to a linear one (KWTK, $p < 0.05$), while the performances of the classifier generally showed no differences (KWTK, $p > 0.5$). The proposed algorithm led to high performance with a minimal EMG set-up and using only a fraction of the gait duration, paving the way for efficient control of powered lower-limb prostheses with minimal set-up burden and a rapid classification output.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

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Topic: E.06. Posture and Gait

Support: Gatsby charitable foundation GAT3755
Wellcome 219627/Z/19/Z

Title: Motor adaptation and anticipatory postural adjustments

Authors: *H. J. MORLEY, A. J. MURRAY;
Sainsbury Wellcome Ctr., Univ. Col. London, London, United Kingdom

Abstract: Transitioning between different voluntary movements has the potential to destabilise balance and posture through disturbance of the body's centre of mass. To prevent this the nervous system makes pre-emptive adjustments to body posture prior to limb movements, termed anticipatory postural adjustments (APAs). These APAs are a form of motor adaptation. Through experience the nervous system learns and updates how destabilising upcoming movements will disrupt postural equilibrium and adjusts the body posture accordingly. However, it is not clear how APAs are adapted during dynamic movements and we have little understanding of the neural circuitry that underlies these adjustments. Inspired by visuomotor transformation experiments in humans, we have developed a novel dual-belt traveller paradigm to induce adaptive APAs in freely moving mice. This behavioural assay allows a mismatch to be created between the expected (visual cue) and actual (belt) speeds of two moving belts as mice locomote between them, giving us the ability to flexibly manipulate the postural requirements of the task within the same experimental context. This provides an exciting avenue to probe the neural circuits involved in performing APAs.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

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Program #/Poster #: 476.06

Topic: E.06. Posture and Gait

Support: NSF Grant 17-572-1815506

Title: Toe clearance over obstacles when locomoting in natural and virtual environments

Authors: *A. H. MASON, A. S. PADILLA, A. PEER, M. TOEPFER, K. PONTO, K. A. PICKETT;
Univ. of Wisconsin - Madison, Madison, WI

Abstract: Virtual Reality (VR) systems have been touted as promising technologies for training, rehabilitation and assessment, particularly of essential motor skills such as locomotion. Because obstacle negotiation is an important component of natural locomotion, understanding how individuals navigate obstacles in VR is important if we are to make effective use of this technology. One important difference between motor performance in natural and immersive virtual environments relates to how the body is seen by the performer. Visual feedback about the body's position can range from no representation, to stick and ball figures, to full body avatars. In many previous studies quantifying locomotor behavior in immersive VR, no representation of the lower limbs has been available. We were interested in quantifying how absence of visual feedback about the lower limbs influences toe clearance over an obstacle in natural and virtual

environments. **Method:** Eleven young adults (mean age = 22.2 years) were asked to walk 20m in three different Visual Conditions: (i) natural environment/normal vision (ii) natural environment/occluded lower body; (iii) immersive virtual environment/no representation of lower body. As participants walked, they were also asked to step over an obstacle (4" X 6" X 9") that was positioned at 25%, 50% or 75% of the walkway length. A 3x3 repeated measures ANOVA was used to examine main effects and interactions of Visual Condition and Obstacle Location on toe clearance height over the obstacle (measured via video). **Results:** Main effects of Visual Condition ($F_{2,20}=9.91$, $p=0.001$, $\eta^2=0.498$) and Obstacle Location ($F_{2,20}=3.86$, $p=0.038$, $\eta^2=0.278$) were found for toe clearance height. Specifically, toe clearance was larger when visual feedback of the lower body was occluded in the natural environment (0.139 ± 0.010 m) than when participants walked with full vision in the natural environment (0.117 ± 0.006 m). Additionally, toe clearance was larger in the virtual environment condition (0.148 ± 0.011 m) than in both natural environment conditions. Participants also used a larger toe clearance height at the 75% obstacle location (0.139 ± 0.009 m) than at the 25% obstacle location (0.129 ± 0.008 m). The 50% obstacle location did not result in significantly different toe clearance heights (0.136 ± 0.007 m) than the other two locations. **Conclusion:** The results of the current study suggest that not being able to see the lower limbs influences obstacle clearance height in both natural and virtual environments. Our results also suggest that a lack of body representation is only partly responsible for differences in performance between natural environments and VR.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

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Program #/Poster #: 476.07

Topic: E.06. Posture and Gait

Support: NSF Grant 1933751
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Title: Neural population dynamics in the hindlimb and trunk motor cortex encode for postural corrective movements in rats

Authors: *G. DISSE¹, B. NANDAKUMAR², K. A. MOXON¹;

¹Biomed. Engin., Univ. of California Davis, Davis, CA; ²Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: Adaptive postural control is critical for independent movements. Brain-machine interface (BMI) technologies are an exciting approach to restore voluntary motor function in individuals with spinal cord injury; however, little work has focused on restoring lower limb

movements and trunk stabilization necessary for postural control. Given previous work demonstrating that the hindlimb motor cortex (HLM1) modulates its response to postural perturbations, we hypothesized that critical postural information can be decoded from these signals and across a larger cortical area (e.g., the trunk representation in M1, or TrM1). Using a linear dynamical systems approach, we characterized the combined TrM1 and HLM1 population response to unanticipated postural perturbations in the rat and determined how well this signal can be used to decode the limbs' corrective behaviors. We implanted rats chronically in one hemisphere with 32-channel microwire arrays centered on TrM1 and HLM1 and recorded isolated single neuron responses while the rats were tilted in the lateral plane in each direction at different speeds. The tilt platform was embedded with force and torque sensors to measure changes in center of pressure (CoP). Using preferential subspace identification (PSID), we observed that a three-dimensional model of behaviorally-relevant latent population activity was able to reproduce ground reaction forces in response to perturbations on a single-trial basis. Plotting of the population activity in two-dimensional latent space revealed specific rotational dynamics in line with other motor tasks. For a given tilt direction, the population traversed the same state space for fast and slow tilts, suggesting that the cortex performs similar computations at different time scales. The shape of these trajectories was supported by unique individual neural firing patterns at different points in the state space. Further evaluation of the model demonstrated that each extracted latent variable heavily weighted a different aspect of the mediolateral shifts in center of pressure. Further work will be needed to determine both if spinal cord injuries alter these population dynamics and the extent to which ground reaction forces can be reproduced based on recorded neural activity.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

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Program #/Poster #: 476.08

Topic: E.06. Posture and Gait

Support: American Heart Association (Postdoctoral Fellowship 20POST34990005, SAH)

Title: Prefrontal activation during inhibition of a balance recovery step

Authors: E. ABUGU, S. A. HARPER, Y. KIM, *D. A. E. BOLTON;
Kinesiology and Hlth. Sci., Utah State Univ., Logan, UT

Abstract: The ability to quickly step is an important strategy to avoid a fall. However, real-world settings often constrain a stepping path. Such constraints necessitate response inhibition to prevent an inappropriate step and select a new course of action to ultimately recover balance. The present study investigated neural mechanisms that underlie this ability to stop a highly automatic balance recovery step. In the field of cognitive neuroscience, response inhibition has

typically been researched using focal hand reaction tasks performed by seated participants. This approach combined with neuroimaging has revealed a neural stopping network, which includes the right Inferior Frontal Gyrus (rIFG) as a key node in this network. It's unclear if the same brain-based stopping networks suppress a prepotent balance reaction since compensatory balance reactions are subcortically triggered, multi-segmental responses that are much faster than voluntary reactions. To test this, we used functional near-infrared spectroscopy (fNIRS) to measure brain activity in 21 young adults (ages 18-30) as they performed a balance recovery task that demanded rapid step suppression following postural perturbation. We hypothesized that the rIFG would show heightened activity when suppressing an automatic balance recovery step. A lean-and-release system was used to impose temporally unpredictable forward perturbations by releasing participants from a supported forward lean. For most trials (80%), participants were told to recover balance by quickly stepping forward. However, on 20% of trials at random, a high-pitch tone was played immediately after postural perturbation signaling participants to suppress a step and fully relax into a catch harness. This allowed us to target the ability to cancel an already initiated step in a balance recovery context. Average oxygenated hemoglobin changes were contrasted between step and stop trials, 2-6 seconds post perturbation. Our results revealed that 8 of 10 fNIRS channels covering the rIFG had significantly greater activation when stopping (paired t-tests, $p < 0.001$). Consistent with our hypothesis, the results showed a greater prefrontal response during stopping trials, supporting the idea that executive brain networks are active when suppressing a balance recovery step. This study demonstrates one way in which higher brain processes may help us prevent falls in complex environments where behavioral flexibility is necessary. This study also presents a novel method for assessing response inhibition in an upright postural context where rapid stepping reactions are required.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

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Topic: E.06. Posture and Gait

Support: NIH R37-NS090610 to AJ Bastian.
American Heart Association predoctoral fellowship to C Rossi

Title: Why does locomotor adaptation generalize only partially to overground walking?

Authors: *C. ROSSI, R. T. ROEMMICH, A. J. BASTIAN;
Johns Hopkins, Johns Hopkins Univ., BALTIMORE, MD

Abstract: Adaptation is an error driven learning process that recalibrates movement in response to predictable changes in the environment. For example, when people walk on a split belt treadmill, they learn a new gait pattern that accounts for the belt speed difference. Perception of

belt speed also recalibrates such that people do not perceive the full split after learning. Motor and perceptual recalibrations are not 1:1; motor changes are larger. Thus, at least two mechanisms are operating, one purely motor and one linked to perceptual changes. After adaptation, people exhibit both motor and perceptual aftereffects when the belt speeds are equal: they take *unequal* steps and perceive the belt speeds as *unequal*.

Previous work has shown that adaptation generalizes partially to overground walking. Motor aftereffects are smaller during overground walking and are reduced upon return to the treadmill. Here we ask why adaptation is partially generalizable and if *perceptual* aftereffects generalize to overground walking. We tested two hypotheses regarding limited generalization: 1) differential generalizability of different locomotor learning mechanisms or 2) delayed switching between context-specific locomotor memories.

In Experiment 1, we tested for generalization of motor and perceptual aftereffects from treadmill to overground walking. We hypothesized that the generalizable portion of adaptation may coincide with the learning mechanism that changes both movement and perception. Instead, we found partial generalization of perceptual aftereffects: people perceived their leg speeds as unequal overground, but they still exhibited reduced perceptual aftereffects upon return to the treadmill. This result was consistent regardless of the duration or walking speed of the overground washout. This indicates that the generalizable portion of adaptation is not accounted for by the mechanism that also alters perception.

In Experiment 2, we tested whether people store separate locomotor memories for treadmill vs overground, but are slow at switching between two. Subjects adapted on the treadmill, fully washed out overground, and then alternated between bouts of treadmill and overground walking. We hypothesized that slow switching may result in reemergence of overground aftereffects and continued decay of treadmill aftereffects with each alternation. Instead, we found no evidence of reemergence or decay of aftereffects. Taken together, our results suggest that limited generalization is explained by a portion of the locomotor memory being shared between treadmill and overground. Yet, this portion is not 1:1 with the mechanism that involves perceptual recalibration.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 476.10

Topic: E.06. Posture and Gait

Title: The power of balance: Task-specific neural adaptations in response to balance skill learning

Authors: *L. BAKKER¹, A. ABUD DA SILVA COSTA², M. P. VELDMAN¹, C. LAMOTH¹, T. HORTOBAGYI¹;

¹Univ. Med. Ctr. Groningen, Univ. of Groningen, Groningen, Netherlands; ²Escola de Educação Física e Esporte de Ribeirão Preto, Univ. de São Paulo, Ribeirão Preto, Brazil

Abstract: Background and aims: Balance training interventions (BAL) induce rapid but task-specific improvements in balance skill performance. Adaptations in alpha power derived from electroencephalographic (EEG) oscillatory signals correlate with manual skill learning. Whether these adaptations in balance performance are accompanied by task-specific neural adaptations remains unclear. The aims of this study were to determine 1) the effects of a 20-minute BAL on balance performance assessed in a trained and an untrained balance task and 2) to examine if the adaptations in balance performance are accompanied by task-specific neural adaptations. We hypothesize that balance skill learning increases balance performance in the trained but not the untrained balance task that is accompanied by reductions in task-related alpha power during performance of the trained but not the untrained balance task. **Methods:** Twenty-four healthy younger adults (12 males, age: 23 y) were randomly assigned to BAL on an unstable board or to the control intervention of seated rest (CON). Balance performance was assessed by determining the standing time in balance on an unstable balance board and by the center of pressure velocity while standing on foam before and immediately after the intervention session. During the performance of the balance tasks, EEG was recorded. After pre-processing the EEG data, task-related alpha power in electrodes representing the motor cortex was calculated as a contrast between power during the balance tasks relative to power during quiet standing. **Results:** An exploratory analysis revealed that BAL increased time in balance on the unstable board by 38% ($p < 0.05$, Cohen's effect size $d = 0.83$) without change in CON (8%; n.s.). Performance in the untrained balance transfer task did not change in either group (BAL: +0.1%, $p > 0.05$, $d = 0.20$; CON: +0.0%, $p > 0.05$, $d = -0.08$). The adaptations in balance performance were accompanied by reductions in task-related power measured during the trained task but not during the transfer task. However, these reductions did not reach significance in this initial data set (all $p > 0.05$, $d = -0.06$ -0.28). **Conclusions:** BT improved performance in the trained balance skill. As hypothesized, these changes were accompanied by reductions in task-related alpha power. However, there was no transfer of the acquired dynamic balance skill to the performance in the untrained balance task, i.e., sway while standing on foam. The task-related alpha power measured in this transfer task also did not change. A better understanding of task-specificity of learning and transferring a balance skill and its neural correlates requires additional studies.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 476.11

Topic: E.06. Posture and Gait

Title: Spatial navigation in projected virtual reality reduces human frontoparietal electrocortical spectral power

Authors: *Y. CHENG¹, A. D. NORDIN^{1,2,3};

¹Texas A&M Inst. for Neurosci., College Station, TX; ²Dept. of Hlth. and Kinesiology, ³Dept. of Biomed. Engin., Texas A&M Univ., College Station, TX

Abstract: While navigating environmental terrain on foot, the human cerebral cortex processes multisensory information during gait. Elocortical spectral power measured from mobile high-density electroencephalography (EEG) provides insight into the neural dynamics of natural human behaviors. Measuring human electrical brain activity during locomotion has uncovered spectral power fluctuations in alpha (8-13) and beta bands (13-30Hz) from the sensorimotor cortex that reduce at faster gait speeds, and event-related spectral power synchronizations and desynchronizations tied to environmental stimuli. Treadmill locomotion is a common form of exercise often used during rehabilitation, but navigating real-world environments requires additional visual processing to navigate complex terrain. To study human electrocortical spectral power dynamics during treadmill locomotion, we used a force-measuring treadmill, motion capture system, and high-density mobile EEG during gait at a range of speeds (0.6 m/s, 0.8 m/s, 1.0 m/s), with and without visual spatial navigation in a projected virtual reality environment. To analyze our EEG data, we filtered and cleaned the raw data to remove motion, eye, and muscle artifacts and decomposed the EEG channel data into independent components that we fit to a human brain model as equivalent current dipoles using EEGLAB. We performed spectral power comparisons among conditions and evaluated time-frequency spectral power fluctuations throughout the gait cycle using stepping events detected from the force-measuring treadmill. Congruent with previous results, alpha and beta band sensorimotor electrocortical spectral power fluctuated with the step sequence, increasing during stance and decreasing during contralateral limb swing, and decreased at faster gait speeds. Compared to walking on a treadmill without a projected virtual reality environment, visual spatial navigation within a simulated nature scene significantly reduced alpha, beta, and low gamma (30-50 Hz) spectral power from frontal and parietal cortical regions. Reduced electrocortical spectral power identifies increased cortical processing that is influenced by gait speed and visual spatial navigation within specific cortical networks. Although lateralized sensorimotor processing is tied to the step sequence, reduced frontoparietal electrocortical spectral power uniquely occurred during spatial navigation in projected virtual reality. These results show that it is possible to identify changes in sensory processing using mobile high-density EEG, uncovering human frontoparietal network dynamics during locomotor spatial navigation.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

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Program #/Poster #: 476.12

Topic: E.06. Posture and Gait

Support: R01 HD091184

Title: Gait asymmetry does not impair reactive balance control during split-belt walking in neurotypical older adults

Authors: *T. CORNWELL¹, R. NOVOTNY², J. M. FINLEY³;

¹Dept. of Biomed. Engin., ²Neurosci. Grad. Program, ³Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA

Abstract: Motor control theories propose that people adapt their gait to reduce sensory prediction errors, reduce energetic cost, and/or increase stability^{1,2}. The adaptive control of walking is often studied on a split-belt treadmill, where people reduce their step length asymmetry (SLA) over time. Here, we tested the hypothesis that the changes in SLA observed during split-belt adaptation lead to improvements in reactive balance control. Our sample consisted of older adults due to the increasing importance of balance control in this population. Sixteen neurotypical adults (70.0 ± 5.3 years old; 6 males) walked on an instrumented treadmill during five randomized SLA target conditions. SLA targets were 0%, ±5%, and ±10%, where $SLA = 100\% * (Left\ SL - Right\ SL) / (Left\ SL + Right\ SL)$. Participants wore reflective markers for 3D motion capture, and the left and right belts moved at 1 and 0.5 m/s, respectively. We provided real-time visual feedback of achieved and target step lengths, as well as intermittent single-belt accelerations to trigger reactive balance control strategies (10 trips/side). To quantify measures of reactive control, we computed the minimum margin of stability (MOS) and whole-body angular momentum (L) in the sagittal plane during the perturbation and recovery steps (the subsequent contralateral step). We expected that more symmetric gait patterns would be associated with increases in the MOS and reductions in peak forward angular momentum. We fit separate linear mixed-effects models to test for differences in perturbation and recovery step MOS and L across levels of achieved SLA (0%, ±5%, ±10%) and to determine if any differences varied with the perturbation side (fast belt, slow belt).

Our results demonstrated that SLA was not associated with minimum MOS or L during the perturbation step (p=0.938 and p=0.520, respectively) or recovery step (p=0.754 and p=0.400, respectively). The interaction effects were also not significant (p>0.400). Overall, older neurotypical adults successfully used biofeedback to vary their gait asymmetry during split-belt walking, but doing so did not affect their reactive control of balance. Taken with recent work^{3,4}, our findings suggest that split-belt adaptation is not driven by stability maximization but may instead result from a combination of minimizing effort and sensory prediction errors.

References: ¹Reisman, D. et al. 2005. *J Neurophysiol*. ²Finley, J. et al. 2013. *J Physiol*.

³Roemmich and Bastian 2018. *Annu Rev Neurosci*. ⁴Sánchez, N. et al. 2019. *J Physio*.

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Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

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Topic: E.06. Posture and Gait

Support: McPherson ERI: Kenzie Valentyn Grant

Title: The Effects of Dual-Task Interference on Gait and Visual Attention in Young Adults

Authors: *R. W. NELSON¹, K. A. PICKETT², B. G. TRAVERS³, A. H. MASON⁴;

¹Univ. of Wisconsin, Madison, Madison, WI; ²Kinesiology, Univ. of Wisconsin, Madison, WI;

³Kinesiology, Univ. of Wisconsin-Madison, Madison, WI; ⁴Kinesiology, Univ. of Wisconsin - Madison, Madison, WI

Abstract: Introduction: Previous studies have suggested that children and adults experience significant motor performance decline when simultaneously performing two motor tasks, such as walking while carrying an object. This phenomenon is known as dual-task interference. While several studies have measured motor declines during dual-tasks, the role of visual attention on performance is not well understood. We conducted the current study to measure the effects of dual-task interference in four different walking and bimanual carrying tasks, ranging from simple to complex. We wanted to determine how parameters of gait and visual attention were impacted by the increased complexity of the secondary task. **Methods:** Sixteen healthy young adults (10 male, $M = 24.55 \pm 1.2$ years) participated in the study. Participants were fitted with a Pupil Core GNU eye-tracking headset and were asked to walk across a Zeno Walkway™ (4.87m) while performing ten trials in each of four motor tasks: A) simple, overground walking (baseline), B) walking while carrying an empty tray, C) walking while carrying a tray with unstacked wooden blocks, and D) walking while carrying a tray with stacked wooden blocks. We quantified spatiotemporal gait parameters and the number and duration (ms) of visual fixations per trial. The locations of each fixation (tray/blocks, floor, wall, starting light, or other) were determined during posthoc analysis. **Results:** Gait and visual attention were both affected by increased task complexity. Significant gait differences ($p < 0.05$) in step length (cm) occurred between conditions A and D ($M_A = 66.45$, $M_D = 57.81$) and conditions B and D ($M_B = 64.66$). Additionally, significant differences ($p < 0.01$) were found in velocity (cm/s) between condition D ($M_D = 100.70$) and all other conditions ($M_A = 120.99$, $M_B = 117.38$, $M_C = 114.43$). For visual activity, the overall number of fixations in condition D ($M_D = 433.8$), as well as the total number of fixations on the tray/blocks ($M_D = 269.31$), were significantly higher ($p < 0.01$) than in all other conditions (overall: $M_A = 336.4$, $M_B = 341.8$, $M_C = 363.0$, tray/blocks: $M_B = 40.44$, $M_C = 92.44$). Moreover, the duration of fixations (ms) in condition D was significantly longer ($p < 0.01$) both overall ($M_D = 161.32$) and on the tray/blocks ($M_D = 150.8$) than in all other conditions (overall: $M_A = 136.35$, $M_B = 147.67$, $M_C = 148.39$, tray/blocks: $M_B = 132.8$, $M_C = 130$). **Conclusions:** Increasing the complexity of a secondary task performed while walking results in slower, more conservative gait and more visual attention toward the secondary task. These findings directly address attention allocation in complex motor tasks and may help us to better understand neural processing.

Disclosures: R.W. Nelson: None. K.A. Pickett: None. B.G. Travers: None. A.H. Mason: None.

Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 476.14

Topic: E.06. Posture and Gait

Support: NSF Award 2001222

Title: Increased intramuscular coherence in the tibialis anterior during dual-task split-belt walking

Authors: *M. E. MULVEY¹, S. D. SATO², J. T. CHOI¹;

¹Univ. of Florida, ²Univ. of Florida, Gainesville, FL

Abstract: Introduction: Walking patterns must be constantly adapted in the environment and often times with a concurrent dual task that requires prefrontal cortical activity (i.e., talking on the phone), which increases fall risk in older adults. During split-belt treadmill walking, corticospinal drive to leg muscles is increased, but the influence of a dual task on the role of corticospinal drive during walking adaptation is unknown. Here we examined changes in beta-band intramuscular coherence in the tibialis anterior (TA), a marker of corticospinal drive, during dual-task split-belt adaptation. **Hypothesis:** Coherence in the beta band (12-30 Hz) of the slow leg would decrease during dual-task compared to single-task walking, reflecting decreased corticospinal drive with less voluntary (more automatic) control. **Methods:** Participants (N=10, 21 ± 4.62 years) walked in two conditions: single- and dual-task. The paradigm for both conditions included three phases: 1) baseline tied-belt walking at 0.5 m/s, 1.0 m/s, and 0.5 m/s for 5 minutes each; 2) three 5-minute adaptation phases with a 1:2 split-belt walking speed ratio where the dominant leg moved at twice the speed as the non-dominant leg; 3) three 5-minute de-adaptation phases with tied-belts at 0.5 m/s, 1.0 m/s, and 0.5 m/s respectively. In the dual-task condition, participants were asked to count backwards by seven from a random number as quickly and as accurately as possible concurrently with the walking paradigm. Electromyography was recorded bilaterally from the proximal and distal muscle bellies of the TA with surface electrodes. Coherence for each 5-minute phase was calculated from the proximal and distal TA signals during the early swing phase of gait (0-400ms after toe-off). The natural logarithm of the summed coherence in the beta band was used for statistical analysis. **Results:** There was a significant condition x phase interaction (p=0.010). The increase of beta-band coherence in the slow TA during adaptation compared to baseline was prolonged with the dual-task condition. However, there were no differences in beta-band coherence in the slow TA between conditions for the baseline (p>0.05) or the de-adaptation phases (p>0.05). **Conclusion:** Beta-band TA coherence is increased with a cognitively demanding dual task, specifically during the novel split-belt walking task (but not during tied-belt walking). This may suggest that a dual task could potentially be used to train an increase TA coherence during walking adaptation tasks.

Disclosures: M.E. Mulvey: None. S.D. Sato: None. J.T. Choi: None.

Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 476.15

Topic: E.06. Posture and Gait

Support: UF University Scholars Program award

Title: Validation of Rover Walk for remote gait measurements

Authors: A. N. CHERTOK¹, *J. T. CHOI²;

²Univ. of Florida, ¹Univ. of Florida, Gainesville, FL

Abstract: Aging individuals often experience changes in gait parameters which are too subtle to be noticed through observation alone. Routine tracking and analysis of gait parameters can be useful for detecting abnormalities and assessing fall risk. Currently, regular fall risk assessment may require visits to healthcare facilities and can be time-consuming and expensive, or inaccessible. Rover Walk, a gait analysis and fall risk assessment system worn on the ankle, aims to deliver precise and continuous gait parameter data through an app outside of clinical settings. While existing systems with wearable accelerometers have used various algorithms to detect fall risk, Rover claims to be the first Remote Therapeutic Monitoring (RTM) system that specifically collects data on gait and balance. However, there is a lack of research comparing data collected by Rover with 3D motion capture, the gold standard for lower limb kinematics data collection. The purpose of this study is to validate gait parameters collected from Rover by comparing them to lower limb kinematics data recorded at 100 Hz using a 9-camera Miquis system. A total of ten healthy subjects participated in the study: five older participants (4 male, age 76 ± 3.29 years) and five younger participants (2 male, age 26.6 ± 9.6 years). Kinematics and Rover data were both collected as subjects walked on a split-belt treadmill under various conditions: subjects walked at speeds of 0.6 m/s, 1.2 m/s, at preferred walking speed (between 0.6 - 1.3 m/s), and under split-belt conditions (left = 0.6 m/s, right = 1.2 m/s). The variety of treadmill speeds and conditions and the varied ages of subjects served to investigate the accuracy of the system for a wide range of potential Rover users and to ensure scientific rigor and reproducibility. Kinematic data were low-pass filtered using MATLAB, and heel-strike and toe-off events (defined at the time at which vertical ground reaction force crossed a threshold of 8% of the subject's body weight) were reviewed and manually corrected when necessary. Rover demonstrated an excellent level of correlation (Pearson's $R > 0.94$) when measuring cadence, gait cycle time, and stance time, and a high level of correlation (Pearson's $R = 0.86$) for stride length. However, Rover demonstrated poor accuracy (Pearson's $R < 0.2$) for swing time and swing velocity. These results are novel because the Rover system has not been directly compared with 3D motion capture data; while its limitations must be recognized before expanding its use, an accurate remote gait analysis system has the potential to improve outcomes for at-risk populations and make healthcare more accessible.

Disclosures: A.N. Chertok: None. J.T. Choi: None.

Poster

476. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 476.16

Topic: E.06. Posture and Gait

Support: NSF Award 2001222

Title: Changes in leg speed perception following visuomotor gait adaptation

Authors: *B. ROBERTS¹, J. T. CHOI²;

²Dept. of Applied Physiol. and Kinesiology, ¹Univ. of Florida, Gainesville, FL

Abstract: Accurate sensory processing is critical for walking, especially in complex environment. The nervous system can recalibrate sensory estimates in the presence of sensorimotor errors. Perceived leg speed (measured using a speed matching task) has been shown to be altered following split-belt walking adaptation, where one belt moves unexpectedly faster than the other. It is unclear whether this type of perceptual change is dependent on the speed difference experience by the two legs, or other factors such as the asymmetric joint kinematics induced by the split-belt perturbation. Here we determined changes in leg speed perception following a visuomotor walking task that leads to asymmetry in spatiotemporal gait parameters without changing the treadmill speed. Healthy young adults (n=12; 3 male; ages 20 ± 1.1 yrs) participated in the study. A screen in front of treadmill displayed stepping targets and continuous feedback of the foot position during walking. Subjects were instructed to hit the targets by changing step length. During adaptation, the screen-to-treadmill ratio (visuomotor gain) for the right leg was reduced from 1 to 0.9 which induced stepping errors that was gradually reduced with visuomotor adaptation. Pre- and post-adaptation leg speed perception threshold were determined using an adaptive staircase algorithm that assessed point of subjective equality (PSE, defined as the speed with 50% response accuracy). Participant's responses to "Is the right leg faster than the left?" were used to compute the expected entropy for a given probe value, and the probe that was expected to minimize overall expected entropy was set as the next right belt speed. PSE was equal to the mean of the posterior probability distribution after 10 probes. This was repeated with the assumed minimum expected entropy set at the last PSE, and the mean was reported as participants' perceptual threshold. Participants adapted to the visuomotor task with longer right step lengths (p = 0.001), step times (p = 0.001), and increased PSE by 0.063 m/s (p = 0.031). During late adaptation, peak right limb extension, hip extension, knee flexion, and ankle extension angles were lower compared to left peak joint angles and, except hip extension, occurred ~4% earlier in the gait cycle. In conclusion, adapting to a visuomotor task with lower right leg gain induced significant adaptations to step length, time, peak joint angle magnitude, peak joint angle timing, and speed perception despite participants having never experienced changes to treadmill belt speed.

Disclosures: B. Roberts: None. J.T. Choi: None.

Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.01

Topic: E.09. Motor Neurons and Muscle

Title: Structural plasticity of presynaptic voltage-gated calcium channel nanoclusters

Authors: *F. KOMMA¹, S. DANNHÄUSER¹, M. M. PAUL^{1,3}, M. PAULI¹, P. KOLLMANNBERGER², A.-L. SIRÉN^{1,4}, M. HECKMANN¹, A. MRESTANI^{1,5};

¹Inst. for Physiology, Dept. of Neurophysiol., ²Ctr. for Computat. and Theoretical Biol., Julius Maximilian Univ. of Würzburg, Würzburg, Germany; ³Dept. of Orthopaedic Trauma, Hand, Plastic and Reconstructive Surgery, ⁴Dept. of Neurosurg., Univ. Hosp. of Würzburg, Würzburg, Germany; ⁵Dept. of Neurol., Leipzig Univ. Med. Ctr., Leipzig, Germany

Abstract: Active zones (AZs) are specialized sites of the presynaptic terminal where vesicle fusion takes place. Voltage-gated calcium channels (VGCCs) are assumed to reside in the center of the AZs at the neuromuscular junction (NMJ) of *Drosophila melanogaster* [1, 2] and to be essential for evoked transmitter release. Their absolute numbers within individual AZs and their distribution within the AZ (i.e. clustered versus homogenous) remain to be determined. To further investigate these questions, we performed two-channel *direct* stochastic optical reconstruction microscopy (*d*STORM) of Ib boutons at the *Drosophila melanogaster* NMJ of muscles 6/7 in segments A2 and A3 of male third instar larvae in combination with hierarchical density-based spatial clustering of applications with noise (HDBSCAN). We co-stained the AZ scaffold protein Bruchpilot and the sfGFP-tagged VGCC cacophony (Cac) [3], using primary IgG antibodies and fluorophore-coupled secondary F(ab)₂ fragments. Our analysis revealed a median of 8 Cac subclusters per AZ that were not clustered at the AZ center, but instead formed a circular structure with a median distance of about 100 nm to the AZ centers (n = 565 AZs from 12 NMJs of 6 animals). These results suggest a new structural model for the nanotopology of VGCCs at the AZ. Furthermore, Presynaptic Homeostatic potentiation (PHP) might cause a rearrangement of VGCCs. There are two forms of PHP: Acute PHP can be induced pharmacologically and chronic PHP genetically. For both of these PHP forms an increase in Cac signal intensity was reported on confocal resolution level [3, 4]. Our experimental setup will permit imaging of PHP-induced changes at the sub-AZ level to resolve the structural dynamics of individual Cac subclusters.

References 1. Newman, Z.L., et al., *Determinants of synapse diversity revealed by super-resolution quantal transmission and active zone imaging*. Nat Commun, 2022. **13**(1): p. 229. 2. Fouquet, W., et al., *Maturation of active zone assembly by Drosophila Bruchpilot*. J Cell Biol, 2009. **186**(1): p. 129-45. 3. Gratz, S.J., et al., *Endogenous Tagging Reveals Differential Regulation of Ca(2+) Channels at Single Active Zones during Presynaptic Homeostatic Potentiation and Depression*. J Neurosci, 2019. **39**(13): p. 2416-2429. 4. Li, X., et al., *A Glutamate Homeostat Controls the Presynaptic Inhibition of Neurotransmitter Release*. Cell Rep, 2018. **23**(6): p. 1716-1727.

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Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.02

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Gertrude Flora Ribble Scholarship (C.H.)
University of Kentucky A&S Summer Fellowship (C.H.)
Chellgren Fellows Program (C.H.)
Chellgren Endowed Professor fund (R.L.C)

Title: How the permeability varies in relation to external K^+ concentration and temperature in fitting the curves to the Goldman Hodgkin Katz equation for muscle

Authors: *C. HADDAD¹, R. L. COOPER²;

¹Biol., ²Univ. of Kentucky Dept. of Biol., Univ. of Kentucky, Lexington, KY

Abstract: Temperature has effects on the membrane potential of cells in various ways. The membrane potential of a cell is complex due to the varying types of ion channels, permeability of ion channels, ionic pumps, and ionic exchangers which can all potentially vary with temperature changes. It is important to understand the effects of temperature on the membrane potential and ionic flux as cells and tissues are stored and transported for research and healthcare needs. In addition, acute exposures to various temperatures are used for medical treatments such as cardiac arrest, sepsis, and other maladies. It is well established that passive properties of cell membranes are generally related to extracellular K^+ concentrations ($[K^+]_o$). However, few studies address the effect of altering the $[K^+]_o$ along with variation in temperature. This study addresses this topic by using muscles of crayfish and larval *Drosophila* and using an intracellular electrode to measure the membrane potential while changing $[K^+]_o$ and temperature from 5°C to 30°C. Considering a detailed study using muscle from barnacles, it is predicted that the permeability of K^+ will vary with temperature, and the effects of $[K^+]_o$ will not be a linear relationship. Based on the raw data collected thus far, the equilibrium potential for potassium may not be feasible to fit the data present in relationship to temperature and membrane potential. The data collected shows a curved relationship between membrane potential (mV) and the $[K^+]_o$ especially at lower $[K^+]_o$ concentrations due to sodium being the dominant ion at potassium concentrations. The Goldman Hodgkin Katz (GHK) equation would be more fitting with considering in the changes of permeability for the ions. It is important to determine if this is also the case for other organisms as a commonality. Medical induced hypothermia can be used as a type of therapeutic treatment for brain injuries in which the local extracellular K^+ may not be in a physiological range, so it is important to have a better understanding of the potential of combinational effects. In addition, in

the preservation and culturing of cells from various tissues and organs, it is important to have a better understanding of how varying media affects cellular properties and survival.

Disclosures: C. Haddad: None. R.L. Cooper: None.

Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.03

Topic: E.09. Motor Neurons and Muscle

Support: NSF DBI2015317
NSF IOS1754869
NIH R01 NS118606

Title: Full Hill-type Muscle Model of a Key Feeding Retractor Muscle (I1/I3 muscle) from *Aplysia californica*

Authors: *R. SUKHNANDAN¹, Q. CHEN⁴, J. SHEN⁴, S. PAO⁴, J. P. GILL⁴, H. J. CHIEL^{4,5,6}, V. A. WEBSTER-WOOD^{1,2,3};

¹Mechanical Engin., ²Biomed. Engin., ³McGowan Inst. for Regenerative Med., Carnegie Mellon Univ., Pittsburgh, PA; ⁴Biol., ⁵Neurosciences, ⁶Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: The ability of animals to engage in sophisticated behaviors like feeding, locomotion and manipulation requires the coordination of both neural and muscular systems. The marine mollusk *Aplysia californica* exhibits such complex multifunctional behavior during feeding as it (1) attempts to grasp food (biting), (2) successfully grasps and ingests food (swallowing) and (3) egests inedible food (rejection). In addition, the tractable neural circuitry of *Aplysia* makes it an excellent organism to study the neuromechanical mechanisms that allow such multifunctional behavior. However, to date only the muscle responsible for protraction of the grasper (I2) has been characterized with a Hill-type muscle model [1]. The I3 retractor muscle plays an important role in all three feeding behaviors. This study characterizes the I1/I3 retractor muscle complex and presents a full Hill-type muscle model for use in neuromechanical studies of *Aplysia* feeding behavior. One half of the cylindrical I1/I3 complex was isolated from a fully anesthetized adult *Aplysia*, with intact innervation via Buccal Nerve 2 (BN2). Stimulation was applied via a suction electrode connected to BN2, with a hook electrode providing a backup pathway for stimulation and recording of neural firing on BN2. The muscle was submerged in a bath of *Aplysia* saline containing glucose. The muscles were mounted to a length-controlled servo-motor along the circumferential contraction direction. We have previously presented work characterizing the Force-Frequency and Length-Tension properties [2]. To complete the Hill-type model, we carried out Force-Velocity experiments where the active force generated was measured as it passed through the rest length of the muscle for constant velocity shortening or lengthening.

Velocities from 0.25 mm/s to 8 mm/s were tested. The obtained normalized force-velocity profiles were similar to the previously characterized I2 muscle, where there was an increase in contractile force with increasing lengthening velocity and a reduction in contractile force with increasing shortening velocity. These studies will help to clarify neuromechanics of feeding in *Aplysia*.

[1] S.-N. Yu, P. E. Crago, and H. J. Chiel, "Biomechanical properties and a kinetic simulation model of the smooth muscle I2 in the buccal mass of *Aplysia*," *Biol. Cybern.*, vol. 81, no. 5-6, pp. 505-513, Nov. 1999, doi: 10.1007/s004220050579.

[2] Q. Chen, R. Sukhnandan, S. Pao, J.P. Gill, H.J. Chiel, V.A. Webster-Wood.

"Characterization and creation of a linear Hill-type muscle model of the *Aplysia californica* I1/I3 retractor muscle complex". Neuroscience 2021. Chicago, Illinois. Virtual. November 8-16, 2021.

Disclosures: R. Sukhnandan: None. Q. Chen: None. J. Shen: None. S. Pao: None. J.P. Gill: None. H.J. Chiel: None. V.A. Webster-Wood: None.

Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.04

Topic: E.09. Motor Neurons and Muscle

Support: VA grants: I01 RX002462 and IK6 RX003351

Title: Direct intraoperative measurement of human gracilis muscle specific tension

Authors: *B. I. BINDER-MARKEY¹, L. S. PERSAD², A. Y. SHIN², W. J. LITCHY², K. R. KAUFMAN², R. L. LIEBER³;

¹Drexel Univ., Philadelphia, PA; ²Mayo Clin., Rochester, MN; ³Shirley Ryan AbilityLab, Chicago, IL

Abstract: Quantifying muscle structure and function is critical to understanding motor control. However, most physiology experiments used to predict human performance are performed in small animal models and then extrapolated to predict human function. Here we leverage a unique surgical technique, in which a human gracilis muscle is transferred from the thigh to the arm to restore elbow flexion after brachial plexus injury. This allows direct measurement of gracilis in situ properties to quantify its optimal fiber length and specific tension. After IRB approval, 29 patients were consented and enrolled, from which 13 complete data sets were obtained. Prior to harvesting the gracilis, the lower limb was gradually lengthened through its anatomical ranges. At each length muscle-tendon unit (MTU) length was measured by threading a suture parallel to the MTU from the muscle's origin to its insertion. To induce active contraction, we isolated the obturator nerve's anterior branch for stimulation and directly measured muscle force using a buckle force transducer on the distal gracilis tendon. To minimize intraoperative movement artifacts, the nerve was stimulated at 20 Hz at 50% of the current that produced a maximum

compound muscle action potential (CMAP). To quantify maximum muscle force, we measured spatial and temporal summation using twitch forces at 50% and 100% CMAP and tetanic forces at frequencies of 5, 10, 20, and 50 Hz. On average, muscle twitches at 50% max CMAP intensity produced 82% of the force of max CMAP, and tetanic forces at 20 Hz produced 94% of the force of 50Hz. These data demonstrate that the tetanic force of a 20Hz tetani at 50% CMAP is 77% of the true muscle maximum force. After in situ data collection, the MTU was excised and muscle length, tendon length, and volume measured. Each subject's optimal fiber length was calculated based on the width of their active force vs. muscle length curve at half maximum force. Using measured volume and calculated optimal fiber length, each muscle's physiologic cross-sectional area was calculated and used to determine the muscle specific tension. Average optimal fiber length was 12.0 cm (± 1.2 SEM) and average specific tension of the gracilis was 167 kPa (± 24.8). This specific tension is less than the typical value of 225 kPa obtained from animal studies, but within the large range of ~100-660 kPa from indirect human studies and consistent with 152-157 kPa of muscle with predominantly type I fibers. The value of 167 kPa value thus represents a new gold standard specific tension for human muscle and represent the first direct measurement of in situ human muscle properties.

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Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.05

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant 5R01AR074988

Title: The Function of Spectrins in Skeletal Muscle

Authors: ***O. SERT**¹, M. N. RASBAND²;

¹Development, Dis. Models & Therapeut. Grad. Program, Baylor Col. of Med., Houston, TX;

²Neurosci., Baylor Col. of Med. Dept. of Neurosci., Houston, TX

Abstract: Spectrins are submembranous cytoskeletal proteins that maintain cell shape and link ankyrin and ion channel protein complexes to the actin cytoskeleton to participate in signal transduction. Although ankyrins and muscle-specific voltage-gated sodium channels (Nav1.4) are enriched at the neuromuscular junction (NMJ), the role of spectrins in the muscle and at the NMJ are unknown. There are two α and five β spectrin subunits in mammals. Among these five β spectrins, pathogenic human $\beta 4$ -spectrin (SPTBN4) variants are proposed to cause both motor neuropathy and myopathy. To determine the role of $\beta 4$ -spectrin in muscles, we generated skeletal muscle-specific $\beta 4$ -spectrin deficient mice. However, we did not detect any pathology, suggesting $\beta 4$ spectrin does not contribute to muscle function. To determine which β -spectrin is

important for muscle function, we performed immunoblotting and immunostaining and we found high enrichment of β 1-spectrin in the muscle and at the NMJ. To determine the role of β 1-spectrin in the muscle, we generated skeletal muscle-specific β 1-spectrin deficient mice. We did not detect muscle pathology or impaired synapse function, but we found that β 1-spectrin is necessary for Nav1.4 maintenance at the NMJ. In neurons, two α and two β spectrin subunits form a functional spectrin heterotetramer. To investigate the role of spectrins in muscle, we generated skeletal muscle-specific α 2-spectrin deficient mice. Surprisingly, we found no change in muscle health, NMJ morphology, synapse function, or NMJ clustering of β 1-spectrin and Nav1.4 channels. Thus, among muscle spectrins, β 1-spectrin maintains the Nav1.4 channels at the NMJ and contrary to accepted models, may function independently of α 2 spectrin.

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Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.06

Topic: E.09. Motor Neurons and Muscle

Title: Functional role of posterior digastric muscle during deglutition

Authors: *Y. TSUTSUI, T. TSUJIMURA, K. PIRIYAPRASATH, T. CHOTIRUNGSAN, J. MAGARA, K. OKAMOTO, K. YAMAMURA, T. MAEDA, M. INOUE; Niigata Univ., Niigata city, Japan

Abstract: Backgrounds and objectives During swallowing, many muscles are activated. The activity pattern of related muscles varies among species although swallowing neural network is located in the brainstem. The posterior belly of digastric (post Dig) muscle is known to be one of the suprahyoid muscles. So far, no studies have evaluated activity pattern of the muscle during swallowing and contribution to the swallowing movements. The aim of the present study was to clarify if the post Dig muscle is involved in swallowing function. **Method** Experiments were carried out on 7-9 w Sprague-Dawley male rats anesthetized with urethane. First, swallowing responses were recorded from the post Dig and thyrohyoid electromyograms (EMGs) to determine the activity pattern of deglutition. Second, to identify the location of post Dig motoneurons, immunohistochemical staining using choline acetyltransferase (ChAT) was performed. Third, post Dig motoneurons were retrogradely identified using 4% solution of Fluoro-Gold (FG). For this purpose, FG was injected into the post Dig muscle on both sides. On one side, the post Dig motor nerve was dissected. Ten days later, retrogradely labeled FG-positive cells were examined. Fourth, swallowing reflexes were repetitively evoked by laryngeal mechanical stimulation using a von-Frey filament every 10 sec for 60 min and the number of c-Fos-like immunoreactive (LI) cells was counted. Finally, swallowing was evoked by the mechanical tracheal stimulation in the FG-injected rats, followed by the double staining (c-Fos protein and FG) of brainstem section. **Result** EMG bursts were observed in the post Dig and

thyrohyoid muscles during swallowing. Onset time was not different between them, but the peak of the former always preceded the latter. FG labeled cells were clearly found in the intact side of the accessory facial nucleus (Acs7) which was immunohistochemically identified. The c-Fos-LI cells were detected in the Acs7 following repetitive swallows and the number of those cells was larger than the sham-operated group. Furthermore, several c-Fos/FG double-labeled cells were observed in the Acs7. **Conclusion** We conclude that post Dig muscle is one of the major swallowing muscles. In our next study, the functional role of this muscle will be examined.

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Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

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Program #/Poster #: 477.07

Topic: E.09. Motor Neurons and Muscle

Support: Conacyt 857159SDS
UATLx-CA-237OLG.

Title: Changes in the frequency of bulbospongiosus muscle activation during the urethro-genital reflex by maternal and postnatal sugar water consumption in the offspring of adult male rats.

Authors: S. DÁVILA SANTACRUZ¹, D. CORONA-QUINTANILLA², E. ORTIZ ORTIZ³, L. NICOLAS⁴, M. MARTINEZ-GOMEZ⁵, *J. RODRIGUEZ⁶;

¹Posgrado en Ciencias Biológicas, ²Ctr. Tlaxcala de Biología de la Conducta, ³Facultad de Odontología, Univ. Autónoma de Tlaxcala, Tlaxcala, Mexico; ⁴Ctr. Tlaxcala de Biología de la Conducta, Univ. Autónoma de Tlaxcala, Tlaxcala DE Xicohtencatl, Mexico; ⁵Inst. de Investigaciones Biomédicas UNAM, Tlaxcala, Mexico; ⁶Univ. Autonoma De Tlaxcala, Tlaxcala, Mexico

Abstract: The development of striated muscle fibers takes place during gestation and the nutritional change during this stage modifies the phenotype of muscle fibers, the strength and the frequency of activation of striated muscles, such as pelvic-perineal muscles, in the offspring. Subsequently, in the adult stage, the affectations of said musculature, specifically that of the bulbospongiosus muscle, could cause male sexual dysfunctions, such as premature ejaculation. Therefore, the aim of this study was to determine changes in the frequency of bulbospongiosus muscle activation during the urethro-genital reflex, which simulates erection and ejaculation, by maternal and postnatal sugar water consumption in the offspring of adult male rats. For this, males from mothers with consumption of plain water and with consumption of sugared water (5 %) were used. In the prenatal stage they were divided into males from mothers with plain water consumption and continued in the same way (CM-CO) and those who continued with sugar

water (CM-SO), males from mothers with sugar water and continued with plain water (SM-CO) and males with consumption of sugar water (SM-SO). Adult males from all groups were anesthetized with urethane (1.5 g/kg, i.p.) and electromyographic recording of the bulbospongiosus muscle was performed during mechanical stimulation of the perigenital and penile skin and urethrogenital reflex. From the electromyograms, the activation frequency of the bulbospongiosus muscle was analyzed through Fourier Transform analysis. The results showed that the consumption of sugar water during critical stages, such as pregnancy and lactation, generate a decrease in the activity of the bulbospongiosus muscle during stimulation of the perigenital and penile skin in SM-CO and SM-SO males. However, the bulbospongiosus muscle significantly increases its frequency of activity during the urethrogenital reflex of the CM-SO, SM-CO and SM-SO males; specifically in the bursts of activity that are generated during the expulsion of fluid. Our results suggest that a combination of a high-sugar diet during pregnancy, lactation, and post-weaning modifies the frequency of bulbospongiosus muscle activation during penile and ejaculatory reflexes, such as the urethrogenital reflex. Also, the change in muscle activity could cause male sexual dysfunctions, such as premature ejaculation.

Disclosures: **S. Dávila Santacruz:** None. **D. Corona-Quintanilla:** None. **E. Ortiz Ortiz:** None. **L. Nicolas:** None. **M. Martinez-Gomez:** None. **J. Rodriguez:** None.

Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.08

Topic: E.09. Motor Neurons and Muscle

Support: R01-NS121374-02
K01-NS116119-01

Title: Novel high-throughput optogenetics system to assess functional human neuromuscular junction properties.

Authors: D. CHEN, P. PHILIPPIDOU, B. BRENHA, A. SCHAFFER, ***H. C. MIRANDA**;
Case Western Reserve Univ., Cleveland, OH

Abstract: Neuromuscular junctions (NMJs) are specific synapses that connect motor neurons to skeletal muscle and therefore are primarily responsible for voluntary movement. The degeneration of this system can lead to symptoms that are observed in motor neuron and neuromuscular diseases. There is a substantial amount of studies on the cellular and molecular composition of NMJs in animal models, such as mouse and fly, whilst the knowledge on human NMJs is scarce due to obvious ethical concerns and relative inaccessibility of samples. In this work, we use human induced pluripotent stem cells (iPSCs) to generate motor neurons and skeletal muscles and co-culture these two cell types to form an in vitro humanized NMJ system. iPSC-derived NMJs were characterized morphologically by a-bungarotoxin staining. We also

observed that when iPSC-derived skeletal muscles and motor neurons were co-cultured in the presence of agrin, there was a significant increase in the expression of acetylcholine receptor (AChR). Furthermore, we performed functional analysis of the iPSC-derived motor neuron and skeletal muscles separately by a multielectrode array (MEA) system using the Maestro instrument (Axion Biosystems). Both isolated cultures display spontaneous spike activity that increases over time, indicative of maturation of the cultures. The quantification of the spontaneous contractions of the iPSC-derived skeletal muscle cultures enabled the further optimization of the iPSC-derived NMJs functional analyses using the MEA system. In the iPSC-motor neuron and skeletal muscle co-culture, we observed increased spike frequency when compared to skeletal muscle alone. We have also taken advantage of optogenetics and transduced the iPSC-derived motor neurons with a lentivirus harboring the humanized channelrhodopsin (ChR2) under the control of the synapsin promoter. Transduced motor neurons co-cultured with skeletal muscle displayed an increase in number the of Bursts when subjected to light stimulation using the LUMUS apparatus (Axion Biosystems). To our knowledge, we are the first laboratory to optimize the MEA system for quantitation of iPSC-derived skeletal muscle activity and to measure functional human NMJs. We are currently using this system to investigate NMJ phenotypes associated with motor neuron disease pathogenesis.

Disclosures: **D. Chen:** None. **P. Philippidou:** None. **B. Brenha:** None. **A. Schaffer:** None. **H.C. Miranda:** None.

Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.09

Topic: E.09. Motor Neurons and Muscle

Title: Tissue engineered motor units featuring spinal motor neurons innervating myofiber bundles

Authors: *M. HILMAN^{1,3}, S. DAS^{2,3}, D. CULLEN^{2,1,3};

¹Bioengineering, ²Neurosurg., Univ. of Pennsylvania, Philadelphia, PA; ³Ctr. for Neurotrauma, Neurodegeneration & Restoration, Corporal Michael J. Crescenz Veterans Affairs Med. Ctr., Philadelphia, PA

Abstract: A motor unit is the functional unit of muscle contraction and comprises a motor neuron and the population of skeletal muscle fibers innervated by the neuron's axon terminals. Tissue engineering strategies are being pursued to treat severe neuromuscular injuries by mimicking aspects of native myofascicular architecture and providing topographical guidance to regenerating motor units. Our group has previously developed pre-innervated tissue engineered muscle sheets comprising planar myofibers in co-culture with spinal motor neurons grown on a nanofiber scaffold. This co-culture system showed that the presence of motor neurons facilitated myofiber maturation *in vitro* as well as promoted a pro-regenerative microenvironment following

implant in a rat model of neuromuscular injury. While promising, the planar nature of this pre-innervated muscle inhibited the ability of this system to mimic the three-dimensional (3D) bundled architecture of myofibers *in vivo*. To address this challenge, we have developed novel Tissue Engineered Motor Units (TEMUs) comprising centimeter-scale aligned bundles of myofibers encased within a 3D collagen hydrogel and innervated by axons projecting from a discrete population(s) of spinal motor neurons. Specifically, skeletal myoblasts were mixed with a collagen solution and seeded inside 3D printed polydimethylsiloxane (PDMS) micro-scale channels paired with aggregates of motor neurons on the endpoints. This system facilitated the self-assembly of myoblasts into aligned 3D bundles of myofibers measuring millimeters in diameter and spanning centimeters in length. Using this platform, we investigated the combined effects of channel topography and innervation on the maturation and elongation of myocytes. Through immunocytochemistry and confocal microscopy, myofiber bundle thickness, fusion index, axon ingrowth, and neuromuscular junction formation were visualized and quantified. The presence of aggregated motor neurons and axonal integration were observed to enhance myofiber maturation and contractility. Cross-sectional histological analysis showed that TEMUs exhibited fascicle-like organization with spatial segregation between myofiber bundles and the neuronal compartment on the periphery, with axons projecting inward to innervate the myofibers. To date, we have successfully generated TEMUs up to 8cm long and 750 μ m in diameter, grown to at least 21 days in culture. Ongoing studies are focused on using the TEMU platform to further establish the critical role of axon innervation on the development, maturation, functionality, and regenerative potential of large-scale 3D myofiber bundles.

Disclosures: **M. Hilman:** None. **S. Das:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Patent App. PCT/US2019/060585 titled “ Engineering of Innervated Tissue and Modulation of Peripheral Organ Activity ”. **D. Cullen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Patent App. PCT/US2019/060585 titled “ Engineering of Innervated Tissue and Modulation of Peripheral Organ Activity ”. Other; D.K.C. is a co - founder of two University of Pennsylvania spin - out companies concentrating in applications of neuroregenerative medicine: Innervace , Inc. and Axonova Medical, LLC.

Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.10

Topic: E.09. Motor Neurons and Muscle

Support: U.S. Department of Defense through the Medical Research and Materiel Command (W81XWH-19-1-0867)

Title: Human innervated tissue engineered muscle for neuromuscular recovery following volumetric muscle loss

Authors: *S. DAS^{1,3}, M. C. HILMAN^{2,3}, F. A. LAIMO^{1,3}, D. K. CULLEN^{1,3};
¹Neurosurg., ²Bioengineering, Univ. of Pennsylvania, Philadelphia, PA; ³Michael J. Crescenz
Veterans Affairs Med. Ctr., Philadelphia, PA

Abstract: Innervation plays a critical role in skeletal myocyte development, function, and regeneration. Severe musculoskeletal injuries like Volumetric Muscle Loss (VML) are characterized by traumatic or surgical loss of skeletal muscle tissue accompanied by chronic axotomy that generally lead to debilitating and persistent motor deficits. Tissue engineering strategies for VML repair need to achieve bulk restoration as well as promote re-innervation of injured area to facilitate functional recovery. Our group has previously developed Innervated Tissue Engineered Muscle (INTEM) by coculturing myocytes and motor neurons of rodent origin on nanofiber sheets. The INTEMs showed the effectiveness of pre-innervation in promoting muscle maturation *in vitro* as well as in maintaining a pro-regenerative microenvironment in host muscle tissue following implantation in a rat model of VML. In this study, we have advanced the INTEM technology by: (a) developing a human INTEM by coculture of primary human skeletal myocytes and human induced pluripotent stem cell (iPSC) derived motor neurons on aligned nanofiber sheets, and (b) using a clinically relevant delayed repair model of VML in rats to evaluate functional efficacy of the human INTEM. In INTEMs built using all-human cell source(s), we found that the presence of motor neurons/axons significantly increased myocyte length and fusion index while also reducing myocyte width, suggesting that innervation enhances human myofiber formation and maturation *in vitro*. Human INTEMs also demonstrated upregulation of neuromuscular genes such as *MUSK*, *DOK-7*, *RAPSYN*, *LRP-4* as well as led to higher secretion of Agrin, a proteoglycan stabilizing neuromuscular junctions, as compared to non-innervated TEMs. Human INTEMs were then implanted in a rat VML model either immediately post-injury or with a 3 week delay to better mimic clinical repair scenarios. Immunohistochemistry and confocal microscopy revealed survival of implanted myocytes and motor neurons to at least 4 weeks post-implantation following both immediate as well as delayed repair VML models. Ongoing studies are focused on further analyses of histological and electrophysiological data at chronic time points post-repair to investigate the efficacy of human INTEMs in augmenting functional recovery in clinically relevant models of VML.

Disclosures: **S. Das:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Patent App. PCT/US2019/060585 titled “Engineering of Innervated Tissue and Modulation of Peripheral Organ Activity”.. **M.C. Hilman:** None. **F.A. Laimo:** None. **D.K. Cullen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder of two University of Pennsylvania spin-out companies concentrating in applications of neuroregenerative medicine: Innervace, Inc. and Axonova Medical, LLC; U.S. Patent App. PCT/US2019/06058.

Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.11

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant R01HD084009
NIH Grant R01NS058667
NIH Grant R03HD094615
NIH Grant T32EB025766

Title: Quantification of forearm musculoskeletal micro- and macro-structural adaptations in children with hemiparetic cerebral palsy using MR-based diffusion tensor imaging

Authors: *D. JOSHI^{1,2}, M. H. SOHN², J. P. A. DEWALD^{1,2,3}, C. INGO^{2,4};

¹Dept. of Biomed. Engin., Northwestern Univ., Evanston, IL; ²Dept. of Physical Therapy and Human Movement Sci., ³Dept. of Physical Med. and Rehabil., ⁴Dept. of Neurol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Hemiparetic cerebral palsy (HCP), caused by a unilateral brain injury that occurs around birth, results in progressive secondary musculoskeletal changes that have a profound impact on lifelong development and function. Changes may include (1) shortening of muscle fascicles, which reduces the force-generating capacity of a muscle, and/or (2) proliferation of extracellular matrix (ECM), which may increase passive stiffness and decrease passive range of motion (PROM). In this study, we implement diffusion-weighted magnetic resonance (MR) based diffusion tensor imaging (DTI) to comprehensively quantify *in vivo* micro-structural (fractional anisotropy, an indirect metric sensitive to ECM proliferation) and macro-structural (fascicle length) properties. Specifically, we focused on interlimb differences in the flexor carpi ulnaris (FCU), a wrist flexor muscle important for hand function. We performed DTI and T1 MRI analyses on 3 children with HCP (10.4±1.9 years, 1 female, reduced wrist PROM severity: HCP1<HCP2<HCP3) and 1 typically developing (TD) child (10.3 years, female). T1-weighted (voxel=0.78x0.78x3mm³) and diffusion-weighted (voxel=1.25x1.25x6.5mm³, b=400s/mm², 12 directions) MR images were acquired in both forearms of each participant. The ulna bone and FCU muscle were segmented using the T1-weighted images. From the diffusion-weighted images, fractional anisotropy (FA) was calculated and probabilistic tractography was performed to estimate the fascicle lengths of the FCU. Fascicle lengths were normalized to the bone length (the 3D Euclidean distance between the ulnar styloid and olecranon processes) to correct for interlimb differences in skeletal development. The TD individual showed similar bone lengths between arms, but all three individuals with HCP had shorter ulna bones on the paretic arm as compared to the non-paretic arm. The fascicle lengths were similar between arms in TD (interlimb difference: 7.35%) but decreased in the paretic limb in HCP (interlimb differences for HCP1: 6.45%, HCP2: 49.3%, and HCP3: 40.4%, positive indicates non-paretic limb is higher) even after correction for a difference in bone lengths. Furthermore, the FA was also similar between arms in TD (interlimb difference: 3.72%) but increased in the paretic limb in HCP (interlimb differences for HCP1: -18.0%, HCP2: -36.3%, HCP3: -69.3%, negative indicates paretic limb is higher). Preliminary results show decreased fascicle lengths and increased FA in the FCU of children with HCP. These musculoskeletal morphological changes between upper limbs will be linked with changes in passive stiffness and PROM in subsequent experiments in individuals with HCP.

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Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.12

Topic: E.09. Motor Neurons and Muscle

Title: The electrical impedance myography detects dystrophin-related muscle changes in *mdx* mice

Authors: *T. HIYOSHI¹, F. ZHAO², R. BABA¹, T. HIRAKAWA¹, R. KUBOKI¹, K. SUZUKI¹, Y. TOMIMATSU¹, C. WINKELMANN², P. O'DONNELL³, S. HAN⁴, N. ZACH³, M. NAKASHIMA¹;

¹Neurosci. Drug Discovery Unit, Takeda Pharmaceut. Co. Limited, Kanagawa, Japan;

²Preclinical and Translational Sci., ³Neurosci. Drug Discovery Unit, ⁴Neurosci. Therapeut. Area Unit, Takeda Pharmaceut. Co. Limited, Boston, MA

Abstract: Duchenne muscular dystrophy (DMD) is caused by disruption of dystrophin protein levels, resulting in muscle injury and chronic skeletal muscle inflammation. The muscular dystrophy X-linked (*mdx*) mouse model that shows a deficiency of dystrophin is one of the widely used preclinical models for DMD. Electrical impedance myography (EIM) is an emerging tool that provides a non-invasive biomarker to longitudinally and frequently monitor muscle conditions. Although magnetic resonance imaging (MRI) of skeletal muscles is becoming a standard measurement in DMD clinical development, a unique benefit of EIM such as portable and inexpensive natures of the devices enables monitoring disease progression or response to therapy remotely, including at home. This allows us to utilize the EIM as a quantitative biomarker easily in clinical trials. Toward practical application of the EIM as a biomarker for DMD, it was necessary to investigate whether this method is sensitive to alterations in the muscle condition with the deficiency or restoration of dystrophin. In this study, we characterized disease progression in *mdx* mice, looking at the longitudinal alteration of EIM parameters in comparison with MRI measurements. Furthermore, we investigated whether EIM could detect dystrophin-related muscle changes in *mdx* mice after administration of cell-penetrating peptide conjugated phosphorodiamidate morpholino oligomer (PPMO). In the natural history study with *mdx* mice (6-18 weeks old), MRI imaging in the hindlimbs showed higher T2 intensity at 6 weeks compared to WT mice although it gradually decreased with aging. In contrast, in a longitudinal EIM measurement in the gastrocnemius muscle (6-24 weeks old), EIM reactance in *mdx* mice began to decrease at 12 weeks and reached the peak at 18 weeks. EIM resistance and phase also decreased in an age-dependent manner. In addition, the muscle fiber analysis revealed that *mdx* mice had an increased small muscle fiber fractions. Repeated intravenous doses of PPMO (10 mg/kg, every 2 weeks, 4 times) reversed the change in EIM reactance in *mdx* mice at 24 weeks in company with a robust increase in muscular dystrophin protein. These results suggest that EIM is a useful tool for non-invasively monitoring dystrophin-deficient muscle

pathology associated with morphological skeletal muscle changes. EIM has the potential to evaluate dystrophin-related muscle changes by exon skipping therapies.

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Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.13

Topic: E.09. Motor Neurons and Muscle

Title: Characterization of redox environment and tryptophan catabolism through kynurenine pathway in military divers' and swimmers' serum samples

Authors: *L. SANCHEZ-CHAPUL^{1,5}, G. PÉREZ-DE LA CRUZ⁹, L. A. RAMOS-CHÁVEZ¹⁰, J. F. VALENCIA-LEÓN⁶, J. TORRES-BELTRÁN², E. ESTRADA-CAMARENA¹¹, P. CARRILLO-MORA¹, D. RAMÍREZ-ORTEGA¹², J. U. BAÑOS-VÁZQUEZ⁷, G. MARTÍNEZ-NAVA³, A. LUNA-ANGULO¹, C. MARTÍNEZ-CANSECO⁴, T. WENCES-CHIRINO¹, J. RÍOS-MARTÍNEZ⁸, V. PÉREZ DE LA CRUZ¹³;

¹División de Neurociencias Clínicas, ²Subdirección de Medicina del Deporte, ³Lab. de Gerociencias, ⁴Servicio de Bioquímica, Inst. Nacional de Rehabilitación, Mexico City, Mexico; ⁵Dirección Gen. Adjunta de Sanidad Naval, Secretaría de Marina Armada de México, Cdmx, Mexico; ⁶Dirección Gen. Adjunta de Sanidad Naval, ⁷Escuela de Búsqueda y Rescate y Buceo, ⁸Inst. de Investigación en Ciencias de la Salud de la Secretaría de Marina, Secretaría de Marina Armada de México, CDMX, Mexico; ⁹Dept. of Mathematics, Fac. of Sciences, Univ. Nacional Autónoma de México, CDMX, Mexico; ¹⁰Dept. de Neuromorfología Funcional, ¹¹Lab. de Neuropsicofarmacología, Inst. Nacional de Psiquiatría "Ramón de la Fuente", CDMX, Mexico; ¹²Neuroimmunology Lab., ¹³Neurobiochemistry and Behavior Lab., Natl. Inst. of Neurol. and Neurosurg. "Manuel Velasco Suárez, CDMX, Mexico

Abstract: Endurance and resistance exercises, alone or in combination, induce metabolic changes that affect tryptophan (Trp) catabolism. The kynurenine pathway (KP) is the main route of Trp degradation, and it is modulated by the inflammatory and redox environments. Previous studies have shown that KP metabolites work as myokines that mediate the positive systemic effects related to exercise. However, it is poorly understood how different exercise modalities and intensities impact the KP. The aim of this study was to characterize the effect of two different exercise modalities, military diving and swimming, on the KP and the redox environment. A total of 34 healthy men of the Mexican Navy were included in the study, 20 divers and 14 swimmers, who started and stayed in military training consistently during the six months of the study, and 12 Mexican men without fitness training were used as the control group. Physical fitness criteria; body composition; serum levels of Trp, kynurenine (KYN),

kynurenic acid (KYNA) and 3-hydroxykynurenine (3-HK); the glutathione ratio (GSH/GSSG); and malondialdehyde (MDA) were determined at the beginning and after 6 months of training. Results showed a significant loss of body fat in both the diver and swimmer groups. Considering the comparison with the control group, divers showed a decrease in Trp and 3-HK levels, but no changes were observed in the KYN/Trp, KYNA/Trp or 3-HK/Trp ratios, while swimmers showed a decrease in KYN levels and an increase in the KYNA and 3-HK levels. Additionally, divers showed a decrease in the GSH/GSSG ratio and an increase in MDA levels, in contrast to the swimmers, who showed a decrease in MDA levels and an increase in GSH/GSSG 55 levels. Our findings suggest a differential shift in the KP and redox environment induced by diving and swimming. Swimming promotes an antioxidant environment and a peripheral overactivation of the KP. These findings pave the way for new studies in which swimming at different intensities could be studied as a potential therapeutic adjuvant in many diseases presenting fluctuations in brain KP metabolites with neuroactive properties

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Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

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Program #/Poster #: 477.14

Topic: E.09. Motor Neurons and Muscle

Title: The effects of physical tissue feature on muscle reinnervation by peripheral nerves in a clinically-relevant animal model

Authors: *A. L. LOWE¹, K. QUINN¹, M. V. RIVERA-SANTANA³, V. SURESH², S. TUFFAHA², N. V. THAKOR¹;

¹Biomed. Engin., ²Plastic & Reconstructive Surgery, Johns Hopkins Univ., Baltimore, MD;

³Biol., Univ. of Puerto Rico, Mayaguez, Puerto Rico

Abstract: After limb amputation, cut peripheral nerves can become entangled with scar tissue during their natural regeneration, creating a painful formation known as a neuroma. To prevent neuroma formation, reconstructive surgeons perform targeted muscle reinnervation (TMR) in which the cut nerve is provided with a new denervated muscle target (DMT) to grow into. Since the inception of TMR, no two DMTs have been constructed the same way or using the same muscles in humans, all without any understanding of how physical variations in the DMT creation process might affect the tissue health or efferent/afferent signaling after reinnervation is completed months later. To study these effects, we created a highly standardized model of TMR

in the rat hindlimb that uses 3 distinct and clinically-relevant variations of DMTs all created from the soleus muscle: nerve-coapted targets (N-DMTs), vascularized grafts (V-DMTs), and devascularized mobile grafts (D-DMTs). We found that muscle atrophy was significantly different between the 3 groups at 100 days of reinnervation ($p < 0.05$) and that muscle atrophy was indirectly correlated with signal amplitude from anesthetized rats ($R^2 > 0.7$, $p < 0.05$). We are currently deploying a fully wireless, implantable EMG recording system for chronically recording from different DMT tissues as they are reinnervated over time. This implantable system is being used for monitoring the increase in DMT electrical activity of freely behaving rats on a week-to-week basis, as well as for classifying EMG signals from reinnervated muscle for possible control of a virtual prosthesis.

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Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

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Topic: E.09. Motor Neurons and Muscle

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Title: Age-associated reduction of survival motor neuron protein as a potential target for sarcopenia

Authors: *M. BALCH¹, P. BOBBILI¹, H. HARRIS¹, R. RODRIGO¹, D. CHUGH¹, C. C. IYER¹, F. ROUSSEL², A. HARLAUB², A. J. BLATNIK, III¹, A. H. BURGHESE¹, W. ARNOLD¹;

¹The Ohio State Univ., Columbus, OH; ²Ctr. for Gene Therapy, Abigail Wexner Res. Inst. at Nationwide Children's Hosp., Columbus, OH

Abstract: Ubiquitously-expressed Survival Motor Neuron protein (SMN) is vital for motoneuron function. SMN is produced in humans by *SMN1* and *SMN2* genes. Unlike full-length SMN produced by *SMN1*, *SMN2* mostly yields the truncated - and quickly degraded - SMN-delta7 splice variant. Homozygous loss of *SMN1* causes Spinal Muscular Atrophy (SMA), an autosomal recessive disorder characterized by insufficient SMN and, consequentially, motoneuron loss. Notably, age-induced reduction of muscle size and strength (sarcopenia) is associated with motoneuron dysfunction, and prior data show mice with higher SMN demonstrate increased longevity and resiliency and improved repair after nerve injury. Here, we assessed SMN protein levels in wildtype mice throughout aging and interrogated associations between SMN levels and neuromuscular function. C57BL/6 mice, balanced for sex, were grouped by age: mature (6-10mo, n=16), aged (21mo, n=6), and advanced-aged (27mo, n=10).

Compound muscle action potential (CMAP), single motor unit potential (SMUP), and motor unit number estimation (MUNE) were recorded electrophysiologically from the right gastrocnemius after sciatic nerve stimulation. Plantarflexion contractility was assessed following tibial nerve stimulation. In gastrocnemius and spinal cord, including motoneuron-enriched samples obtained by laser-capture microdissection, SMN levels (pg SMN/mg soluble protein) were measured via electrochemiluminescence immunoassay. Age had a significant effect on SMN level ($p=0.019$), motor output (CMAP, $p=0.0003$), number of functional motor units (MUNE, $p=0.007$), and gastrocnemius weight ($p<0.0001$), and a trending effect on tetanic contractility ($p=0.054$). Spinal cord SMN was 22% lower in advanced-age versus mature mice ($p=0.019$) and correlated with gastrocnemius weight ($r=0.484$, $p=0.006$) and MUNE ($r=0.413$, $p=0.019$). Motoneuron-specific SMN was reduced by 55% from mature to advanced-age ($p=0.007$), correlated with gastrocnemius weight ($r=0.747$, $p=0.001$), and trended with tetanic contractility ($r=0.391$, $p=0.121$). Work in SMA has elucidated key roles of SMN protein in motoneuron health. Our data suggest sarcopenia and functional decline in aging are linked to reduced SMN expression in motoneurons. Ongoing studies are investigating how increasing SMN levels - by inducing full-length SMN from an *SMN2* transgene, or overexpressing with AAV - impact sarcopenic phenotypes. In summary, age-associated reduction of SMN protein is a potential target for sarcopenia intervention and, considering the current therapeutic landscape for patients with SMA, represents an easily-translatable approach for application in aging.

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Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

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Topic: E.09. Motor Neurons and Muscle

Support: NIH/NINDS R35NS097212

Title: Presynaptic homeostatic plasticity protects against age-dependent neuromuscular decline in mice

Authors: *B. O. ORR, G. W. DAVIS;

Univ. of California San Francisco, Univ. of California San Francisco, San Francisco, CA

Abstract: Presynaptic homeostatic plasticity (PHP) is a potent adaptive physiological mechanism that stabilizes synaptic function at neuromuscular junctions, from *Drosophila* to human (Cull-Candy et al., 1980; Davis, 2013, 2006; Plomp et al., 1992; Sons et al., 2003). PHP is induced following a perturbation that impairs postsynaptic neurotransmitter receptor function or number (Acetylcholine at the mammalian NMJ and glutamate at the *Drosophila* NMJ).

Impaired receptor function induces an increase in presynaptic release that precisely offsets the postsynaptic deficit and sustains normal neuromuscular function. We recently coined the term 'homeostatic neuroprotection' to describe how the induction of PHP protects mice against the effects of neuromuscular degeneration in mouse models of Amyotrophic Lateral Sclerosis (Orr et al., 2020). Two key results were shown. First, synaptic disassembly induces the expression of PHP. Second, genetic elimination of PHP in mouse models of ALS led to accelerated disease progression and diminished lifespan by ~50%. To determine the generality of homeostatic neuroprotection, we asked whether PHP also protects against age-related neuromuscular decline. Age is the single greatest risk factor for neurodegeneration. In humans, an age-related decline in neuromuscular function is associated with loss of muscle mass (sarcopenia), increased frailty and a degradation of both health and quality of life (Clark, 2019). The only known treatments are life-style based, including exercise and diet (Valdez et al., 2010; Tintignac et al., 2015). Here we demonstrate advanced age is associated with fragmentation of NMJ Acetylcholine receptor fields and the induction of PHP. Mechanistically, age-dependent induction of PHP requires the activity of the epithelial sodium channel (ENaC), previously demonstrated to be required for PHP in ALS-like neurodegeneration. Application of an ENaC channel antagonist (benzamil) to the aged NMJ eliminates age-dependent induction of PHP. Next, we take advantage of a recently generated *Scnn1a* conditional knockout to eliminate *Scnn1a* expression (and ENaC function) in motoneurons. The conditional knockout mice were aged for more than 2 years. We document precocious age-related anatomical neuromuscular decline, accelerated age-dependent sarcopenia and accelerated muscle weakness over the lifespan. There is no change in animal lifespan, consistent with the conclusion that the induction of PHP contributes substantially to organismal health-span.

Disclosures: B.O. Orr: None. G.W. Davis: None.

Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.17

Topic: E.09. Motor Neurons and Muscle

Title: Effect of aerobic exercise on the glucose uptake and contractile properties of the tibial anterior and gastrocnemius muscle of the rat.

Authors: *B. SEGURA¹, E. REQUENA-ISLAS², E. GUTIERREZ-POSADAS², C. ALVAREZ², K. P. GARCIA-PELAGIO³;

¹FES-Iztacala, UNAM, Tlalnepantla, Mexico; ²Univ. Nacional Autonoma de Mexico, Tlalnepantla, Mexico; ³Physics, Univ. Nacional Autonoma de Mexico, Mexico, city, Mexico

Abstract: Exercise allows the remaining body to function in optimal condition. Aerobic exercise, such as swimming, improves functions of the cardio-respiratory system and regulates glucose homeostasis; it also promotes mitochondrial biogenesis and the transformation of fibers

type of skeletal muscles. However, little information is available about the relation between glucose homeostasis, and strength developed by skeletal muscle after a long time of exercise, flexors, and extensor muscles. Establishing the relationship between the exchange of energy and contractile properties is essential to understanding the function of the flexion and extension of whole muscles. This research aimed to establish the effect of aerobic exercise (swimming) on glucose uptake and the isometric force developed by the tibialis anterior (TA; flexor of the foot) and gastrocnemius (G; extensor of the foot) hindlimb muscles of the rat. Forty young male rats (Wistar strain) under similar conditions of temperature, humidity, and light-dark cycle, were randomly divided into two groups: not trained or control (C) and trained (T), three weeks swim program, 30min/day. Shift reagent dye and the force transducer -isometric myograph- measured the presence of glucose and the force developed by the muscles, respectively. Our results show that both muscles of animals subjected to endurance training performed by aerobic exercise during three weeks increased glucose uptake by 63%. Additionally, TA showed an increment of between 35% and 70% in force development, contrary to G which, remains the same. We conclude that aerobic exercise, explicitly swimming, had a differential effect on the mechanical properties of young rat's flexors and extensors muscles, but not on glucose uptake.

Disclosures: **B. Segura:** A. Employment/Salary (full or part-time);; Profesor full time. **E. Requena-Islas:** None. **E. Gutierrez-posadas:** None. **C. Alvarez:** A. Employment/Salary (full or part-time);; profesor full time. **K.P. Garcia-Pelagio:** A. Employment/Salary (full or part-time);; Profesosr full time.

Poster

477. Motor Neurons: Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 477.18

Topic: E.09. Motor Neurons and Muscle

Title: Exploring the Effects of Muscle Conditioning on the Golgi Tendon Organ

Authors: ***K. NOGI**¹, T. KOKUBUN²;

¹Saitama Prefectural Univ., Koshigaya-shi, Japan; ²Physical Therapy, Saitama Prefectural Univ., Koshigaya-Shi, Japan

Abstract: Proprioceptive sensation, which is responsible for motion and position sensation, profoundly influences the neural control system. The Golgi tendon organ, believed to contribute to force sensation, is desensitized after maximal voluntary contraction (MVC), causing sensory errors. However, how this intrinsic sensory error affects the motor control system (MCS) is unclear. This study aimed to determine how the Golgi tendon organ affects voluntary muscle activity at the neuromuscular junction after muscle conditioning from a neurophysiological perspective using motor unit (MU) changes. Four healthy adult males (A, B, C, and D) without central nervous system disorders participated in this study. 4-ch wireless EMG was attached to the biceps brachii muscle of the dominant arm to measure muscle activity. At the same time,

elbow flexion torque was measured at 90 degrees of elbow flexion using a load cell. The control condition performed 4% MVC for 15s, and the intervention condition performed 4% MVC for 5s, followed by 4% MVC for 15s. In both tasks, the force was reproduced with the eyes closed. The reproducibility of 4% MVC was evaluated from the torque data. The muscle activity data were analyzed using Neuro Map to examine the mean and standard deviation of the number of motor unit (MU) mobilizations and the mean firing rate (MFR) of the three times. The mean MFR of each MU was calculated for the middle and the second half. For all subjects, the 4% MVC reproducibility of the control condition was within $\pm 5\%$ error. Except for subject C, the first half of the time, the torque was more significant than the torque at 4% MVC. The error tended to decrease from the middle to the second half. In the intervention condition, subject A was the only subject who demonstrated less torque than the control condition. The other subjects showed similar trends. The number of MU detected tended to be smaller in the intervention condition than in the control, and the MFR of the intervention condition tended to be larger than that of the control from the middle to the latter half of the exercise period for all subjects except Subject B. It has been reported that after MVC, the Golgi tendon organ becomes desensitized and overestimates the torque of 4% MVC, resulting in more than twice as much torque. The results of this study contradict those of previous studies. However, muscle conditioning with MVC for 5s decreased the number of MU mobilizations during the 15s 4% MVC reproduction. The results also suggested an increased firing rate during the 4% MVC reproduction. This indicates that EMG can be used to analyze the effects of proprioceptive sensations such as the Golgi tendon organ on neuromuscular activity in MCS.

Disclosures: **K. Nogi:** None. **T. Kokubun:** None.

Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.01

Topic: F.02. Neuroendocrine Processes and Behavior

Support: NIH Grant 1R15MH124042-01

Title: Are mice pup ultrasonic vocalizations relevant signals for adult pup retrieval?

Authors: J. BUCHANAN¹, B. Y. LAU², D. CASENHISER⁴, K. REILLY⁴, ***K. KRISHNAN**³; ²Biochem. & Cell. and Mol. Biol., ³Univ. of Tennessee At Knoxville, ¹Univ. of Tennessee At Knoxville, Knoxville, TN; ⁴Univ. of Tennessee Hlth. Sci. Ctr., Knoxville, TN

Abstract: During pup retrieval task, an experienced adult female (surrogate, Sur) mouse is tasked with retrieving scattered pups to the nest. Studies from the 1960s showed that rat dams use a combination of olfactory, tactile, and auditory information to locate, identify and retrieve pups. Isolated pups emit ultrasonic vocalizations (USVs), thought to act as 'distress calls' which prompt retrieval from adults. However, in our studies with female mice, we noticed that

retrievals are fast, and can occur without USVs. This prompted us to inquire if pup USVs are indeed ‘distress calls’ and broadly, if they communicate socially relevant information useful for retrieval. Though pup calls in isolation have been reported for decades, fewer studies have reported characteristics of pup calls during physical interactions with adults. We analyzed ~15,000 calls emitted by different cohorts of pups, aged postnatal days 0-5 with manual curation and DeepSqueak analysis. The pup calls are from three different phases of the pup retrieval task; (1) pup isolation, (2) during physical interactions with adults during retrieval, and (3) habituation phase, when the pups are in the nest, with the adult in the cage, before retrieval. We found significant developmental changes in spectral and temporal properties of pup calls, regardless of different conditions (phases, genotype of retrieving adults and successful or failed retrievals). Furthermore, we identified ~13 single call types using two different hierarchical clustering analysis. In preliminary analyses, we do not observe significant differences in spectral and temporal call characteristics produced specifically during retrieval interaction. These results suggest that these USV features from the pups are not important for pup retrieval by adult female mice, and likely do not function as specific signals for social communication by the pups.

Disclosures: **J. Buchanan:** None. **B.Y. Lau:** None. **D. Casenhiser:** None. **K. Reilly:** None. **K. Krishnan:** None.

Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.02

Topic: F.02. Neuroendocrine Processes and Behavior

Support: UMass PBS Graduate Student Research Funds

Title: Selective recruitment of offspring-responsive medial preoptic area networks modulates caregiving behavior attuned to the needs of offspring

Authors: ***K. COPELAS**, N. CELESTIN, M. PEREIRA;
Univ. of Massachusetts, Amherst, Amherst, MA

Abstract: The medial preoptic area (mPOA) is a critical integration site where hormonal signals and offspring-related sensory information converge to facilitate maternal caregiving behavior. The mPOA mediates flexible caregiving behaviors through projections to areas including the infralimbic cortex (IL) and the ventral tegmental area (VTA). The objective of this study is to map out the distribution of offspring-responsive mPOA→IL- and mPOA→VTA-projecting neurons and evaluate how recruitment of these regions changes during interactions with pups of varying needs. To this aim, I used fluorescent retrograde axonal tracers and cFos immunohistochemistry to visualize the subregional origins of cFos-expressing mPOA→IL and mPOA→VTA neurons in the maternal mPOA during mother-litter social interactions. In order to investigate which mPOA networks are selectively recruited during exposure to offspring of

various needs, a second study used compartment analysis of temporal activity by fluorescent *in situ* hybridization (catFISH) to evaluate temporospatial mPOA recruitment during delayed dual-exposure events with low-needs (non-demanding) and high-needs (demanding) litter conditions. Results thus far show that the proportion of mPOA→IL and mPOA→VTA neurons recruited is greater under demanding pup conditions compared to non-demanding conditions, and that certain mPOA subregions (MnPO, MPA, VMPO) may be more sensitive to offspring need signals than others. By identifying the circuits of mPOA neurons that are specifically responsive to offspring signals we can gain greater insight into distinct aspects of maternal sensitivity and how mothers mother.

Disclosures: K. Copelas: None. N. Celestin: None. M. Pereira: None.

Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.03

Topic: F.02. Neuroendocrine Processes and Behavior

Support: NIH-MH119631

Title: Postpartum-induced alterations in colocalization of CRF receptors 1 and 2 with oxytocin neurons

Authors: *K. E. PARRA¹, R. M. DE GUZMAN¹, J. J. LAFRICAN¹, K. A. RYBKA¹, M. SHARIF¹, L. UGARTEMENDIA UGALDE², N. J. JUSTICE², D. G. ZULOAGA¹;

¹SUNY Albany, Albany, NY; ²Univ. of Texas Hlth. Sci. Ctr., Houston, TX

Abstract: The postpartum period is characterized in part by a state of resilience that is unlike any other phase of adulthood. This resilience that mothers experience is adaptive to both survivability and the maintenance of maternal care in the face of stressors and may partially be accounted for by changes in corticotropin releasing factor (CRF) and oxytocin signaling after parturition. Using two transgenic mouse lines (CRFR1-GFP and CRFR1-Cre-tdTom) that report current expression levels of corticotropin releasing factor receptor 1 (CRFR1), we found that CRFR1 uniquely appears in oxytocin (OT) neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of postpartum mice. Male mice and reproductively naïve (nulliparous) female mice do not exhibit this colocalization of CRFR1 and oxytocin neurons, and CRFR1 is entirely absent from the SON of nulliparous females. The expression of colocalized CRFR1/OT neurons increases in relevant brain regions as the postpartum period progresses, peaking at the time of weaning (postpartum day 21). Using c-Fos immunohistochemistry, we determined that CRFR1/OT neurons are activated in response to an acute stressor. We also confirmed the physiological implications of these neurons through in-vitro electrophysiology which verified that they are activated in response to bath application of CRF. Additionally, we have used several different hormonal and experiential manipulations to pinpoint what aspect(s) of the

postpartum period contribute to this co-expression of CRFR1 within OT neurons. Using a third transgenic mouse line (CRFR2-Cre-tdTom) that reports another receptor for CRF, CRFR2, we have gathered preliminary evidence to suggest that CRFR2 colocalization with OT also changes in hypothalamic brain regions of postpartum mice compared with nulliparous females and males. Taken together, these stress-related receptors likely play a role in the adaptive behavioral alterations seen in maternal mice.

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Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.04

Topic: F.02. Neuroendocrine Processes and Behavior

Support: Leon Levy Foundation Postdoctoral Fellowship
Brain & Behavior Research Foundation NARSAD Young Investigator Award
NSF Graduate Research Fellowship
DP1MH119428
Program Projects Grant (NS074972)
BRAIN Initiative (NS107616)
NICHD (HD088411)

Title: Neural circuitry for oxytocin release and maternal behavior

Authors: ***S. VALTCHEVA**^{1,2}, H. A. ISSA³, C. BAIR-MARSHALL³, K. A. MARTIN³, K. JUNG⁴, H.-B. KWON⁴, R. C. FROEMKE³;

¹Univ. of Cologne, Univ. of Cologne, Cologne, Germany; ³New York Univ., ²New York Univ., New York, NY; ⁴Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Parenting behaviors emerge from complex neural circuits conferring sensitivity to infant needs to ensure survival of the species. One important molecular signal for the maternal brain is oxytocin (OT), a nine amino acid peptide produced mainly in the hypothalamus (Althammer 2017; Jurek 2018; Valtcheva 2019). Oxytocin is believed to powerfully enhance parental behaviors by increasing the salience of sensory cues from the offspring (Dölen 2015; Grinevich 2018; Froemke 2021). However, it remains unexplored what sensory cues from infants can activate OT neurons in new mothers. Here we describe a neural circuit routing auditory information about infant vocalizations to the OT system in maternal mice (dams). By performing in vivo cell-attached and whole-cell recordings from optically-identified OT neurons, as well as fiber photometry of OT cells in awake dams, we found that OT neurons, but not other hypothalamic cells, are activated following playback of pup distress vocalizations. Using

anatomical tracing and channelrhodopsin-assisted circuit mapping (Petreanu et al., 2007), we identified the projections and brain areas (from inferior colliculus and auditory cortex to the posterior intralaminar thalamus, PIL) relaying auditory information to OT neurons. We identify the PIL as a major auditory input to OT cells. In hypothalamic brain slices, we found that optogenetic activation of PIL fibers led to long-term depression of synaptic inhibition in OT neurons mediated by postsynaptic internalization of GABAARs via dynamin signaling. Therefore, persistent activation of OT neurons following pup calls in vivo is likely mediated by disinhibition. Using a genetically-encoded OT sensor (Mignocchi et al., 2020), we demonstrated that pup calls, but not pure tones, were efficient in triggering OT release in downstream areas such as the VTA. Finally, inhibiting PIL projections to hypothalamus with chemogenetics, perturbed pup retrieval behavior in dams by decreasing the amount of pups retrieved and increasing the latency of retrieval over multiple trials. These findings suggest that the thalamus-hypothalamus noncanonical auditory pathway may be a specific circuit for the detection of social sounds, important for disinhibiting OT neurons, gating OT release in downstream brain areas, and sustaining maternal performance over time.

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Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.05

Topic: F.02. Neuroendocrine Processes and Behavior

Support: UMass Amherst Graduate School Research Grants, Pre-dissertation Research Grant
UMass Amherst Graduate School

Title: Chemogenetic activation of the medial preoptic area ameliorates deficits in maternal motivation in the wistar-kyoto rat model of depression

Authors: *A. A. ANDERSON¹, M. P. HESTER², M. PEREIRA³;

¹Dept. of Psychological and Brain Sci., ²Biol., Univ. of Massachusetts, Amherst, Amherst, MA;

³Dept. of Psychological and Brain Sci., Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Drug use in new mothers is a serious clinical problem that affects postpartum women's health and the health of their children severely. Accumulating evidence strongly suggests that cocaine use during the postpartum period is significantly impacted by the competing motivation to care for the child. However, this relapse-resistant phase is not achieved in new mothers experiencing postpartum depression, suggesting that depression-related deficits in maternal motivation underlie the higher rates of drug relapse rates. Given the demonstrated importance of the medial preoptic area (mPOA) in maternal motivation, the present study

investigated the functional sufficiency of the mPOA in preventing relapse of cocaine seeking in new mothers. To accomplish this goal, we used the well-validated Wistar-Kyoto (WKY) rat model of depression, which prior studies in our lab demonstrated exhibits reduced maternal motivation, and employed a novel adaptation of the extinction-reinstatement conditioned place preference (CPP) model of relapse. We used Gq-coupled designer receptors exclusively activated by designer drugs (DREADDs) to investigate the effect of chemogenetically activating the mPOA in preventing relapse to cocaine seeking in new WKY mothers. Virgin WKY and control Sprague-Dawley (SD) females were trained to acquire a cocaine CPP, which afterwards was extinguished during pregnancy, and subsequently retested during early postpartum, when the new mothers were concurrently faced with a highly salient new stimulus in their environment, their offspring, by re-exposure to cocaine (subthreshold challenge dose). Consistent with the clinical literature, WKY mothers exhibited high reinstatement rate, with the majority (80+%) preferring the cocaine- over the pup-associated option during cocaine-primed reinstatement testing, highly contrasting the distribution of preferences of control SD mothers. Results thus far suggest that chemogenetic activation of the mPOA biased the decision making of WKY mothers toward pup-associated cues. In addition, mPOA activation ameliorated the caregiving deficits of WKY mothers. Together, results suggest that altered activity of mPOA circuits underlies depression-related deficits in maternal motivation. Considering the impact of maternal cocaine use on both mother and child health, it is of major clinical significance to understand how maternal motivation reduces relapse to drug seeking.

Disclosures: **A.A. Anderson:** None. **M.P. Hester:** None. **M. Pereira:** None.

Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.06

Topic: F.02. Neuroendocrine Processes and Behavior

Support: NIMH Grant RO1MH106656
Feil Family Foundation

Title: Parvalbumin-positive interneuron regulation of maternal pup retrieval behavior

Authors: ***A. PAGLIARO**¹, D. RUPERT^{1,2}, S. D. SHEA¹;
¹Cold Spring Harbor Lab., Cold Spring Harbor, NY; ²Stony Brook Univ., Stony Brook, NY

Abstract: Learning requires the formation or modification of neural circuits - an extraordinary showcase of the brain's plasticity. The goal of this work is to uncover how circuitry of the auditory cortex (AC) facilitates learning of an auditory-driven maternal retrieval behavior in mice. Mouse pups emit ultrasonic vocalizations (USVs) when they are separated from the nest which cues maternal retrieval - a learned response to these distress cries. Interestingly, the transcription factor methyl-CPG binding protein 2 (MeCP2) is required for successful retrieval.

Furthermore, a subpopulation of inhibitory cells, parvalbumin-positive (PV) interneurons, is particularly susceptible to MeCP2 perturbations. Females with an MeCP2 mutation (a Rett Syndrome model) exhibit deficits in pup retrieval, have elevated AC PV expression, and more perineuronal nets encompassing AC PV cells - a hallmark signaling the closure of plasticity periods. This suggests that disruptions to the AC PV inhibitory circuitry may underlie deficits in retrieval by limiting the cortical plasticity necessary to learn this behavior. However, PV activity has never before been probed *during* retrieval. Here, we aim to uncover the real-time PV network contributions to retrieval, and how disruptions to the network impair behavior. Using fiber photometry, we found AC PV activity was highly dynamic throughout retrieval sessions in wild types, but not MeCP2 mutants. In wild types, there are pronounced peaks in AC PV activity during both auditory events (USV occurrences) and retrieval behavioral epochs such as the mouse contacting pups. We hypothesize that the dynamic nature of the wild type signal reflects significant retrieval motifs and facilitates successful behavior, whereas the overall absence of such signal flexibility in MeCP2 mutants reflects circuit dysregulation underlying retrieval deficits. Interestingly, we have also found that the AC PV signal is highly correlated with pupillary diameter (a proxy for attention) in wild types, but not MeCP2 mutants. This result informs a non-mutually exclusive hypothesis that the well-defined peaks in the wild type AC PV signal reflect an attentional PV network state that may facilitate retrieval. Our ongoing work aims to investigate this potential attentional state and probe how it may modulate responses to pup USVs, and/or prompt retrieval-associated behavioral events.

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Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.07

Topic: F.02. Neuroendocrine Processes and Behavior

Support: NIH-MH119609

Title: Oxytocin receptor-expressing neurons in anteroventral periventricular nucleus regulate pupretrieval.

Authors: *A. ABDOLLAHI GOVAR¹, K. SHARMA¹, B. GHIMIRE¹, K. NISHIMORI², R. TERUYAMA¹;

¹Dept. of Biol., Louisiana State Univ., Baton Rouge, LA; ²Dept. of Obesity and Intrnl. Inflammation, Fukushima Med. Univ., Fukushima, Japan

Abstract: Mother-infant bonding is crucial for the survival and development of offspring. The oxytocin system has an essential role in mother-infant bonding. Oxytocin modulates maternal behavior by binding to oxytocin receptors (OXTRs) in various parts of the brain. Previously, we showed that OXTR is expressed in the anteroventral periventricular nucleus (AVPV) of females,

but not in males, using OXTR reporter (OXTR-Venus) mice. This finding suggests that OXTR neurons in the AVPV are involved in the expression of female-specific behaviors. The present study was conducted to investigate if OXTR cells in the AVPV are involved in the regulation of maternal behaviors. The total number of the OXTR-Venus cells was significantly greater in postpartum dams (1371 ± 89 cells) than in virgin females (644 ± 23 cells, $p < 0.0001$). Since dopaminergic neurons in the AVPV are involved in the regulation of maternal behaviors, immunocytochemistry of tyrosine hydroxylase (TH) antibody was performed on brain slices from OXTR-Venus mice. Only ~25% of OXTR-Venus cells were TH⁺ in postpartum dams and virgin females, although the number of TH⁺-OXTR-Venus cells in postpartum dams was significantly higher (339 ± 41) than in virgin females (198 ± 7 cells, $p = 0.0004$). To assess if activity of the OXTR cells is involved in expression of maternal behaviors, the Designer-Receptors Exclusively Activated by Designer Drugs (DREADDs) technique was used. A Cre-recombinase-dependent adeno-associated virus vector containing inhibitory DREADD (hM4D(Gi)) or control DREADD (DIO) was injected into the AVPV of OXTR-Cre mice. A series of maternal behavior tests were conducted on the postpartum day one: first without Clozapine-N-Oxide (CNO: a ligand for the DREADD) and subsequently with i.p. injection of CNO. None of hM4D(Gi) dams retrieved any pups following the CNO injection. The CNO injection did not affect the ability of DIO dams to retrieve pups. After the CNO injection, hM4D(Gi) dams spent significantly less time (915.3 ± 285.9 s) crouching over pups compared to themselves without CNO (1993.5 ± 86.08 s, $p = 0.008$). The effect of CNO was not observed in the time spent inside the nest, pup-directed licking, self-grooming, eating, or drinking behaviors in hM4D(Gi) or DIO dams. These findings demonstrate that OXTR cells in the AVPV are involved in the expression of specific maternal behaviors namely pup retrieval and crouching over pups.

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Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.08

Topic: F.02. Neuroendocrine Processes and Behavior

Title: Neurobiology of pregnancy and the postpartum period in the common marmoset

Authors: *T. M. DRAZAN¹, K. M. COLE^{2,3}, S. P. BRADLEY⁴, C. C. YEN⁵, K. F. BERMAN², P. J. SCHMIDT³, Y. CHUDASAMA^{1,4},

¹Section on Behavioral Neurosci., ²Section on Integrative Neuroimaging, ³Section on Behavioral Endocrinol., ⁴Rodent Behavioral Core, Natl. Inst. of Mental Hlth., Bethesda, MD; ⁵Reproductive Biol. and Genet. Core, Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Preclinical data suggest pregnancy is associated with neurobiological changes in brain regions relevant to maternal behavior. Rodent studies have modeled some mood- and anxiety-like symptoms related to parturition (Suda et al., 2008, *Biol Psychiat.* 2008 64:311-9; Maguire and Mody, 2008, *Neuron* 59:207-13), but there is a virtual absence of studies that directly examine changes in behavior or in critical brain circuits accompanying pregnancy and parturition in nonhuman primates. Given their phylogenetic relatedness to humans and the complexity of their social organizations, we chose the common marmoset (*Callithrix jacchus*) as a nonhuman primate model to determine both normal and adverse consequences of pregnancy and the postpartum period, and its effects on maternal behavior. Eight nulliparous females (4 pregnant; 4 non-pregnant controls) were studied at four time points: pre-pregnancy baseline, gestation 1 (50-70 days), gestation 2 (105-125 days), and postpartum (3-14 days). Infant-directed behavior was assessed using a custom-built T-Maze designed specifically to assess preferences or choices for one infant over another. The number of times the female approached either infant and the overall pattern of movement was tracked using EthoVision and compared with non-pregnant multiparous females or postpartum controls. Structural (T1-weighted and DTI) and functional (awake resting-state) MRI scans were collected on a 7T Bruker scanner. Our preliminary behavioral data confirm nulliparous females were less sensitive to infants across gestation relative to their pre-pregnancy baseline behavior. During the postpartum period, the now primiparous females became more sensitive to infant calls, and displayed behaviors similar to those observed in postpartum female controls. Thus, our findings support previous reports of infant avoidance in nulliparous females prior to parturition and increased infant responsiveness during the postpartum. Future analysis of our multimodal neuroimaging data will help us elucidate the relationship between pregnancy and the postpartum, and elucidate putative brain changes that might impact the emotionality of the female toward the infant in the postpartum period.

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Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.09

Topic: F.02. Neuroendocrine Processes and Behavior

Support: Princeton University New Ideas in the Natural Sciences (Dean for Research Innovation Fund)

Title: Neural basis of male parenting in the African striped mouse (*Rhabdomys pumilio*)

Authors: *F. D. ROGERS¹, A. KASPER³, S. S. WANG¹, R. MALLARINO², C. J. PENA¹;
¹Princeton Neurosci. Inst., ²Dept. of Mol. Biol., Princeton Univ., Princeton, NJ; ³Rochester Inst. of Technol., Rochester, NY

Abstract: It remains largely unknown what neural mechanisms subserve naturally occurring, mammalian male parenting. It is also unknown by which mechanisms environmental influences drive variation in male parenting. In a series of experiments, we leverage the naturally occurring parental behavior of adult male African striped mice (ASM, *Rhabdomys pumilio*) to address this knowledge gap. In an initial set of behavioral experiments, we established that sexually naïve male ASM display experience-dependent variation in parental care for novel pup stimuli. Manipulation of peri-adolescent social experience (i.e., rearing in group housing or social isolation), reflective of natural conditions, promotes distinct behavioral phenotypes of male parenting. Socially isolated males demonstrate more licking/grooming ($p < .001$) and maintain more physical contact ($p < .001$) with pup stimuli than group housed males. Using this variation, we then identified possible neural correlates of male parenting by analyzing patterns of immediate early gene expression using both immunolabeling-enabled 3D imaging of solvent-cleared organs (iDISCO) and immunohistochemistry (IHC). In a pilot sample using iDISCO, we identified that expression of cFOS+ cells is greater in the medial preoptic area (MPOA) of ASM males interacting with a novel pup than those in an empty cage ($p < .001$). In two subsequent cohorts, we applied IHC for the unbiased, global characterization of cFOS activity in sexually naïve ASM males exposed to novel pup stimuli ($n = 22$) or an empty cage ($n = 20$). In pup-exposed males, several regions demonstrate appreciable cFOS expression, including the MPOA. Within this group, we compare cFOS expression in these regions between socially isolated and group housed males, by generalized phenotype (e.g., parental, ambivalent, infanticidal), and in correlation with specific parental care behaviors (e.g., contact time). In a subsequent experiment, we then applied bulk RNA-sequencing to tissue from the MPOA for the identification of candidate genes, possibly involved in paternal care behavior. We compared gene expression in the MPOA from a sample of males demonstrating care toward novel pup stimuli ($n = 5$) and those demonstrating infanticide and ambivalence ($n = 5$). We are identifying differentially expressed genes and altered gene network patterns related to rearing experience and paternal vs infanticidal behavior. These sequencing results may inform selection of genes of which activity might be manipulated in future experiments. Together, our findings further elucidate the neural basis of male parenting in ASM, and provide potential candidates for such mechanisms in other species.

Disclosures: F.D. Rogers: None. A. Kasper: None. S.S. Wang: None. R. Mallarino: None. C.J. Pena: None.

Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.10

Topic: F.02. Neuroendocrine Processes and Behavior

Support: NIH Grant F31MH123025
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Title: Sex differences in the transcriptional networks underlying playfulness suggest a distinct function for play in males compared to females

Authors: *A. E. MARQUARDT¹, J. W. VANRYZIN², M. BASU³, B. ALTAS², S. A. AMENT⁴, M. M. MCCARTHY²;

¹Program in Neurosci., ²Dept. of Pharmacol., ³Inst. for Genome Sci., ⁴Dept. of Psychiatry and Inst. for Genome Sci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Social play is a dynamic, well-conserved behavior known to be sexually differentiated; in most species, males play more than females, a sex difference driven by the medial amygdala (MeA). To investigate whether the transcriptional signatures underlying play also differ by sex, we performed RNA-sequencing of MeA samples from high- and low-playing juvenile rats of both sexes. Using Weighted Gene Co-expression Network Analysis (WGCNA), we identified 22 co-expression modules, or networks of genes highly correlated in expression. Of the 12 modules (for $p < 0.05$) associated with play, almost all (11/12; ~92%) are sex-specific in expression, correlating with expression of play in one sex only. These data suggest there is a distinct transcriptomic profile associated with playfulness in the MeA of males compared to females, a noteworthy finding given the MeA regulates many sex-typical adult behaviors. We propose this is no coincidence: play-associated gene networks in the MeA are sex-specific because play modulates circuitry driving different adult behaviors in males compared to females. To test this hypothesis, we constructed Plexiglass cage dividers to separate juvenile cagemates and thus prevent juvenile play, predicting that this would result in impairments in later-life behavior that would differ by sex. Supporting our hypothesis, preventing juvenile play impaired object memory and copulatory behavior and resulted in a hypersocial phenotype in adulthood in males only. Surprisingly, females were largely resilient to this manipulation. To investigate whether expression of our sex-specific gene modules is causally related to juvenile playfulness and to expression of later-life behavior, we are currently developing viruses to drive module overexpression via CRISPR activation. Together, these analyses will provide novel insight into the ultimate function of play and how and why this may differ by sex.

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Poster

478. Sexual and Parental Behavior

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.11

Topic: F.02. Neuroendocrine Processes and Behavior

Support: NIH R01NS082179
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Title: Neuroestrogen regulation of inhibitory synaptic transmission in genetically-identified auditory neurons

Authors: *H. KANG¹, Y. YAZAKI-SUGIYAMA², Y. MOROHASHI², L. REMAGE-HEALEY¹;

¹Neurosci. and Behavior, Univ. of Massachusetts Amherst, Amherst, MA; ²Okinawa Inst. of Sci. and Technol. (OIST) Grad. Univ., Okinawa, Japan

Abstract: Neuroestrogens, brain-derived estrogens, are synthesized by the enzyme aromatase, which is abundant in the forebrain auditory circuits of vocal learners including humans and songbirds. In songbirds, the local synthesis of neuroestrogens in the forebrain is responsive to song stimuli and regulates neuronal firing and synaptic transmission to shape song-evoked responses and auditory learning. However, the synaptic mechanisms and identified cell types that account for these rapid actions of neuroestrogens remain unclear. To address this gap, we used cell-type specific viral vectors and whole-cell voltage clamp electrophysiology to understand the synaptic mechanisms of acute neuroestrogen actions in the zebra finch auditory forebrain. We particularly examined the caudomedial nidopallium (NCM) which is important for auditory processing and memory, and used viral targeting of either calcium/calmodulin-dependent protein kinase II (CaMKII) expressing primary excitatory neurons or glutamate decarboxylase 1 (GAD1) expressing inhibitory interneurons. We hypothesize that neuroestrogens acutely modulate inhibitory synaptic transmission by regulating GABAergic input onto principal excitatory and inhibitory cell types. Our results thus far indicate that inhibitory synaptic transmission onto CaMKII neurons is a primary target of neuroestrogen modulation. Ongoing experiments are examining the G-protein coupled estrogen receptor1 (GPER1) as a primary molecular mediator for these acute actions. Broadly, these findings will reveal how neuroestrogens specifically modulate genetically-identified neurons, and further elucidate the mechanisms of neuroestrogen regulation of sensory processing in a vocal learning species.

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Poster

478. Sexual and Parental Behavior

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Topic: F.02. Neuroendocrine Processes and Behavior

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AMB is student PhD on Physiological Sciences and fellowship from CONACYT No. 662091

Title: Ultrasonic vocalizations as a sexual incentive in high- and low- yawning rats

Authors: *A. MORA-BOLAÑOS¹, C. CORTES¹, J. R. EGUIBAR²;

¹Behavioral Neurophysiology, Inst. of Physiol., ²Behavioral Neurophysiology, Inst. of Physiology; Intl. Office., Benemérita Univ. Autónoma de Puebla, Puebla, Mexico

Abstract: During sexual interactions the male and female rats emit 50-KHz ultrasonic vocalizations (USVs), as a form of socio-affective communication that promoted copulation. Those USVs can be subdivided into flat, frequency-modulated and trill calls with different communication values. The aim of this study was to evaluate the USVs emitted by male rats during a mate choice paradigm for copulation with different male partners. We used high-yawning rats (HY) with a mean of 20 spontaneous yawns/h, low-yawning (LY) and outbred Sprague-Dawley (SD) with less than 2 yawns/h. All subjects were maintained under standard conditions. We used a specific designated maze that consist of 5 dark boxes, the central one (40 cm x 30 cm x 45 cm) and 4 peripheral ones (40 cm x 30 cm x 30 cm) that communicate through PVC tubes that do not allow touching among the subjects. Hearing and olfactory stimuli are possible. Sexually receptive female was placed in the central cage and three sexually experienced HY, LY, SD males were randomly assigned in each box and one empty box as a control. The subjects were habituated for 5 min for basal recordings of USVs, then a receptive female added in the central box and additional 5 min of recording of USVs were taken using ultrasonic microphones (UltraVox™, Noldus Technologies, The Netherlands). All experiments were done during the dark phase and the acoustical analysis was done using UltraVox™ software. Our results showed that the duration and number of USVs were not different among the different male tested. However, trill calls in LY males were significantly higher with respect to HY and SD male rats ($P \leq 0.05$), there were not significant differences in the frequency-modulated and trill calls among different male rats. These results showed that LY male rats are the only capable of emit trills that could be useful for a more efficient sexual interaction.

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Poster

478. Sexual and Parental Behavior

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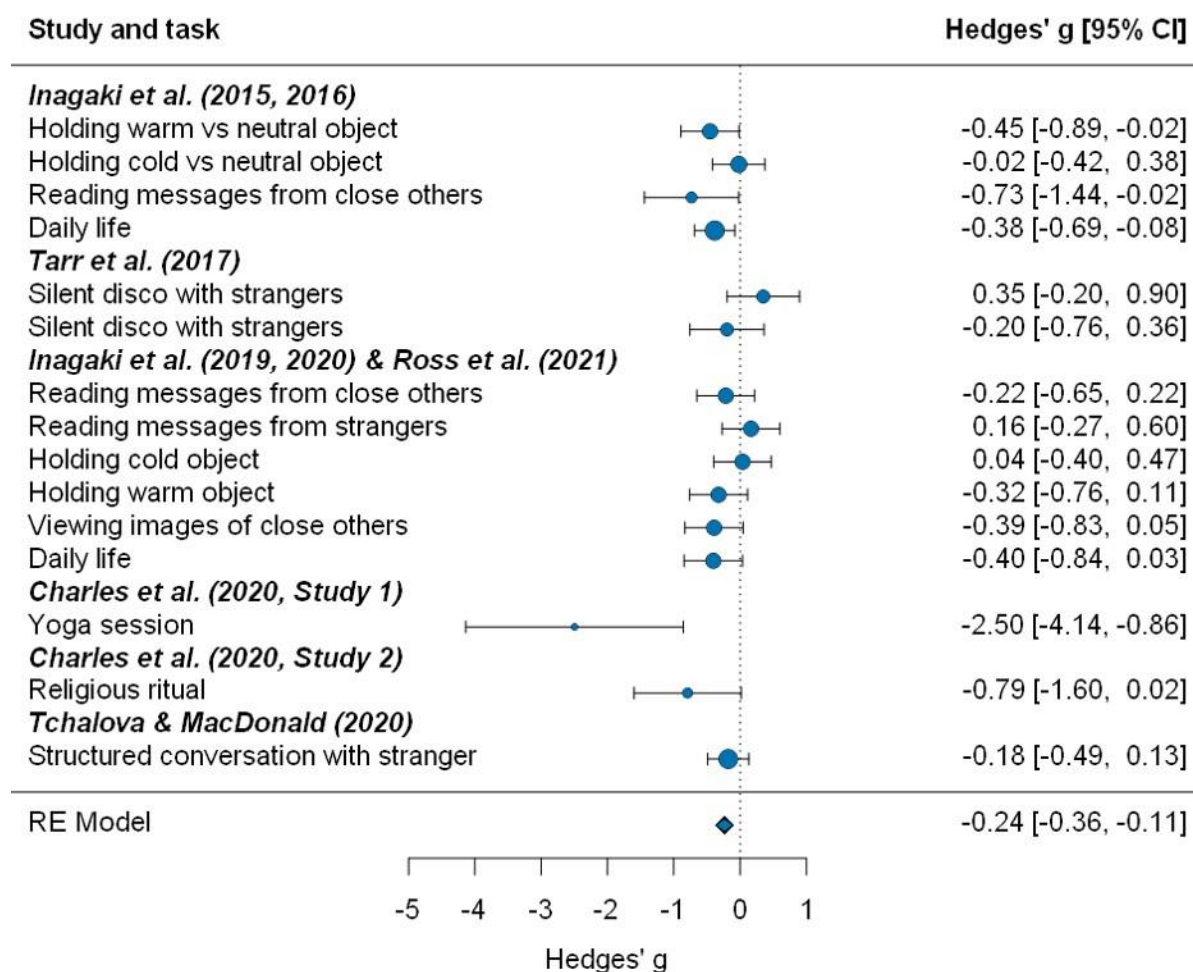
Topic: F.02. Neuroendocrine Processes and Behavior

Support: ERC Starting Grant 802885 to Siri Leknes
South-Eastern Norway Regional Health Authority grant number 2020087 to Siri Leknes
South-Eastern Norway Regional Health Authority grant number 2018035 to Marie Eikemo

Title: Opioid Modulation of Social Bonds in Humans - a Meta-Analysis

Authors: *G. E. LØSETH¹, M. TRØSTHEIM^{2,1}, M. EIKEMO¹, S. LEKNES¹;
¹Dept. of Psychology, Univ. of Oslo, Oslo, Norway; ²Dept. of Diagnos. Physics, Oslo Univ. Hosp., Oslo, Norway

Abstract: Social motivation and bonding processes in non-human animals rely on and are modulated by opioid signalling (e.g. Moles et al. 2004, *Science*; Løseth et al. 2014, *Front.Behav.Neurosci.*). An emerging body of literature suggests opioid modulation of social connectedness also in humans. We conducted a preregistered random-effects meta-analysis of randomized double-blind studies comparing the effects of a centrally active mu-opioid antagonist and an inert substance (placebo) on social connectedness in healthy humans (see osf.io/x5wmq for preregistered methods). Data from eight publications reporting a total of fifteen outcomes from six different studies were included (N = 379). The studies all used naltrexone (25-100mg) to block the endogenous opioid system. Outcomes were self-reported measures of social connectedness. On average, naltrexone slightly reduced feelings of social connectedness (Hedges' g [95% CI] = -0.24 [-0.36, -0.11]). Results were highly consistent within and between studies ($I^2 = 16\%$), but there was some indication of publication bias in favour of larger negative effects (Egger's test: $B = -2.48$, $SE = 0.99$, $z = -2.51$, $p = 0.01$). These results indicate that the endogenous opioids system does play a role in modulating or fine-tuning human feelings of social connectedness. However, the findings clearly demonstrate that the human experience of social connectedness is not dependent on intact opioid signalling, with robust ratings of social connectedness even during pharmacological opioid blockade. The small effect size observed in human studies compared to non-human animal findings could relate to the nature of the measure (subjective versus behavioural/motivation-related) or to differences in the neural representation of social connections in humans compared to e.g. macaques or rodents.



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Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

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Topic: F.02. Neuroendocrine Processes and Behavior

Support: R01 GM140415
UR SPIN pilot award

Title: Sex differences in nutrient-dependent behavior in *C. elegans*

Authors: *C. BAINBRIDGE, D. PORTMAN;
Univ. of Rochester, Rochester, NY

Abstract: Animals must meet nutritional requirements by coordinating cues about nutrient availability in the environment and their nutritional status. Often this is achieved through changes in foraging strategies that reflect current metabolic demands. Despite the ubiquitous requirement for nutrient intake, many animals show sexually dimorphic nutrient-dependent behaviors. However, the mechanisms by which biological sex acts to regulate neuronal function to produce sex-specific responses to nutrient status is poorly understood. *C. elegans* exhibit well-defined locomotor behaviors for food search and foraging. Past work from our lab and others indicates that *C. elegans* exhibit sexually dimorphic behavioral and neuronal responses to nutrient availability. Here, we investigate sex differences in satiety quiescence (nutrient-dependent cessation of locomotion and feeding) in *C. elegans* by recording both sexes on high quality bacterial food. From these recordings, we used a Hidden Markov Model to determine changes in nutrient-dependent locomotor behaviors. Preliminary results show that male worms exhibit nutrient-dependent strategies distinct from hermaphrodites. By manipulating the genetic sex-determination pathway, we found that the sexual state of the nervous system is essential for sex-specific nutrient-dependent behaviors. To determine if nutritional status might be modulated by biological sex, we tested insulin and IGF signaling (IIS) pathway mutants for changes in nutrient-dependent locomotor behavior in both sexes. Preliminary results suggest that increased insulin signaling in males may promote male nutrient-dependent behavior. This approach will serve to generate a framework to understand the genetic and neuronal basis of sex-specific nutrient-dependent behaviors. Moreover, this approach provides an opportunity to explore potentially conserved mechanisms by which sex can regulate neuronal and behavioral responses to nutritional status.

Disclosures: C. Bainbridge: None. D. Portman: None.

Poster

478. Sexual and Parental Behavior

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Topic: F.02. Neuroendocrine Processes and Behavior

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Title: Differential changes in brain functional networks by monoparental rearing in the prairie vole

Authors: *F. LÓPEZ-GUTIÉRREZ¹, Z. GRACIA-TABUENCA², J. J. ORTIZ¹, F. J. CAMACHO¹, R. G. PAREDES¹, L. J. YOUNG³, N. F. DIAZ⁴, S. ALCAUTER¹, W. PORTILLO¹;

¹Behavioral and Cognitive Neurobio., Inst. De Neurobiología, UNAM, Querétaro, Mexico;

²Dept. of Neurol. and Neurosurg., McGill Univ., Montreal, QC, Canada; ³Ctr. for Oxytocin and Social Cognition, Emory Univ., Atlanta, GA; ⁴Inst. Nacional de Perinatología Isidro Espinosa de los Reyes, Mexico City, Mexico

Abstract: The prairie vole (*Microtus ochrogaster*) is an animal model that enables the study of the neurobiology of complex social behaviors that are analogous to human behavior. Previous studies have shown that variation in early life parental structure in the prairie vole induces long term differences in brain neurochemistry and social behavior, likely producing several changes in the modulation of socio-sexual neural circuits. Since brain functional networks in voles are also known to undergo plastic changes due to social bonding, it is yet to be studied how these networks are influenced by monoparental rearing in juvenile and adult stages.

Behavioral observations revealed that overall, monoparental-reared pups were left alone for longer periods of time and received less licking/grooming than biparental pups. At postnatal (PND) day 21 and 70, male and female voles were subjected to resting-state functional magnetic resonance imaging (rsfMRI) acquisition. Network-based statistic (NBS) analysis comparing juvenile ($n=29$) vs adult ($n=25$) rsfMRI data identified significant changes in a functional network of 9 brain regions, suggesting plastic changes as a consequence of sexual maturity ($p=0.03$). The same voles ($>PND 70$) were placed in cohabitation for pair bond formation. When adult rsfMRI baseline data was compared, NBS analysis showed monoparental rearing induced a significant alteration in a network with 21 functional connections that correlated with the display of affiliative behavior (huddling latency) during the first hours of cohabitation ($p=0.01$), finding a distinct modulation in reward-related regions compared to biparental-reared voles. Subjects were scanned again for rsfMRI data 24 hr after the onset of cohabitation. In contrast to biparental-reared subjects ($n=8$, $p=0.03$), monoparental-reared voles didn't show a significant preference for their partner at 48 h of cohabitation ($n=8$). Interestingly, at 24 h of cohabitation, a functional network that significantly correlated with the amount of social interaction during the partner preference test was not found to be influenced by early life family structure ($n=16$, $p=0.018$), and a significant correlation was also found between the partner preference index and the medial preoptic area - periaqueductal gray functional connectivity in all subjects ($n=16$, $p=0.02$, $r=-0.8$).

These preliminary results suggest that the type of parental care in the prairie vole may influence the modulation of particular brain functional networks related to socio-sexual behavior, suggesting differences in social-reward processing, and also showing that such modulation may be temporally dynamic and context-dependent.

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Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.16

Topic: F.02. Neuroendocrine Processes and Behavior

Support: NIH MH114994

Title: Topical oxytocin influences brain response to licking and grooming

Authors: *A. GONZALEZ, N. FERNANDEZ, E. RODRIGUEZ FORTI, E. A. HAMMOCK;
Psychology, Florida State Univ., Tallahassee, FL

Abstract: The neuropeptide, oxytocin, plays a role in many neurobiological processes across species, including social behavior and maternal care. In many rodents, licking and grooming behaviors are a significant component of parental care. Deficits in parental licking of neonatal offspring alters social behavior in later life. Licking of the anogenital area not only contributes to typical social and sexual behavior development but is also crucial for the survival of the offspring by promoting waste elimination. Artificial stimulation of the anogenital area in neonatal rats and rabbits increased c-Fos immunoreactivity in the hypothalamus. We observed oxytocin receptors (OXTR) in the anogenital area of neonatal mice and prairie voles, raising the possibility that parental salivary oxytocin may activate OXTR during licking and grooming behaviors. Therefore, the goal of the present study is two-fold: 1) determine if exogenous oxytocin applied to the anogenital area of neonatal mouse pups modulates brain activity and 2) determine if OXTR expression is localized to sensory neurons innervating the anogenital area. To accomplish the first aim, male and female postnatal day 8 OXTR-EGFP mice were randomly assigned to either a “holding” control or stimulation group. Artificial stimulation of the anogenital area was accomplished with a paint brush dipped in one of three solutions per animal: saline vehicle, 1 μ M oxytocin, or 1 μ M OXTR antagonist - atosiban. Stimulation sessions were video recorded for behavioral responses. Brain tissue was collected 90 minutes after stimulation and processed for c-Fos immunohistochemistry. Analyses indicate c-Fos activity in the hypothalamus of animals in each group, with the greatest number of c-Fos positive cells identified in animals that received oxytocin treatment. Additionally, preliminary analysis of OXTR-EGFP postnatal day 0 male and female mice collected and prepared for immunohistochemistry indicates OXTR expression in a small population of lower dorsal root ganglia sensory neurons. Together, these data suggest exogenous oxytocin applied to the anogenital area augments sensory-dependent activity of hypothalamic neurons. Funding: NIH MH114994

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Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.17

Title: WITHDRAWN

Poster

478. Sexual and Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 478.18

Topic: F.02. Neuroendocrine Processes and Behavior

Support: Simons Collaboration on the Global Brain
HHMI
Wenner-Gren Foundation

Title: Mesoscale hypothalamic network dynamics during innate behaviors

Authors: *S. STAGKOURAKIS¹, G. SPIGOLON¹, M. FEYDER², J. KIM¹, M. PACHITARIU³, D. J. ANDERSON¹;

¹Caltech, Pasadena, CA; ²Stanford Univ., Stanford, CA; ³Janelia Res. Campus, Ashburn, VA

Abstract: Understanding the neural basis of innate behaviors has relied significantly on our ability to record and perturb the neural activity of single brain nuclei. The hypothalamus attracted early interest, as stimulation of specific hypothalamic sites leads to the triggered expression of instincts, such as aggression, mating, or fear. A promising approach towards understanding better the role of the hypothalamus in behavior is to investigate how dynamic spatio-temporal activity patterns throughout the hypothalamus correlate with behaviors of interest. Yet it has been challenging to measure neural activity with single-cell resolution simultaneously in multiple hypothalamic sites in freely behaving animals. In the present study we performed mesoscale neural recordings from ~20 hypothalamic subregions covering ~400 units/animal in freely moving mice performing 3 different innate behaviors (mating, fighting and predator defense), using custom silicon probes. Our observations suggest that neural activity during specific behaviors is distributed across many recorded sites along the A-P axis, and that activity sampled sparsely within single hypothalamic nuclei (~20 cells recorded/nucleus/animal) correlates with diverse behavioral activities. In addition, we identify behavior-specific mesoscale features, such as the expression of a sharp, panhypothalamic “ignition” peak in neural activity across all recorded sites at the onset of attack, as compared to other behaviors. Application of clustering methods to single-unit data revealed that: 1) the majority (>50%) of hypothalamic units exhibit mixed selectivity, while only a fraction of cells (typically 5-10%) exhibit behavior-specific activity, which fluctuates across behavioral episodes; 2) neural activity clusters have a strong spatial bias, typically composed of cells originating from a few nearby subregions; and 3)

clusters with similar activity can be found at opposite ends of the A-P axis. Our findings identify mixed selectivity as a dominant mode of hypothalamic neural activity during innate behaviors. Specific behavior-tuned neurons detected by this method are rare and exhibit activity of low fidelity across trials. These data suggest that behavior may be encoded through population dynamics, rather than via deterministic “labelled lines” (although they do not exclude that rare behavior-specific cell types were missed by our relatively sparse sampling). Future work is required to elucidate the functional role of distributed coding, as well as of spatially segregated hypothalamic ensembles which nevertheless exhibit similar activity profiles during behavior.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.01

Topic: F.03. Stress and the Brain

Title: Sex differences in the anti-depressant-like effect of Cannabidiolic acid methyl ester in a genetic rat model of depression

Authors: *D. HEN-SHOVAL^{1,2}, L. MOSHE^{1,2}, T. NAIMER², N. KOGAN³, H. ZAIDAN⁴, I. GAISLER-SALOMON⁴, R. MECHOULAM³, A. WELLER^{1,2};

¹Psychology Dept., ²Gonda Brain Res. Ctr., Bar-Ilan Univ., Ramat Gan, Israel; ³Med. Fac., Hebrew Univ. Inst. for Drug Res., Jerusalem, Israel; ⁴Sch. of Psychological Sci. and the Integrated Brain and Behavior Res. Ctr., Univ. of Haifa, Haifa, Israel

Abstract: The underlying pathophysiology of major depressive disorder (MDD) is diverse, yet treatment strategies remain limited. Cannabinoids and the endocannabinoid system have been linked to depression in clinical and pre-clinical studies. Cannabidiolic Acid-Methyl Ester (EPM-301) demonstrated more potent anti-nausea/anti-emetic effects than Cannabidiol (CBD) *in vitro* and *in vivo*, however, it has undergone only minimal psychopharmacological assessment. We explored the acute and chronic properties of EPM-301 using a depressive-like genetic rat model; the Wistar-Kyoto (WKY) rat, which presents various behavioral and physiological endophenotypes frequently observed in MDD. In the first study, male and female WKY rats underwent the Forced swim test (FST) following acute EPM-301 oral ingestion (1,5,10 mg/kg). In the second study, male and female WKY rats underwent the FST, Open Field test (OF) and Saccharine Preference Test (SPT) after chronic (14 days) oral ingestion of EPM-301 (0.5 mg/kg-males; 1/2.5/5 mg/kg-females) or 15 mg/kg of imipramine. Corticosterone blood serum levels and mRNA gene expression of CB1/CB2 receptors, Fatty Acid Amid Hydrolase (FAAH), the Serotonin transporter (SERT), Corticosterone Releasing-Hormone (CRH) and CRH Receptor type 1 (CRHR1) in the hippocampus were evaluated. In the third study, male and female WKY rats were injected with CB1 (AM-251) and CB2 (AM-630) antagonists 30 min before acute

EPM-301 ingestion (1 mg/kg for males and 5 mg/kg for females) followed by FST. Brain-Derived Neurotrophic Factor (BDNF) levels in blood serum were then measured. Results indicated sex differences in drug effects; the lower dosages produced a larger anti-depressant-like effect in males (1 mg/kg acutely and 0.5 mg/kg chronically) compared to females (5 and 10 mg/kg acutely in the FST and no chronic effect in response to any of the dosages). The chronic drug effect in males was accompanied by lower corticosterone levels and upregulated CB1R, FAAH, SERT and CRHR1 mRNA expression in the hippocampus. Furthermore, AM-630 blocked the acute anti-depressant-like FST effect in females, and elevated BDNF serum levels were detected in the EPM-301 treated group compared to the other groups. These findings expand the limited knowledge on the antidepressant effects of EPM-301, opening a path for cannabinoid-mediated treatment venues in MDD and related disorders.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.02

Topic: F.03. Stress and the Brain

Title: Prenatal Stress-induced Depressive-like Behavior is Associated with Brain Metabotranscriptome Remodeling

Authors: *S. ALHASSEN;
Univ. of California, Irvine, Irvine, CA

Abstract: Traumatic stress transmission from pregnant mothers to the offspring increases the life-course susceptibility to depression and is a major risk factor for developing different neuropsychiatric disorders. Whether the intergenerational trauma transmission and its deleterious outcomes are a consequence of in-utero fetal neurodevelopment disruptions or from poor maternal care by traumatized mothers are still largely unknown. The complexity, superposition, and inseparability between the prenatal and postnatal mechanisms, which are not mutually exclusive, make it difficult to test the mechanistic involvement of the prenatal stress independent of stress effects on the mother's care. On day 17 of gestation, female mice were exposed to a predator scent to induce stress to the animal. Pups of control (C) and stress (S) mice were raised by their biological mother (C/C and S/S) or were cross-fostered to a female of the other treatment (C/S and S/C for S pups with C mothers and C pups with S mothers respectively) within 24h of birth. The male mice were then selected for the study and underwent a series of behavioral assays to understand the effects of the prenatal and postnatal stress conditions. In the study, we were able to show that the exposure to traumatic stress during pregnancy induces in the offspring depressive-like behavior and social deficits through divergent and convergent mechanisms of both in-utero and early life parenting environments. Metabolomics, transcriptomics and

bioinformatics analyses reveal mechanisms that involve acute but robust stress- and hypoxia-response energy metabolic pathways (mitochondrial ATP production). These acute responses lead to long-lasting adaptations in glycolysis, homeostasis of energy lipids, membrane and signaling lipids, and epigenetic processes pertaining DNA and chromatin modifications. We were able to further demonstrate that by providing early pharmacological intervention through the use of acetyl-L-carnitine supplementation to correct the mitochondria and lipid metabolism as well as the epigenetic modifications, the mice are able to produce outlasting protection against the observed behavioral deficits.

Disclosures: S. Alhassen: None.

Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.03

Topic: F.03. Stress and the Brain

Support: NIH Grant T32 121780

Title: Pramipexole modulates pro-plasticity proteins, attenuates inflammation, and improves depressive-like behaviors in Balbc female mice

Authors: *K. GILBERT, K. CONANT;
Neurosci., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Major Depressive Disorder (MDD) is a pervasive, and often comorbid, mental disorder with a lifetime prevalence of approximately 20% in the United States and affecting roughly twice as many females than males. About 30% of individuals with MDD do not respond to traditional antidepressants. This presents a pressing need to identify novel therapeutic mechanisms for the treatment of MDD. Pramipexole (PPX), a dopamine 2/3 receptor (D2/3R) agonist, has been recently explored as a potential antidepressant; its 8-fold selectivity for D3Rs over D2Rs may be pertinent to its antidepressant effects. Previous studies have shown that D3R^{-/-} mice have a depressive behavioral phenotype, including light avoidance and lack of sucrose preference. In a mouse model of PD, 2 weeks of 1mg/kg PPX significantly improved depression-like behavior in D2R^{-/-} mice following chronic mild stress, but was not effective in D3R^{-/-} mice. In vitro, PPX increased plasticity, making it a promising antidepressant candidate for the improvement of neuroinflammatory and cognitive dysfunction mechanisms in MDD. We hypothesize that 2 weeks of treatment with 1mg/kg PPX will induce an anxiolytic and antidepressant-like phenotype in naturally anxious 6-week-old female Balbc mice by modulating pro-plasticity kinases, increasing dendritic arborization, and decreasing inflammation compared to controls or corticosterone treated mice. Anxiety and depressive-like behaviors are assessed using behavioral tests including the elevated plus maze and sucrose splash test, followed by biochemical analysis of the prefrontal cortex (PFC) and striatum and Golgi-Cox stain

quantification. Pilot data comparing PPX treated mice (N=10) to water controls (N=10) has indicated that protein levels of chemokines CCL5 (p=0.045) and CCL2 (p=0.011) were significantly decreased in the striatum of PPX-treated mice. PPX increased PSD-95 (p=0.051) and significantly decreased TIMP-1 (p=0.005) in the PFC compared to control mice, indicative of increased plasticity. We anticipate an increase in dendritic arborization from the Golgi stained hemi brains of the PFC. PPX-treated mice traveled significantly more in the closed arm of the EPM (p=0.035), which was strongly associated with striatal IL-6 levels ($r^2=0.3$, p=0.07). PFC TIMP-1 levels strongly negatively correlated with distance traveled ($r^2=0.3533$, p=0.0699) and significantly positively correlated with time spent in the closed arm ($r^2=0.6156$, p=0.0072) in control mice. Repurposing PPX to attenuate depressive-like behavior, inflammation, and cognitive impairment in female mice may have important implications on treatment outcomes for patients living with MDD.

Disclosures: K. Gilbert: None. K. Conant: None.

Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.04

Title: WITHDRAWN

Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.05

Topic: F.03. Stress and the Brain

Support: F31- MH123041
R01-MH101729
R01-MH048698

Title: Chronic Stress Alters Mitochondrial Function in Prefrontal Parvalbumin Interneurons

Authors: *N. NAWREEN¹, M. A. SMAIL², N. MAHI¹, R. E. MCCULLUMSMITH⁴, R. C. THOMPSON⁵, J. P. HERMAN³;

¹Univ. of Cincinnati, Cincinnati, OH; ²Pharmacol. and Systems Physiol., Univ. of Cincinnati, Independence, KY; ³Dept Pharmacol. and Systems Physiol., Univ. of Cincinnati, Cincinnati, OH;

⁴Neurosciences, Univ. of Toledo, Toledo, OH; ⁵Univ. of Michigan, Ann Arbor, MI

Abstract: Hypofunction of the prefrontal cortex (PFC) contributes to stress-related neuropsychiatric illnesses. Previous findings from our lab indicate that chronic stress enhances inhibitory neurotransmission in the PFC, however the mechanisms leading to the prefrontal hypoactivity is still unclear. Mounting evidence suggests that chronic stress leads to an increase in activity of parvalbumin (PV) expressing GABAergic interneurons (INs) in the PFC. As a result, the goal of this study was to elucidate the translational gene expression profile of parvalbumin interneurons in the prefrontal cortex following exposure to chronic stress. Transgenic mice Rosa26L10a-eGFP/L10a-eGFP; PV^{cre/+} were generated expressing an EGFP-L10a ribosomal fusion protein under control of a mouse PV promoter. Male mice were subjected to the chronic variable stress (CVS) paradigm, comprised of a series of randomly alternating stressors administered twice daily over a period of 14 days or served as non-stressed controls. 24 hours after cessation of the stress protocol, we isolated translating ribosomes specifically from prefrontal cortex parvalbumin interneurons using the Translating Ribosome Affinity Purification (TRAP) technique, followed by RNA-seq analysis. Data were analyzed using targeted pathway analysis using Enrichr and full transcriptome analysis using GSEA. Immunohistochemistry combined with Imaris volumetric analysis was utilized to confirm the findings. Our cross-pod analysis showed several pathways to be affected in PV INs following chronic stress, including regulation of lipid metabolism, adrenergic receptor signaling and alteration in mitochondrial functions. Specifically, mitochondria associated pathways such as mitochondrial respiratory chain complex, electron transport and oxidative phosphorylation were altered in PV INs following chronic stress. Importantly, mitochondrial DNA (mtDNA) encoded mitochondrial genes were upregulated and nuclear DNA (nDNA) encoded mitochondrial genes were downregulated in PV INs following chronic stress. Validation studies using immunohistochemistry confirmed enhanced mitochondrial size and volume following chronic stress. Collectively, these results provide new insights into the potential molecular mechanisms associated with chronic stress induced changes in prefrontal GABAergic PV INs, highlighted by alterations in mitochondrial function. Our data are consistent with recent findings noting changes in mitochondria following chronic stress and in the cortex of individuals with stress-related conditions.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.06

Topic: F.03. Stress and the Brain

Support: Swedish Research Council
R01 60203255

Title: Targeting prefrontal cortical somatostatin-interneurons to control antidepressant efficacy

Authors: *H. MUNGUBA, V. GUTZEIT, M. KRISTT, P. VADDI, C. M. LISTON, J. LEVITZ;
Weill Cornell Med., New York, NY

Abstract: Somatostatin-expressing (Sst+) interneurons in prefrontal cortex (PFC) were recently linked to the antidepressant effects of ketamine (KET) in rodents, suggesting these neurons to play a central role in antidepressant mechanisms. One of KET main pharmacological effects is via activation of mu-opioid receptors (MOR), an inhibitory G protein-coupled receptor enriched in PFC Sst+ interneurons. In accordance with previous reports in depressed patients, MOR blockage also attenuates KET antidepressant properties in rodents. In vivo imaging confirmed that global KET acutely inhibits PFC Sst+ neurons and whole-cell patch-clamp recordings in adult PFC slices confirmed that MOR activation leads to prominent somatic and presynaptic inhibition in Sst+ interneurons, but not pyramidal neurons. To test the hypothesis that PFC Sst+ interneurons is a key circuit component for antidepressant efficacy, we employed a cre-dependent viral vector to express pertussis toxin, to block Gi-coupled receptor signaling specifically in this population. This elicited a depression-like state of decreased active behaviors, resembling the phenotype produced after chronic stress, a central risk factor for the development of depression. To test whether PFC Sst+ interneurons are stress-sensitive, we performed bulk RNA-sequencing, synaptic recordings and morphological reconstructions after four weeks of chronic unpredictable stress (CUS). Our converging data support Sst+ interneurons' presynaptic properties to be stress-sensitive and an critical circuit component to be targeted to control antidepressant efficacy.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.07

Topic: F.03. Stress and the Brain

Support: VA (I01BX003858-01)

Title: Spatial Transcriptomic Analysis of Gene Expression in the Amygdala and Prefrontal Cortex of Adult Male Rats after Single Prolonged Stress

Authors: *R. K. PARIKH¹, C. CLOSSON¹, M. E. FITZGERALD¹, J. B. CHAMBERS^{1,2}, N. NAWREEN^{1,2,3}, M. A. SMAIL^{1,2,3}, C. MOORE^{1,2}, J. P. HERMAN^{1,2,3};
¹Pharmacol. and Systems Physiol., ²Veterans Affairs Med. Ctr., ³Neurosci. Grad. Program, Univ. of Cincinnati, Cincinnati, OH

Abstract: Post Traumatic Stress Disorder (PTSD) is a long-lasting anxiety disorder that can develop after exposure to a traumatic event. One rodent model for PTSD is Single Prolonged

Stress (SPS), in which rodents are exposed to 3 different stressors over a single, continuous time period: psychological (restraint), physiological (forced swim), and pharmacological (ether anesthetization), in order to elicit a trauma response. Recently, our group has discovered sex differences in behavioral responses to a standard SPS model. While male rats are consistently responsive to SPS, females seem to be behaviorally resistant. To follow up on this observation, we designed a study that aims to identify changes in mRNA expression in the basolateral amygdala (BLA) and infralimbic cortex (IL) following SPS in male rats. Using multiplex RNA scope (HiPlex), we are assessing changes in the expression of 12 different genes, including those encoding glucocorticoid signaling, various GABAergic interneurons, nonneuronal proteins, and genes that are known to correlate or interact with regulation of stress responses. Our goal was to determine whether SPS drives cell-specific regulation of one or more potential regulatory pathways. In this project, we are using spatial transcriptomics to quantify gene expression in both brain regions. Following staining for 12 different probes, we merge and pseudo-color the fluorophores to differentiate them within the same image, and individually measure the expression of each gene by thresholding nuclei and counting puncta using Imaris, a 3d imaging software. In addition, we have taken images that allow us to look at potential colocalization between related genes in the same region. In the IL, our data suggest strong colocalization between glucocorticoid, (GR) and mineralocorticoid, (MR) and downstream gene products (FKBP5, Per1) as well as between GR and VGluT1 in both SPS and control rats. GR expression appears to be less pronounced in GAD neurons and the interneuron populations (parvalbumin, somatostatin, CCK. GR and MR are also colocalized with IBA1, a marker of microglia. Ultimately, this project aims to understand changes in gene expression as a function of SPS in males and females.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.08

Topic: F.03. Stress and the Brain

Support: NSFC 32130045
NSFC 31522028

Title: Stress hormone rhythmicity proactively promotes emotional memory suppression via prefrontal control over hippocampal-striatal circuitry

Authors: ***B. XIONG**¹, **C. CHEN**², **Y. BIAN**¹, **Y. TIAN**³, **H. SU**¹, **S. ZHANG**⁴, **C. LIU**¹, **J. WU**⁵, **S. QIN**¹;

¹Beijing Normal Univ., Beijing, China; ²Chongqing Normal Univ., Chongqing, China; ³Fac. of

Engin. IT, Univ. of Technol. Sydney, Sydney, Australia; ⁴Capital Med. Univ., Beijing, China; ⁵Shenzhen Univ., Shenzhen, China

Abstract: Psychiatric pathologies, including rumination in depression and intrusive memories in posttraumatic stress disorder (PTSD), involve the inability to suppress unwanted emotional memories and aberrant rhythmicity of stress hormone cortisol. However, it remains unclear how neurocognitive and endocrinological substrates interact to underly these symptoms. Combining memory suppression and consolidation paradigm with comprehensive neuroendocrine measurements, we investigated whether and how cortisol awakening response (CAR) in the morning, a crucial feature of cortisol circadian rhythm believed to exert proactive preparation effects on the human brain, modulates voluntary suppression of emotional memory during the upcoming day. We measured the CAR of eighty-six young healthy male participants by saliva cortisol. During fMRI scanning in the afternoon of the same day, participants actively suppress their emotional memories either newly acquired (recent) or overnight consolidated (remote). We found that a robust CAR is selectively predictive of more efficient suppression of recent but not remote emotional memory, which is accompanied by higher activation in the bilateral dorsolateral prefrontal cortex (dlPFC), higher deactivation in the left hippocampus and right caudate, as well as stronger effective connectivity from the dlPFC to the hippocampus and caudate pathways. These findings suggest that the CAR promotes efficient suppression of emotional memory via proactively enhancing the prefrontal-originated top-down control over the hippocampal-striatal circuitry, which may advance the development of promising neuroendocrine and rhythmical treatments of stress-related mental disorders.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

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Program #/Poster #: 479.09

Topic: F.03. Stress and the Brain

Support: R21 MH116353
R01 MH050479
R01 DA047443

Title: Behavioral control over stress recruits a distinct circuit in females

Authors: ***C. MCNULTY**¹, I. P. FALLON⁴, J. AMAT⁵, R. J. SANCHEZ², D. H. ROOT², S. F. MAIER³, M. V. BARATTA²;

¹Psychology & Neurosci., ³Dept. of Psychology and Neurosci., ²Univ. of Colorado, Boulder, CO;

⁴Neurobio., Duke Univ. Sch. of Med., Durham, NC; ⁵Psychology & Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Instrumental control over stress blunts many of the neurochemical and behavioral outcomes that typically follow exposure to physically identical uncontrollable stress. Current evidence indicates that the stress-buffering effects of behavioral control require engagement of a corticostriatal circuit, involving the prelimbic cortex (PL) and dorsomedial striatum (DMS). Interestingly, the protective effects of control are completely absent in female rats. Here we demonstrate that females selectively engage the dorsolateral striatal (DLS), rather than DMS, system during the acquisition of behavioral control. Following intra-DLS excitotoxic lesions, control over stress now provided protection in females (EXP1). Dopamine is critical to a number of PL functions, however high levels such as those driven by stress can lead to their impairment. Thus, we examined the contribution of PL dopamine on the differential impact of control in males and females. Behavioral control over stress potently reduced prefrontal extracellular dopamine levels in males but had no effect in females, in which dopamine remained elevated (EXP2). Multiplex fluorescent in situ hybridization revealed that PL GABA interneurons preferentially express the D1 receptor subtypes, independent of sex (EXP3). Intra-PL infusions of the D1 antagonist SCH-23390 (1 µg/ hemisphere) led control to be protective in females. In addition, Blockade of PL D1 receptors shifted striatal activation from the DLS to the DMS (EXP4). These findings suggest that that behavioral control at a procedural level is not the critical factor in determining its impact on stress outcome, rather it depends on the circuitry that is recruited during its acquisition. Furthermore, reduced benefit from a resilience factor may present a novel approach to understanding sex differences in stress-linked psychiatric disorders.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.10

Topic: F.03. Stress and the Brain

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Title: Voluntary wheel running normalizes hippocampus response and urogenital hypersensitivity following water avoidance stress exposure in adult mice that underwent neonatal maternal separation

Authors: *T. E. MCQUILLAN¹, A. D. BRAKE², O. C. ELLER¹, B. M. LEVASSEUR¹, J. M. FRICK¹, R. M. FORIGHT¹, J. A. CHRISTIANSON¹;
¹Cell Biol. and Physiol., ²Sch. of Med., Univ. of Kansas Med. Ctr., Leavenworth, KS

Abstract: Incidence of early life stress (ELS) exposure has been correlated with increased pain and symptom severity in patients with urologic chronic pelvic pain syndrome (UCPPS). Neuroimaging studies in UCPPS patients revealed changes in hippocampal gray matter volume, neurochemical concentrations, and functional connectivity that correlate with pain symptomology. Our lab established an ELS mouse model using neonatal maternal separation (NMS) that recapitulates many clinical features of UCPPS including increased urogenital sensitivity, smaller left hippocampal volume, and reduced neuronal integrity. Water avoidance stress (WAS) exacerbates our phenotype particularly by increasing bladder hypersensitivity and altering neurochemical concentrations in the hippocampus. We have also shown voluntary wheel running can attenuate NMS-induced negative health outcomes. Here we use our mouse model to test the hypothesis that exercise can prevent/attenuate WAS-induced hippocampal changes and urogenital hypersensitivity in NMS mice. NMS was performed from postnatal day 1 (P1) to P21 by placing pups in an incubator for 3h/day. Naïve mice remained unhandled. Mice were weaned P22 then given access to a running wheel (Ex) or remained in sedentary housing (Sed) on P28. For WAS, mice were placed on a platform surrounded by water for 1h. Hippocampal integrity and urogenital sensitivity were evaluated at 4-5 months of age prior to and 24-hrs post WAS. Hippocampal volume and neurochemical concentrations were evaluated by magnetic resonance imaging and spectroscopy (MRI/MRS). Urogenital sensitivity was evaluated by perigenital Von Frey (males) and by measuring visceromotor response (VMR) during urinary bladder distention (UBD, females).

In females, we previously reported reduced hippocampal volume in NMS mice and altered neurochemical content due to NMS and WAS. We are carrying out similar analyses in Sed/Ex male/female NMS/naïve groups. Preliminary UBD results have recapitulated previously observed NMS-induced changes in Sed-NMS mice which exhibit significantly higher VMR than naïve-Sed and NMS-Ex mice. Male mice showed no impact of NMS or Ex on perigenital sensitivity at baseline, however only naïve-Sed mice exhibited significantly lower withdrawal thresholds post WAS, suggesting that exercise may not protect from stress-induced allodynia. This work furthers investigation of NMS-induced impairments in hippocampal integrity and urogenital hypersensitivity. Successful completion will provide evidence for effectiveness of exercise as a long-term treatment intervention to attenuate hippocampal decline and urogenital pain associated with ELS.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.11

Topic: F.03. Stress and the Brain

Support: NIH ES 100221

Title: Spatial transcriptomic analysis reveals that mineralocorticoid receptors are required for the distinct gene expression profile of hippocampal area CA2

Authors: P. SINGH¹, *E. P. HARRIS¹, T. WANG², S. PROAÑO¹, X. XU², V. CATALAN GALLEGOS¹, S. DUDEK¹;

¹Neurobio. Lab., ²Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC

Abstract: Mineralocorticoid receptors (MRs) are essential for promoting resilience to stress, but despite several detailed studies over the past decades, much less is known about MR functionality in specific brain regions. In the hippocampus, MR expression is highest in hippocampal area CA2 of both mice and humans. We have previously shown that CA2 pyramidal neurons have a distinct molecular makeup resulting in a plasticity-resistant phenotype and that this is disrupted by MR knockout (KO) (McCann, et al., 2021). To better understand the nature of this disruption in the specific hippocampal regions, we used a spatial transcriptomics approach (Ståhl, et al. 2016; Visium slides by 10x Genomics). Fresh frozen mouse brain sections were placed on a proprietary microscope slide spotted with spatially barcoded mRNA-binding oligonucleotides, which were used to map RNA sequencing reads to specific brain regions (CA1, CA2 and CA3). For this study, sections from two cre-negative and two cre-positive MR KO mice were analyzed (EMX-cre:MR fl/fl). In line with our previous work, genes known to be enriched in CA2 (e.g., Pcp4, Amigo2) were substantially reduced in MR-KO brains. Interestingly, many genes that were normally low in CA2 (Prkcb, Egr1, and CA1 genes Nr3c1 and Wfs1) were found to be upregulated in CA2 of MR knockout tissue. Hierarchical clustering and principal component analyses showed that the gene expression profile in the CA2 of MR-KO brains was more similar to CA1 than what is seen in WT brain (correlation of 0.968 vs 0.839 for KO and WT respectively). Normally, CA2 neurons are more closely related to neurons in CA3 than those in CA1 (Farris, et al., 2019). These data suggest that MR-KO CA2 neurons may be gaining expression of CA1 genes rather than CA3 genes. Our findings provide a greater understanding of transcriptional regulation by MR in CA2 and may provide insight into its role in social behavior, stress regulation, and autism associated with disruption of the NR3C2 gene.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

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Program #/Poster #: 479.12

Topic: F.03. Stress and the Brain

Support: NIH DK124727
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Loma Linda University School of Medicine GRASP Seed Funds

Title: Neural Circuit Alterations Driving Psychosocial Stress Induced Obesity and Sexually Dimorphic Coping Behaviors

Authors: ***T. SIMON**¹, P. ONTIVEROS-ANGEL², B. NOARBE⁴, M. FEBO⁵, J. H. P. COLLINS⁶, A. OBENAU⁴, J. D. FIGUEROA³;

¹Loma Linda Univ., ²Fig Neuro Lab., ³Loma Linda Univ., Loma Linda, CA; ⁴Pediatrics, Univ. of California, Irvine, Irvine, CA; ⁵Psychiatry Dept., Univ. of Florida Dept. of Neurosci., Gainesville, FL; ⁶Univ. of Florida, Gainesville, FL

Abstract: Background: Psychosocial stress and obesity are steadily increasing worldwide with intensified upsurges due to the Coronavirus 2019 outbreak. We reported that these components work bidirectionally to sculpt adolescent neurocircuits implicated in stress reactivity and aberrant feeding habits. Additionally, our animal model corroborates human epidemiological studies revealing sexual dimorphisms, with females being more susceptible to stress-induced binge eating episodes. Here, we aimed to identify sexually dimorphic neural circuits mediating psychosocial stress-induced obesogenic behaviors and maladaptive coping. **Methods:** Adolescent Lewis rats (Male = 24, Female = 24) were fed a control diet (*CD*, 13% kcal from fat) beginning at postnatal day (PND) 21 and then subdivided into exposed and unexposed groups. The exposed groups endured a psychosocial stress model (PSS) that includes 30 consecutive days of social instability (PND 31 - 60) and two predator exposures (at PND 31 and 41). After PSS, animals were again subdivided into feeding patterns: *CD*, continuous control diet, continuous Western-like diet (*WD*, 41% kcal from fat), and intermittent access to the *WD* (*WDI*). Brain tissue was harvested at PND 107 and prepared for diffusion tensor imaging (DTI). **Results:** DTI findings indicate that the hippocampus (HPC), bed nucleus of the stria terminalis (BNST), and lateral hypothalamus are especially sensitive to adolescent stress as evidenced by altered fractional anisotropy, mean, axial, and radial diffusivity. Furthermore, psychosocial stress exposure and access to the *WD* resulted in cooperative and competing effects on the CA1 hippocampal subfield and the subiculum. **Conclusions:** Chronic psychosocial stress during adolescence negatively affects emotional neural circuitry underpinning maladaptive feeding behaviors, particularly the HPC and BNST. Disrupted neurodevelopment of these emotional centers may contribute to stress-induced overeating and binge eating episodes. These results can enhance our understanding of neural circuit architecture linking exposure to early life trauma and obesity.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

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Program #/Poster #: 479.13

Topic: F.03. Stress and the Brain

Support: NIMH R01MH123686
Whitehall Foundation

Title: Exposure to repeated multimodal stress in adulthood has no effect on sensory processing in the posterior parietal cortex

Authors: *S. PARK, G. LUR;
Dept. of Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: There is extensive evidence that chronic stress exposure disrupts cognitive functions. Previous work from our group has shown that repeated multimodal stress (RMS) during adolescence impairs cognitive performance that was paralleled with changes in synaptic architecture of the posterior parietal cortex (PPC). To expand on these results, we investigated the synaptic, circuit, and behavior effects of RMS exposure in young adult (P60-70) male mice. Using two-photon microscopy, we recorded sensory (visual, auditory, and multimodal) responses in layers 2/3 of the PPC in awake, naïve animals. Following a ten-day RMS exposure, we imaged neuronal responses to the same stimuli in the same field of view, identifying the same cells as in our baseline recording, allowing for a longitudinal comparison. A separate, control cohort went through the exact same procedure without exposure to stressors. We found that the proportion of sensory responsive neurons was unaffected by stress. Further analysis revealed no significant effect on response magnitudes across the various stimulus types. Additionally, we tested the effect of RMS on locomotion related signals in the PPC as a proxy for potential effects of stress on neuromodulator systems. These results utilizing in vivo imaging aligned with our previous work suggesting that adult mouse PPC is robust against stress-induced alterations.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

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Topic: F.03. Stress and the Brain

Support: NSFC Grant 32130045

Title: Long-term stress promotes the dynamics of functional brain networks related to task demands

Authors: *H. GAO, Y. ZENG, C. LIU, S. QIN;
Beijing Normal Univ., Beijing, China

Abstract: Human beings reorganize functional brain networks in response to stress, and the lack of the brain resources reallocation could cause people vulnerable to psychopathology. Existing evidence mostly demonstrated that acute stress generally reconfigures resources to salience networks (SN), executive control networks (ECN), and default mode networks (DMN) during exposure to a stressor or the followed task. However, it is unknown how long-term stress without a present or direct stressor changes the dynamics of brain networks during different tasks. We propose that contrary to acute stress, long-term stress will specifically influence the dynamics of brain networks related to task demands, which would be possibly to enable an adaptative response in different tasks. Here, we used Hidden Markov Model (HMM) to investigate how long-term stress changes the brain network dynamics across multiple tasks, including resting, emotion matching, and N-back. We found that different tasks have different dominant brain states but with shared modules. Moreover, long-term stress promotes task demands related to brain networks' dynamics when people perform different tasks. In particular, the stress group showed more expression in DMN-centric brain state during the resting phase, whereas they showed a significantly higher probability of transition to SN-ECN-DMN-centric and Visual-SN-ECN-centric brain states during the emotional matching task. Furthermore, stressed people showed a higher occupancy rate and mean lifetime in the Visual-SN-ECN-centric brain state only during the 2-back block when performing the N-back task. Finally, long-term stress also reduced the dynamics of a brain state with relatively uniform networks activity during the emotion matching task. Our results suggest that compared with acute stress, long-term stress interacts with emotion and cognition to influence the dynamics of brain networks related to task demands.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

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Topic: F.03. Stress and the Brain

Support: Science Foundation Ireland SFI/12/RC/2273_P2
Saks Kavanaugh Foundation

Title: Gut microbiota regulates rhythmic HPA-axis function and induces circadian effects on the stress response

Authors: *G. S. S. TOFANI, S. J. LEIGH, C. E. GHEORGHE, P. SEN, T. BASTIAANSEN, G. CLARKE, J. F. CRYAN;
Univ. Col. Cork, Cork, Ireland

Abstract: Glucocorticoid hormones have widespread effects throughout the body that are key to regulating the organism's response to the environment. Such hormones also have a robust

rhythm with a distinct peak around the sleep-wake transition, and this timed signalling is one of the entraining cues from the brain to the peripheral clock gene machinery. Stress-induced glucocorticoid secretion acts on the same tissues, leading to changes in capacity in order to adapt to psychological or physiological stressors. The gut microbiota encompasses trillions of microorganisms that live in our gut, and these microbes have been reported to modulate hypothalamic-pituitary-adrenal (HPA) axis function and the response to stress. Although this relationship between gut microbiota and stress response is well-established, how the circadian component of glucocorticoid secretion plays a role in such modulation is still largely unknown. In this study we aim to explore the influence of the gut microbiota in the functional rhythmicity of the HPA-axis, and the possible downstream effects on the stress response, using C57BL/6 male germ-free and microbially-depleted animals. We first assessed circadian oscillations in plasma corticosterone and demonstrated that its rhythmicity is disrupted by microbial status. Gene expression profiling throughout the day of the paraventricular nucleus of the hypothalamus, pituitary and adrenal glands revealed alterations in clock gene expression and in genes related to HPA-axis hormone production, function and signalling. Lastly, we demonstrated that these baseline circadian alterations in microbially-depleted animals led to a blunted stress response in a time-of-day dependent manner. Together, these findings reveal that circadian rhythms may be an important factor in dissecting the role of the gut microbiota in the stress response and shed light into microbial-driven modulation of brain function and behaviour.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.16

Topic: F.03. Stress and the Brain

Support: NSERC
Ontario Veterinary College

Title: Dexamethasone treatment alters DUSP6 expression in mHippo E-14 cell line & intact female rats

Authors: *S. DOBRONYI, J. PACOSZ, L. K. ISAACS, K. C. NICHOLSON, N. J. MACLUSKY;
Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada

Abstract: Stress-induced downregulation of the phosphatase DUSP6 has been implicated in human depression, as well as in animal models of depression (Labonte *et al* 2017 Nat. Med. 23(9), 1102). Due to the antidepressant effects of estrogens, we hypothesized that estradiol might attenuate or reverse glucocorticoid-induced downregulation of DUSP6 expression. Under *in vitro*

tissue culture conditions, immortalized mouse neuronal mHippo E-14 cells were treated with 10 nM dexamethasone (DEX; n=6) with and without 1 nM 17-estradiol (E2) pre-treatment. DUSP6 was downregulated by exposure to 10nM DEX, a response blocked by coincubation with 1nM E2. *In vivo*, in cycling rats, DEX was administered for 16h in the drinking water, at a dose (10 µg/ml) designed to simulate the conditions of mild stress (Miller *et al* 1990 Am. J. Physiol. 259: E405), before the animals were returned to their normal drinking water supply. After treatment with the glucocorticoid during proestrus, on the morning of the preovulatory E2 surge, hippocampal DUSP6 levels were not downregulated by DEX treatment, but instead were significantly upregulated. Hippocampal spine density was, likewise, increased after the DEX treatment. To determine whether this was a response observed only during peak E2 exposure, the DEX treatment was repeated at a stage of the cycle when estradiol levels are low (metestrus). Confirming previous results, at both 1 and 5 days after treatment in metestrus, DUSP6 was upregulated in both the CA1 and CA3 regions of the hippocampus. These data suggest that glucocorticoid downregulation of DUSP6 in the hippocampus may be reversed by endogenous E2. Decreases in circulating estrogen (e.g. post-partum, or at menopause) may exacerbate depressive symptoms by withdrawal of the estrogen-mediated protective effect on DUSP6 expression.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.17

Topic: F.03. Stress and the Brain

Title: Identification of genetic pathways influenced by stress and exercise in the rat striatum by single nuclei RNA sequencing

Authors: *T. J. BUHR¹, A. SHOEMAN², Z. V. JOHNSON⁴, L. CHU³, P. J. CLARK²;
¹Interdepartmental Neurosci. Grad. Program, ²Food Sci. and Human Nutr., ³Statistics, Iowa State Univ., Ames, IA; ⁴Biol. Sci., Georgia Inst. of Technol., Atlanta, GA

Abstract: The World Health Organization lists physical inactivity as the 4th leading risk factor for global mortality. Therefore, identifying the factors that contribute to a reduced willingness to be active may be central to the development of new approaches that increase physical activity. Despite a growing body of evidence implicating psychological stress as a contributing factor to the sedentary lifestyle, little is known about stress-induced neuropathology that leads to reductions in physical activity engagement. We have found that exposing rats to a single episode of uncontrollable tail shocks (acute stress) 36hr prior to receiving access to running wheels potently reduces daily running distances. Interestingly, running deficits outlast depression- and anxiety-like behavior measurements following this stressor by months, including behavioral

tasks that require goal directed motor behavior. Here, we use Single-Nuclei RNA sequencing (snRNAseq) to investigate changes in gene expression in the rat striatum to identify cell-specific genetic pathways that become altered following stress exposure that may contribute to running deficits. The striatum was targeted as it is comprised of motivation and motor circuits, critical to running behavior. Young adult male Sprague Dawley rats were exposed to a single episode of acute stress (stress) or left undisturbed in home cages (no stress) and either did (n=6 stress, 6 no stress) or did not (n=6 stress, 6 no stress) receive running wheel access for 42 days. Rats were euthanized and striatums were dissected, followed by nuclei extraction and sorting by flow cytometry. Nuclei were processed with 10x Chromium kits for RNA library preparation and then sequenced on an S1 NovaSeq lane. Using Seurat single cell analysis 13 genetically distinct cell populations in the striatum were detected. Data collection is still underway, however, we have found physical activity status resulted in 10,654 total differentially expressed genes (DEG) and stress exposure resulted in 12,987 DEGs summed across each cell population (FDR, $p < .05$). Next, we will identify DEGs and genetic pathways in specific cell populations that could be mediating stress-induced reductions in physical activity.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.18

Topic: F.03. Stress and the Brain

Support: NIH Grant 1R21MH119561-01A1

Title: The contribution of angiotensin II signaling through endothelial AT_{1a}R receptors to susceptibility to psychological trauma in a mouse model of PTSD

Authors: *P. LEVESQUE^{1,2}, C. CANIVET^{1,2}, V. RISBROUGH^{3,4}, J. DESLAURIERS^{1,2};
¹Neurosci. axis, CHU Res. Center–Université Laval, Quebec, QC, Canada; ²Dept. of Pharmacology, Univ. Laval, Quebec, QC, Canada; ³Psychiatry, Univ. of California, San Diego, CA; ⁴Veterans Affairs Ctr. of Excellence for Stress and Mental Hlth., La Jolla, CA

Abstract: Background and hypothesis: Posttraumatic stress disorder (PTSD) affects over 9% of the population with rates up to 20% in veterans. The only FDA-approved drugs for PTSD treatment, antidepressants have limited effectiveness. It is therefore urgent to better understand the pathophysiological mechanisms of PTSD in order to develop new therapeutic avenues. Studies support a role of angiotensin II (Ang II) in the response to psychological trauma involving loss of blood-brain barrier (BBB) integrity and neuroinflammation, leading to the development of PTSD symptoms. Still, the mechanisms underlying the relationship between Ang II, BBB, and inflammation in higher risk for PTSD remain poorly understood. We hypothesized

that psychological trauma stimulates Ang II release in the periphery, leading to the activation of endothelial angiotensin type 1a receptors (AT_{1a}Rs), BBB disruption and infiltration of inflammatory molecules into the brain. This increased neuroinflammation then contributes to the onset of PTSD. **Methods:** Male and female wild-type (WT) mice and mice deficient for AT_{1a}R in endothelial cells (eAT_{1a}R^{-/-}) were exposed to predator stress (physical contact with a cat for 10 min). A week after stress, anxiety-like and avoidance behaviors were evaluated using the open field, light-dark box, and trauma-reminder tests. A composite score (Z score) was calculated within each test. Blood pressure was monitored by non-invasive tail-cuff plethysmography at baseline, as well as 1 and 10 days after stress. **Results:** In the open field test, predator stress increased anxiety-like behaviors ($p < 0.05$), independently of phenotype and sex, whereas AT_{1a}R phenotype tended to reduce overall anxiety-like behavior ($p = 0.09$). In the light-dark box test, female mice stress exposure increased anxiety-like behavior only in WT mice ($p < 0.05$). No significant differences were found in male mice. In the trauma-reminder test, stress exposure increased avoidance behaviors ($p < 0.001$), regardless of phenotype and sex. Surprisingly, eAT_{1a}R^{-/-} mice tended to have higher systolic and diastolic blood pressure at baseline ($p = 0.06$ and $p = 0.08$) vs WT mice. No changes (vs baseline) were observed 1 and 10 days after predator stress. **Conclusion:** These findings suggest that peripheral Ang II signaling, through endothelial AT_{1a}Rs plays a role in trauma response that is specific to the development of generalized anxiety, not the avoidance-like behavior. Data on plasma and brain levels of inflammatory markers, as well as on the BBB integrity after stress exposure will also be presented to provide additional key insights on the mechanisms underlying the association between Ang II and PTSD risk.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.19

Topic: F.03. Stress and the Brain

Support: Fulbright Scholarship Pasaporte a la Ciencia - Cohort 2020
ICETEX
NIH the Brain Initiative

Title: Social defeat and oxytocin regulation of social avoidance and stress-related signaling proteins in female prairie voles (*Microtus ochrogaster*)

Authors: *L. NERIO MORALES¹, D. A. BATISTA², A. J. BOENDER³, L. J. YOUNG⁴, A. S. SMITH⁵;

¹The Univ. of Kansas, Lawrence, KS; ²The Univ. of Kansas, Lawrence, KS; ³Silvio O. Conte Ctr. for Oxytocin and Social Cognition, Emory Univ., ATLANTA, GA; ⁴Ctr. for Translational

Social Neurosci., Emory Univ., Decatur, GA; ⁵Dept. of Pharmacol. & Toxicology, Univ. of Kansas, Lawrence, KS

Abstract: Social anxiety disorder (SAD) is one of the most common and disabling anxiety disorders. SAD is highly prevalent in female population and characterized by generalized social avoidance often comorbid with other psychiatric diseases. Despite its high prevalence and adverse prognosis, the molecular mechanism behind this condition is poorly understood. The social defeat (SD) paradigm is the most representative animal model to study SAD and the underlying neural mechanisms. Particularly, SD in prairie voles (*Microtus ochrogaster*) advantageously allows the study of female subjects due to their spontaneous display of aggression towards unfamiliar conspecifics after pair bond. Recently, we have documented that SD progressively reduce oxytocin receptor (OTR) binding in the nucleus accumbens (NAc), basal lateral amygdala (BLA), and anterior cingulate cortex (ACC) of defeated female prairie voles over an eight-week period, suggesting a regulatory role of this neuropeptide in the etiology of social avoidance. OTR stimulation leads to the activation of the mitogen-activated protein kinase (MAPK) pathway, a cascade previously associated with the regulation of the anxiolytic effects of oxytocin and stress response. Here, we characterized the effect of SD on the oxytocin system by analyzing changes in local availability of oxytocin, OTR density and the MAPK pathway activity in the NAc, BLA, and ACC of female prairie voles. Additionally, we determined the effect of social preference/avoidance (SPA) test on these markers to assess their response to a test involving a social stimulus. We found that SD reduced OTR binding in the NAc and induced changes in the MAPK pathway activity and the local availability of oxytocin in a region- and stimuli-dependent manner. Finally, we induced an OTR knockdown in these regions using a CRISPR/Cas9 viral construct to assess the effects on SPA behavior and the MAPK pathway activity. OTR knockdown in the NAc, ACC and BLA induced social avoidance and regional- and stimuli-specific changes in the MAPK signaling pathway that resembled some of the features observed in defeated animals. These results show that dysregulation of the oxytocin system and the MAPK pathway in the NAc, ACC and BLA play an important role in the etiology of SAD.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

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Topic: F.03. Stress and the Brain

Support: VA Merit Award BX004693
VA Merit Award BX004646

Title: Extrasynaptic receptors are necessary for the antipsychotic-like effects of GL-II-73, a positive allosteric modulator of $\alpha 5$ -GABA_A receptors

Authors: *A. M. MCCOY¹, T. D. PREVOT², M. MIAN³, J. COOK³, A. FRAZER⁴, E. SIBILLE⁶, D. J. LODGE⁵;

¹UT Hlth. San Antonio, San Antonio, TX; ²Ctr. For Addiction and Mental Hlth., Toronto, ON, Canada; ³Univ. of Wisconsin-Milwaukee, Milwaukee, WI; ⁴Dept Pharmacol., UTHSCSA, Haverford, PA; ⁵UTHSCSA, San Antonio, TX; ⁶CAMH - Univ. of Toronto, Toronto, ON, Canada

Abstract: EXTRASYNAPTIC RECEPTORS ARE NECESSARY FOR THE ANTIPSYCHOTIC-LIKE EFFECTS OF GL-II-73, A POSITIVE ALLOSTERIC MODULATOR OF $\alpha 5$ -GABA_A RECEPTORS

Alexandra M. McCoy, Thomas D. Prevot, Md Yeunus Mian, James M. Cook, Alan Frazer, Etienne L. Sibille, & Daniel J. Lodge

Psychosis, or the disorder of thoughts and perceptions including hallucinations and delusions, is a debilitating symptom accompanying several psychiatric conditions, such as Post Traumatic Stress Disorder (PTSD). Unfortunately, medications to manage symptoms of psychosis remain inadequate due to limited efficacy and undesirable effects. Evidence from our lab and others suggests decreasing excitatory drive from the ventral hippocampus (vHipp) by targeting extrasynaptic GABA_A receptors, can correct dopamine system dysfunction and alleviate psychosis-like behaviors in a rodent model used to study schizophrenia. However, this approach has yet to be validated in a PTSD model. Here, we examined the ability of GL-II-73, a positive allosteric modulator (PAM) selective for $\alpha 5$ -GABA_A receptors ($\alpha 5$ -GABA_ARs), to reverse stress-induced changes to dopamine system function and sensorimotor gating using the inescapable footshock model of PTSD. We found that intra-vHipp administration of GL-II-73 reversed shock-induced increases in dopamine neuron population activity, as measured by *in vivo* extracellular electrophysiology, and deficits in sensorimotor gating, as measured by prepulse inhibition of the startle response (PPI). Additionally, we demonstrated that these effects are dependent on the extrasynaptic localization of the $\alpha 5$ -GABA_ARs, as vHipp-specific siRNA-mediated knockdown of radixin, an extrasynaptic scaffolding protein, increases synaptic $\alpha 5$ -GABA_ARs and completely abolishes the effects of intra-vHipp GL-II-73. Current work will examine how the efficacy of GL-II-73 and $\alpha 5$ -GABA_AR localization changes in a physiologically relevant model of hippocampal hyperfunction, temporal lobe epilepsy (TLE).

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.21

Topic: F.03. Stress and the Brain

Support: NIMH Grant R56MH125895 to CN
Robertson Therapeutic Development Foundation (RTDF) to CN

Title: A novel mitochondrial target for the rapid regulation of the responses to chronic stress

Authors: *C. NASCA^{1,2}, O. BARNHILL³, D. A. ZELLI³, J. DOBBIN⁴, F. LEE⁵, B. BIGIO¹;
¹Psychiatry, NYU Sch. of Med., New York, NY; ²NYU Sch. of Med., Neuroscience & Physiology, NY; ³Rockefeller Univ., New York, NY; ⁴The Rockefeller Univ., New York, NY; ⁵Psychiatry, Pharmacol., Weill Cornell Med. Col., New York, NY

Abstract: Chronic stress is a primary risk factors for main mental illnesses, including major depressive disorders (MDD). The recent findings of the pivotal mitochondrial metabolite acetyl-L-carnitine (LAC) as epigenetic modulator of neuronal plasticity to stress and a therapeutic target for depression (Nasca et al, Neuron 2017, PNAS 2013-18, Nature Mol Psych 2020) led us to test the novel hypothesis for potential role of the main enzyme involved in the synthesis of LAC in the responses to stress using the well-established and largely used chronic restraint stress (CRS) paradigm. Our new RNAseq data and bioinformatic analysis showed a decrease in the expression of this LAC-related key enzyme in the ventral dentate gyrus (vDG) of CRS male mice as compared to age- and sex-matched not stressed control (Ctrl) mice. This finding was replicated in a second mouse model, i.e.: mice carrying the BDNF Val66Met polymorphism. Next, we showed that induction of expression of this enzyme in vDG glutamatergic granule neurons of male CRS transgenic mice expressing Cre recombinase under the control of the mouse calcium/calmodulin-dependent protein kinase II alpha (Camk2a) promoter blunts CRS-induced social interaction deficits as assessed at the three-chamber social interaction test and the related decrease in vDG expression of mGlu2, which we previously showed is regulated by LAC. Our new findings suggest a novel mitochondrial target for the rapid regulation of the responses to chronic stress. Together with the prior discovery of decreased LAC levels in subjects with MDD, the current findings compel further research on this emerging clinically relevant aspect of mitochondrial metabolism as an innovative target for the rapid regulation of brain plasticity.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.22

Topic: F.03. Stress and the Brain

Support: Funding provided by Ellipse Analytics
Richard Barber Interdisciplinary Research Fellowship

Title: A rodent model of oral cannabidiol use: an evaluation of CBD's pharmacokinetic, behavioral, neurochemical, and microbial outcomes

Authors: *J. SKULLY¹, B. TIMMERMAN¹, K. TRAN¹, A. NOVAK¹, A. TROMBLEY², B. ZAGORAC², M. ANGOA-PEREZ², D. KUHN², S. BOWEN¹, S. BRUMMELTE¹;
¹psychology, ²Sch. of Med., Wayne State Univ., Detroit, MI

Abstract: Cannabidiol (CBD) use continues to rise as research on CBD's therapeutic properties (e.g., anxiolytic, analgesic, anti-stress, etc.) has shown promising results. However, CBD's effects and mechanisms of action require further exploration. Our project aimed to evaluate the pharmacokinetics (PK), the neurochemical and behavioral outcomes, as well as the changes in microbiome diversity after oral administration of hemp-derived CBD (Ellipse Analytics; Denver, CO). The PK profile of CBD was assessed by administering CBD (0, 5, 10, 20, or 40 mg/kg, p.o.) to adult male Sprague-Dawley rats (n=10/group) for 10 days while drawing blood (1hr, 2hr, 4hrs) post-administration on the 1st, 5th and 10th day to measure plasma CBD levels. Then, CBD (0, 20, or 40 mg/kg) was administered to male rats over 10 days (n=6-16/group), with behavioral assays occurring on the 1st, 5th, and 10th day to assess pain, stress, anxiety, and sleep behaviors. Rats were sacrificed and brains were collected for analysis on day 11.

Immunofluorescence was used to analyze levels of Ki67 (cell proliferation marker) and Oligodendrocyte transcription factor 2 (Olig2; oligodendrocyte marker) in the PK cohort. Fecal samples were collected from the behavioral cohort of rodents to investigate changes in microbiome diversity. Our PK analysis showed a dose-dependent increase in CBD bioavailability with levels peaking between the 1st and 2nd-hour post-administration, and significantly decreasing by the 4th hour across all groups. Our behavioral results showed that 20mg/kg CBD increased relative activity levels over 6 hours, while 40mg/kg reduced corticosterone levels following a restraint stress test. We also saw a significant dose-dependent difference in microbiome profiles between groups. Future brain analysis will provide insight on Ki67 and Olig2 expression. These results offer support for CBD's potential therapeutic effect on both sleep and stress regulation, while also signifying CBD's ability to shape the gut microbiome.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.23

Topic: F.03. Stress and the Brain

Support: R21 MH116353
RO1 MH050479

Title: Controllable, but not uncontrollable, stress facilitates winning in a social dominance task

Authors: *G. COSTANZA-CHAVEZ, P. T. COLEMAN, G. J. POTTER, H. N. MARTIN, S. N. MELLERT, R. J. SANCHEZ, J. AMAT, S. F. MAIER, M. V. BARATTA;
Dept. of Psychology and Neurosci., Univ. of Colorado, Boulder, CO

Abstract: Instrumental control over stress (escapable stress, ES) prevents a number of neurochemical and behavioral changes that follow a physically identical uncontrollable stressor (inescapable stress, IS). This control-induced protection requires activation of a corticostriatal circuit involving the prelimbic (PL) region of the prefrontal cortex and the dorsomedial striatum (DMS). Recent data has implicated the PL in other aversively motivated behaviors, namely social dominance. Here we examine the impact of stressor controllability on subsequent performance in the warm spot test (WST), a paradigm in which a triad of rats compete for occupancy of a warm spot on a cold cage floor. In EXP1, ES facilitated dominance in the WST seven days post stress treatment. Interestingly, this effect was absent in females: female ES did not facilitate dominance (EXP2). Subsequently, intra-PL microinfusion of muscimol (GABA-A agonist, 50 ng/hemisphere) during ES in males prevented the facilitation of dominance (EXP3). Next, we investigated whether the “winning effect” (stable dominance across repeated WST competitions) required corticostriatal structures. Inhibition of either the PL with muscimol (EXP4) or NMDA receptor blockade in the DMS (AP5, 3 µg/hemisphere, EXP5) led to a long-term reduction in dominance status. Lastly, we addressed if stable dominance itself would produce resilience against subsequent IS. Prior dominance immunized animals against the behavioral (EXP6) and neurochemical (EXP7) outcomes of subsequent IS. Taken together, these experiments suggest that the experience of social dominance and controllable stress recruit similar circuits in producing resilience in the face of future adversity.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.24

Topic: F.03. Stress and the Brain

Support: NIH Grant MH099851
Penn State University

Title: Elucidating mechanisms underlying stress resilience in mice with disinhibited SST⁺ neurons

Authors: *M. SHAO¹, J. BOTVINOV¹, A. SEBASTIAN², I. ALBERT³, B. LUSCHER¹;
¹Dept. of Biol., ²Huck Inst. of the Life Sci., ³Dept. of Biochem. and Mol. Biol., Pennsylvania State Univ., University Park, PA

Abstract: Chronic stress is a key environmental factor for virtually all neuropsychiatric disorders, including especially major depressive disorder (MDD). Clinical and preclinical research in mice indicates that some individuals develop a maladaptive vulnerability to chronic stress exposure, while others adapt and exhibit resilience to stress, suggesting that mechanisms underlying stress resilience may be exploited in the search for novel antidepressant therapies. MDD and chronic stress are associated with diverse defects in GABAergic synaptic inhibitory transmission. In particular, transcriptomic changes point to a key role of somatostatin (SST)-positive neurons, a major subclass of mostly dendrite targeting GABAergic interneurons. Previously, we showed that mice with disinhibited SST⁺ neurons, induced by deletion of the $\gamma 2$ subunit of GABA_A receptors from these neurons (SSTCre: $\gamma 2^{f/f}$ mice), exhibit enhanced GABAergic synaptic inhibition of pyramidal cells, mimic behavioral changes of anxiolytic and antidepressant drug treatment, and are resilient to the anxiogenic-like effects of chronic mild stress treatment. To extend these studies to reward-related behavior we here have used chronic variable stress (CVS) as a more severe stress protocol. We found that SSTCre: $\gamma 2^{f/+}$ and SSTCre: $\gamma 2^{f/f}$ mice were resilient to CVS-induced neophobia in the Open Field Test, and they showed abnormal CVS-induced reductions in aversive behavior in the Novelty Suppressed Feeding Test. In addition, SSTCre: $\gamma 2^{f/f}$ male mice were resilient to CVS-induced reductions in hedonic drive in the Female Urine Sniffing Test. The stress resilience effects of SST⁺ neuron disinhibition were generally more prominent in male than female mice, and they appeared to scale with stress intensity and disinhibition of SST⁺ neurons. To address the molecular mechanisms underlying this stress resilience phenotype, we performed RNASeq from mPFC bulk tissue and live (calcein⁺, actinomycin D-treated) SST⁺ cells that were highly enriched from mPFC by fluorescence activated cell sorting (FACS). Analyses of CVS-induced gene expression changes by RNASeq of mPFC bulk tissue from SSTCre control and stress resilient mutant mice suggest that stress resilience is associated with robust downregulation of the endoplasmic reticulum stress pathway. Preliminary analyses of RNASeq data from purified SST⁺ cells from SSTCre: $\gamma 2^{f/+}$ and SSTCre: $\gamma 2^{f/f}$ mice revealed virtually no significant (q-value) gene expression changes, consistent with behavioral stress resilience. Moreover, they indicate that stress resilience associated with downregulation of the ER stress pathway in bulk tissue involves non-SST cells.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.25

Topic: F.03. Stress and the Brain

Title: Data-driven analysis in mice reveals whole-brain dynamics along a known anterior-posterior axis that inform the neural basis of behavior

Authors: *Y. XU¹, A. TURNBULL², J. LU³, Y. ZUO³, V. LIN¹;

¹Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA; ²Dept. of Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY; ³Dept. of Molecular, Cell and Developmental Biol., UC Santa Cruz, Santa Cruz, CA

Abstract: Stress is a major risk factor for many psychiatric disorders, and is known to compromise the structure and function of multiple brain regions/circuits. However, it is unknown how stress impacts whole-brain dynamics. Approaches that study whole-brain dynamics, rather than isolating specific regions/circuits, are increasing in human studies, but have yet to be systematically applied to mouse models that can probe the causal effects of stress on brain functions that support behavior. We performed mesoscopic fluorescent calcium imaging (10 Hz, 15 min) across the entire dorsal surface of the cortex in an awake, head-fixed mouse, alongside simultaneous monitoring of its behavior (walking/running and whisking) at four timepoints with various status of stress: the pre-stress baseline (labeled as stress level=0), after 7 days of stress (stress level=7), immediately after the injection of a drug known to reduce the effects of stress (stress level=5), and 1 day after drug treatment (stress level=3). We used an independent component analysis-based preprocessing pipeline to remove hemodynamic artifacts (Weiser et al., 2020), and then applied principal component analysis to the calcium imaging time series. The first component (PC1) separated the mouse brain along an anterior-posterior axis, and explained a large amount of overall variance (0.692), suggesting that shifts between high anterior/low posterior and low anterior/high posterior activity reflect a large proportion of whole-brain dynamics. This corresponds to a known axis in the mouse brain that separates anterior motor regions from posterior visual regions. To better understand these changes, we separated the brain regions into anterior (negative on PC1) and posterior (positive on PC1) parts and assessed the relationships of these timeseries to behavior. Visual inspection and follow-up descriptive analysis showed that activity in posterior (visual) regions increased prior to running, and during running there was a shift from high posterior (visual) to high anterior (motor) activity. Linear mixed effects models with stress status showed that running ($z=10.69$, $p<0.001$) and PC1 activity ($z=-5.88$, $p<0.001$) were significantly related to stress. Follow-up analysis suggested that this was driven mainly by increased posterior (visual) activity ($z=3.03$, $p=0.002$) rather than decreased anterior (motor) activity ($z=-1.70$, $p=0.09$) with stress. These findings suggest that data-driven approaches analyzing whole-brain dynamics in mice can uncover activity patterns that match anatomy and can be used to better understand the links between neural processing and behavior, and how they are impacted by stress.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.26

Topic: F.03. Stress and the Brain

Title: A systematic multi-omics study reveals shared and distinct genomic signatures and regulatory patterns between major depression and PTSD

Authors: *J. WANG¹, R. WILSON², H. LI³, T. LAM², A. C. NAIRN⁴, J. H. KRYSTAL⁴, R. S. DUMAN⁴, H. ZHAO³, M. J. GIRGENTI⁴;

¹Computat. Biol. & Bioinformatics, ²Yale/NIDA Neuroproteomics Ctr., ³Dept. of Biostatistics, ⁴Dept. of Psychiatry, Yale Univ., New Haven, CT

Abstract: Major depression disorder (MDD) and post-traumatic stress disorder (PTSD) are two severe psychiatric diseases with high prevalence in the population worldwide. However, despite its prevalence, our understanding of their molecular determinants is limited and examination across genomic layers is necessary to better understand the etiology of these disorders. Here we present findings from the first proteomics analysis of the post-mortem MDD and PTSD brain that reveal shared and distinct proteomic differential expression and co-expression patterns. Integrated analysis with small RNA sequencing of miRNAs identified hsa-mir-589 as a core regulating miRNA in the translational process of disease-associated protein modules for both MDD and PTSD. In addition, we identify significant enrichment of genetic risk genes for other neurodegenerative and psychiatric disorders within these co-expression modules, indicating a shared molecular pathology. Our findings have begun to unravel the proteomic and small RNA landscape of the human frontal cortex and its pathological alteration in MDD and PTSD, providing novel insights into the molecular mechanisms driving both disorders.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.27

Topic: F.03. Stress and the Brain

Support: R01-NS113104-01

Title: Partner loss increases partner-seeking behavior and alters the mesocorticolimbic dopamine system of male prairie voles

Authors: *E. VITALE¹, A. KIRCKOF², A. S. SMITH¹;

¹Dept. of Pharmacol. & Toxicology, Univ. of Kansas, Lawrence, KS; ²Dept. of Pharmacol. & Toxicology, The Univ. of Kansas, Lawrence, KS

Abstract: Death of a loved one is recognized as one of life's greatest stresses, and 10-20% of bereaved individuals will experience a complicated or prolonged grieving period that is characterized by intense yearning for the deceased. The monogamous prairie vole (*Microtus ochrogaster*) is a rodent species that forms pair bonds between breeding partners and has been used to study the neurobiology of social behaviors and isolation. Male prairie voles do not display distress after isolation from a familiar, same-sex conspecific; however, separation from a bonded female partner increases emotional, stress-related, and proximity-seeking behaviors. Using the prairie vole as a model for social loss, our lab has developed a behavioral test to determine whether prairie voles display motivation for reunion with their lost partner, akin to yearning in humans. In this odor preference test (OPT), subjects were given access to two odor cues representing different motivational states - bedding scented with their partner and bedding scented with their regular chow. We found that males paired with a female for one week and then separated from their partner for one week showed increased investigation of the odor cue from their partner compared to those that remained with their partner, were housed with a male cage mate, or were separated from a male cage mate for one week. Furthermore, within the male-female pairs, males that displayed a strong preference for their partner showed the same increase in partner odor investigation after one week of partner loss while males that did not show a partner preference and lost their partner did not differ from those that remained with their partner. Together, these data suggest that both relationship type and relationship quality affect partner-seeking behavior following short-term social loss. Currently, we are investigating whether this loss-induced partner-seeking behavior persists after longer (four weeks) periods of social loss. We predict that by four weeks of social loss, the partner-seeking phenotype will no longer be present since prairie voles are able to form new partnerships after this time-point. Finally, we are currently assessing changes within the central dopaminergic system in response to social loss and have preliminary evidence that partner loss alters DA receptor mRNA expression in the insula and anterior cingulate (regions with higher activation in grieving humans). With the prairie vole social loss model, we hope to characterize the neurochemistry associated with grief- and loss-related behaviors to determine the neurobiological processes that contribute to normal and divergent grief and loss outcome.

Disclosures: E. Vitale: None. A. Kirckof: None. A.S. Smith: None.

Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.28

Topic: F.03. Stress and the Brain

Support: #821-MR-NB-35541

Title: Ribosome associated long non-coding RNA (lncRNA) in pyramidal neurons; effects of EtOH.

Authors: H. E. GAVIN¹, H. S. RIZAVI^{1,2}, D. P. GAVIN¹, *R. P. SHARMA^{1,2};

¹Dept. of Psychiatry, Univ. of Illinois at Chicago, Chicago, IL; ²Jesse Brown VA Med. Ctr., Chicago, IL

Abstract: Although by definition, long noncoding RNAs (lncRNAs) are not translated to code proteins, their association with ribosomes is unexplained. Ribosome-associated lncRNAs may represent a portion of the more than 50K lncRNA molecules that could encode for small peptides. We examine the effects of ethanol and PARP inhibition on lncRNAs. Mice were administered via intraperitoneal injection (i.p.) twice a day with normal saline (CTL) or ethanol (EtOH, to a final daily dose of 2 g/kg) for four consecutive days. ABT-888 (25 mg/kg) was co-administered with ethanol on the fourth day in a sub-group of mice that received ethanol in the previous three days (EtOH+ABT). We measured lncRNA expression in prefrontal cortical sections (PFC) in whole-cell lysate (INPUT) as well as lncRNA bound to ribosomes in CaMKII α -expressing pyramidal neurons using the Translating Ribosome Affinity Purification (TRAP) technique. Both TRAP and INPUT samples were submitted for sequencing, and lncRNA expression was evaluated to determine the effect of EtOH and the coadministration of EtOH and ABT-888. 308 lncRNAs were identified of which 157 were differentially (more or less) attached to ribosomes (TRAP) when compared to the INPUT in the CTL condition. Of these 157 lncRNAs, 68 were significantly more attached to ribosomes (TRAP/INPUT positive logFC). Further bioinformatics analysis was based on the in-silico results of Zeng et al. 2018 who provide lncRNA identities with sequences consistent with ribosomal affinity. From this analysis, nine lncRNA (Mir124-2hg, 5430416N02Rik, Gm10419, Snhg17, Snhg12, Snhg1, Mir9-3hg, Gas5, 1110038B12Rik) were further validated in the same samples using qPCR. We find significant differences in expression in these specific lncRNAs either using normalized counts from RNA-Seq or from direct qPCR validation. We will report on the effects of EtOH and EtOH+ABT-888 on this population of lncRNAs and will suggest an alcohol-dependent regulation in the PFC.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.29

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R00 DA043573

Title: Effects of isolation housing stress on the striatal transcriptome in male and female C57BL/6J and DBA/2J mice

Authors: *M. LEONARDO¹, J. COMSTOCK², S. A. TOWERS², S. BRUNTY¹, J. HUFFMAN¹, J. FAN¹, D. A. PRIMERANO¹, A. Q. NATO, Jr¹, J. DENVER¹, D. B. LESTER²,

P. E. DICKSON¹;

¹Marshall Univ., Marshall Univ., Huntington, WV; ²Univ. of Memphis, Memphis, TN

Abstract: Stress is a critical factor driving drug addiction in humans, and the genetic mechanisms underlying this effect are unknown. Isolation housing, a preclinical model of chronic environmental stress, has been shown to potentiate intravenous drug self-administration and behavioral phenotypes that predict intravenous drug self-administration. The genetic mechanisms underlying these phenomena are unknown but can be identified using a systems genetics approach in mouse recombinant inbred panels such as the BXD and Collaborative Cross. In this regard, we have recently shown that isolation housing stress produces strain- and sex-dependent potentiation of addiction-predictive behavioral phenotypes in the C57BL/6J and DBA/2J mouse strains (BXD founders). It is unknown whether the striatal transcriptome is similarly strain- and sex-dependently influenced by isolation housing stress. This question is crucial to understanding isolation-induced effects on addiction-relevant behaviors because the striatum and its subregions are critically involved in addiction. Evidence of strain- and sex-dependent behavioral effects (previous study) and striatal transcriptomic effects (present study) following chronic isolation housing stress in the BXD founder strains would confirm that the full BXD panel could be used to discover genetic and epigenetic mechanisms underlying these addiction-relevant phenomena. To this end, the goal of the present study was to determine if striatal gene expression is influenced by strain, sex, housing condition, or interactions among these variables in C57BL/6J and DBA/2J mice. Mice were ordered at three weeks of age from The Jackson Laboratory and, upon arrival, immediately transferred to one of three housing conditions: isolation, standard, or enrichment. After housing in these conditions for 10 weeks, striatum was collected, RNA was extracted, and bulk RNA-Seq was performed. FASTQ files were evaluated for quality, genome alignment was performed, genes differentially expressed among experimental groups were identified, and these gene sets were characterized. Findings from the present gene expression study and previous behavioral studies indicate that the full BXD panel can be used to identify genetic and epigenetic mechanisms underlying isolation-induced effects on addiction-relevant behaviors and the striatal transcriptome.

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Poster

479. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 479.30

Topic: B.05. Synaptic Plasticity

Support: Roche Fellowship

Title: The Effects of Opioids on Stress-Induced Neuroplasticity

Authors: *V. SALTZ¹, E. JEON², L. MASSAC², C. MASESE², C. HAN², C. MALDONADO², S. ROBINSON²;

¹Columbia Univ., Columbia Univ., New York, NY; ²Williams Col., Williamstown, MA

Abstract: Opioid exposure and chronic stress have each been shown to cause changes in brain-wide connectivity. However, the effects of opioid exposure on stress-induced neuroplasticity have not been well characterized. Utilizing C57BL/6-Tac mice, we traced downstream projections of the nucleus accumbens (NAc) by injecting an adeno-associated virus (AAV) anterograde tracer delivering a tdTomato fluorophore into the shell of the NAc. We then exposed the mice to 14 days of an unpredictable chronic mild stress (UCMS) behavioral paradigm, combined with concurrent bi-daily morphine exposure. After this 14-day combined stress plus morphine exposure, we performed a Forced Swim Test (FST), and then sacrificed the mice 90 minutes later to look at neural activity patterns using the immediate early gene, *cFos*. Brains were compared for differences in neural projections from the NAc as well as neural activity in the dentate gyrus and basolateral amygdala (BLA) between mice who underwent the following conditions: a) chronic stress and morphine exposure (UCMS MOR), b) chronic stress alone and saline (UCMS SAL), c) morphine exposure alone (NO UCMS MOR), or d) neither treatment (NO UCMS SAL). We identified downstream neural projections from the NAc to the prefrontal cortex (PFC), paraventricular thalamus (PVT), lateral hypothalamus (LH), and ventral tegmental area (VTA) in all successfully injected animals. However, in all animals that underwent chronic stress alone, projections were seen from the NAc to BLA. In contrast, only half of animals that underwent combined chronic stress and opioid exposure showed this NAc \diamond BLA pathway. Projections from the NAc to habenula were also seen in animals that underwent either chronic stress or opioid exposure alone, or combined treatment. Mice that underwent chronic stress alone also had increased neural activity in the dentate gyrus and BLA, however, this stress-induced hyperactivity was counteracted by morphine exposure. These data highlight the complexity of stress and opioid interactions and suggest a buffering effect of morphine against neuroplastic effects induced by stress.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.01

Topic: C.01. Brain Wellness and Aging

Title: Impact of obesity on heart rate variability and anxiety

Authors: *C. ERDENEBAATAR¹, E. TUMURBAATAR³, T. OKA⁴, E. BAT-ERDENE⁵, G. TUMUR-OCHIR⁶, B. MUNKHBAATAR², E. CHIMEDLKHAM⁷, O. BYAMBASUKH⁸, B. LKHAGVASUREN⁹;

²Clin. neuroscience, ¹Brain and Mind Res. Inst., Ulaanbaatar, Mongolia; ³Dept. of Intl. Cyber Educ., Mongolian Natl. Univ. of Med. sciences, Ulaanbaatar, Mongolia; ⁴Dept. of Psychosomatic Med., Intl. Univ. of Hlth. and Welfare Narita Hosp., Chiba, Japan; ⁵Brain and Mind Res. Inst., Brain and Mind Res. Institute, Mongolian Acad. of Sci., Ulaanbaatar, Mongolia; ⁶Mental Hlth., Mongolian Natl. Univ. of Med. Sci., Ulaanbaatar, Mongolia; ⁷Foreign language Sch., Mongolian Univ. of Sci. and Technol., Ulaanbaatar, Mongolia; ⁸Dept. of Endocrinology, Sch. of Medicine, Mongolian Natl. Univ. of Med. Sci., Ulaanbaatar, Mongolia; ⁹Hlth. Sci. Univ., Ulaanbaatar, Mongolia

Abstract: Background: Obesity is a well-established risk factor for cardiovascular diseases. However, mechanisms underlying the associations between obesity and autonomic functions are less understood. We aimed to assess the potential effects of obesity on heart rate variability parameters and anxiety and depression scores in the Mongolian adults. **Methods:** This cross-sectional study was conducted between August and September 2020 in Ulaanbaatar city. We measured body mass index (BMI), waist circumference, neck circumference, and the time domain of heart rate variability. The Hospital Anxiety and Depression Scale (HADS) was used to investigate anxiety and depression. We recruited participants who did not drink caffeine or ingest other stimulants prior to the autonomic functions testing. Subjects were given instructions to remain silent, awake while breathing normally, and retain an empty bladder during the entire measurement. **Results:** A total of 123 participants (71.5% women) with a mean age of 38.89 ± 12.05 years were enrolled in this study. The standard deviation of the normal-to-normal intervals (SDNN) and the root-mean square of differences between adjacent normal RR intervals (RMSSD) were differed between the obese and non-obese participants measured by BMI ($p = 0.013$ and $p < 0.001$, respectively). Both SDNN and RMSSD were inversely correlated with age ($r = -0.531$; $r = -0.473$), body mass ($r = -0.275$; $r = -0.329$), BMI ($r = -0.378$; $r = -0.391$), and waist circumference ($r = -0.413$; $r = -0.454$), respectively. The anxiety score of HADS showed negative correlations with SDNN ($r = -0.225$, $p = 0.013$) and RMSSD ($r = -0.224$, $p = 0.013$). **Conclusion:** These results suggest that obesity may affect negatively the autonomic functions and anxiety level in the general population.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.02

Topic: C.01. Brain Wellness and Aging

Support: Canadian Institutes of Health Research 142255

Title: Extended and replicated white matter changes in obesity: Voxel-based and region of interest meta-analyses of diffusion tensor imaging studies

Authors: *L. M. F. DIETZE^{1,2}, S. R. MCWHINNEY², J. RADUA^{3,4,5,6}, T. HAJEK^{2,7};
¹Dept. of Med. Neurosci., ²Dept. of Psychiatry, Dalhousie Univ., Halifax, NS, Canada; ³Inst. d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; ⁴Mental Hlth. Res. Networking Ctr. (CIBERSAM), Madrid, Spain; ⁵Dept. of Psychosis Studies, Inst. of Psychiatry, Psychology, and Neurosci., King's Col. London, London, United Kingdom; ⁶Dept. of Clin. Neuroscience, Ctr. for Psychiatric Res. and Educ., Karolinska Institutet, Stockholm, Sweden; ⁷Natl. Inst. of Mental Hlth., Klecany, Czech Republic

Abstract: Obesity has become a global public health issue, which aside from general health may significantly impact the brain. In contrast with gray matter correlates of obesity, associations between obesity and white matter microstructure measured using diffusion tensor imaging have not been analyzed in detail, despite a relatively large number of individual studies. We analyzed location of brain white matter changes in obesity using the AES-SDM method in a voxel-based spatial meta-analysis, with validation in a region of interest (ROI) effect size meta-analysis. Our sample included 21742 individuals from 51 studies. The voxel-based spatial meta-analysis demonstrated associations between obesity and reduced fractional anisotropy (FA) in the genu and splenium of the corpus callosum, middle cerebellar peduncles, anterior thalamic radiation, cortico-spinal projections, and cerebellum. The ROI effect size meta-analysis replicated associations between obesity and lower FA in the genu and splenium of the corpus callosum, middle cerebellar peduncles. The extent of these obesity related brain changes was small to medium. We found robust and replicated associations between obesity and lower FA in white matter tracts within networks corresponding with previously reported gray matter changes in obesity. Better understanding the brain correlates of obesity could help identify risk factors for brain alterations, as well as targets for prevention or treatment of brain changes and associated cognitive or mental health outcomes.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.03

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant T32 MH82174

Title: Age-related lipid peroxidation in human STG is altered in Autism Spectrum Disorder

Authors: *E. C. HALEY¹, E. CARLSON², C. M. SCHUMANN²;

¹Ctr. for Neurosci., UC Davis, Davis, CA; ²Dept. of Psychiatry, UC Davis MIND Inst., Sacramento, CA

Abstract: Age-related lipid peroxidation in human STG is altered in Autism Spectrum

Disorder Authors E. C. HALEY¹, E. L. CARLSON², C. M. SCHUMANN²; ¹Ctr. for Neurosci., UC Davis, Davis, CA; ²Dept. Of Psychiatry, UC Davis MIND Inst., Sacramento, CA
Disclosures E. C. Haley: None. E. L. Carlson: None. C. M. Schumann: None.

Abstract Autism spectrum disorder (ASD) is typically viewed as a condition of childhood, which has resulted in a paucity of research concerning the effects of ASD across the lifespan. Our lab has previously shown alterations in the age-related cellular and molecular trajectory of the brain in ASD; specifically, an increase in cell and spine density in childhood followed by cell and spine loss into adulthood. Given the role of oxidative stress and lipid peroxidation in age-related disorders and recent parallels being drawn between neurodegenerative diseases and ASD, we sought to quantify a lipid peroxidation byproduct, 4-hydroxynonenal (4-HNE) in fresh-frozen postmortem human tissue from 10 male ASD (ages 9-56 years) and 8 male control (ages 22-41 years) samples in superior temporal gyrus (STG) due to its function involving social cognition, a core symptom in ASD, and evidence of altered transcriptomic architecture in adults with ASD. Tissue was isolated from fresh-frozen STG postmortem followed by a lysis and total protein quantification via a BCA assay. We then employed a competitive enzyme linked immunosorbent assay (ELISA) to detect 4-HNE protein adducts between groups. We find that, after protein normalization using a BCA assay, 4-HNE protein adduct concentration increases as a function of age in control STG samples, aligning well with our understanding of the trajectory of oxidative stress in neurotypical aging. However, preliminary data indicates that the trajectory for ASD STG is significantly different compared to the control trajectory with more heterogeneous results. This indicates that ASD disrupts typical aging processes such as oxidative stress and lipid peroxidation, which could serve as a basis for later detriments associated with ASD including cognitive decline and early mortality. This study demonstrates that cellular and molecular alterations of ASD extend beyond childhood and 4-HNE protein adducts can serve as an indicator that the trajectory of aging in ASD differs from that of the neurotypical adult. Research concerning the role of 4-HNE in aging regarding ASD pathology is limited, and this study emphasizes the importance of investigating this cytotoxic molecule given its altered presence in ASD across the lifespan in postmortem human tissue.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.04

Topic: C.01. Brain Wellness and Aging

Title: Effect of hydrogen sulfide on vascular dysfunction induced by type 2 Diabetes Mellitus in rat thoracic aorta

Authors: *D. L. SILVA VELASCO¹, J. H. BELTRAN ORNELAS¹, A. SÁNCHEZ LÓPEZ¹, J. TAPIA MARTÍNEZ¹, A. SÁNCHEZ MENDOZA², L. G. CERVANTES PÉREZ², D. CENTURIÓN PACHECO¹;

¹CINVESTAV, CINVESTAV, Mexico City, Mexico; ²Farmacobiología, Inst. Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico

Abstract: Hydrogen sulfide (H₂S) is a gasotransmitter that has been involved in the regulation of the cardiovascular system. H₂S is synthesized from L-Cysteine by three enzymatic pathways, but cystathionine- γ -lyase (CSE) predominates in the cardiovascular system, especially the myocardium and vascular smooth muscle cells (VSMCs). This study aimed to determine the effect of chronic administration of sodium hydrosulfide (NaHS; inorganic H₂S donor) and DL-Propargylglycine (DL-PAG; CSE inhibitor) on the vascular dysfunction in thoracic aorta obtained from male diabetic Wistar rats. For that purpose, neonatal rats were divided into two main sets that received: (1) citrate buffer (n=6) and (2) a single dose of STZ (70 mg/kg/i.p., n=6) on the third day of birth to induce diabetes. After 12 weeks, oral glucose tolerance test. Then, the diabetic animals were divided into 4 subgroups (n=6 each) which received daily i.p. injections during 4 weeks of: (1) nothing; (2) vehicle (PBS, 1 ml/kg); (3) NaHS (5.6 mg/kg); and (4) DL-PAG (10 mg/kg). After treatments (16 weeks), vascular function by *in vitro* experiments (organ bath) was determined. Aortas were cut into rings of 3 mm length and placed in an organ bath camera. We observed that type 2 Diabetes Mellitus induced by streptozotocin leads to: (1) an increase in glucose levels; (2) a decrease in vasorelaxation to angiotensin 1-7 (Ang-1-7) and vasoconstriction induced by angiotensin II (Ang II) compared to control group. None of the pharmacological treatments modified glucose levels. Interestingly, after four weeks of treatment, NaHS increased vasorelaxation and vasoconstriction to Ang 1-7 and Ang II, respectively, when compared to vehicle. On the other hand, the treatment with DL-PAG did not affect the relaxant responses to Ang-1-7 or contractile responses to Ang II compared to the vehicle. These results suggest that chronic treatment with NaHS improved vascular dysfunction produced by streptozotocin-induced type 2 Diabetes Mellitus and may have a potential therapeutic application.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.05

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01NS10279602
NIH Training Grant T32NS099578

Title: Elevated susceptibility to provoked seizures and hippocampal seizure mechanisms in a mouse model of Leigh syndrome epilepsy

Authors: *A. MANNING^{1,2}, V. HAN², A. STEPHENS², R. WANG², F. KALUME^{2,3};
¹Univ. of Washington, Univ. of Washington Grad. Program In Neurosci., Seattle, WA; ²Seattle Children's Res. Inst., Seattle, WA; ³Neurolog. Surgery, Pharmacol., Univ. of Washington, Seattle, WA

Abstract: Leigh syndrome (LS), a pediatric mitochondrial disorder, is caused by genetic loss-of-function mutations in the NADH dehydrogenase (ubiquinone) iron-sulfur protein 4 (NDUFS4) gene which encodes for a subunit of the mitochondrial CI. Epileptic seizures, which constitute one of the most significant clinical features of LS, are difficult to treat and are often a sign of poor disease prognosis. Mice with whole-body *Ndufs4* KO are a well-validated model of LS; they exhibit epilepsy and several other clinical features of LS. We have previously shown that mice with *Ndufs4* KO in only GABAergic interneurons (*Gad2-Ndufs4-KO*) reproduce the severe epilepsy seen in the global KO mice. This finding indicated that *Gad2-Ndufs4-KO* mice represent an excellent model of epilepsy in LS. Here we investigated the susceptibility to provoked seizures and the cellular mechanisms of epilepsy of this model. *Gad2-Ndufs4-KO* mice and littermate controls were generated by crossing a *Ndufs4^{flx/flx}* mouse with the *Ndufs4^{flx/+}::Gad2cre^{+/-}* mouse. Their susceptibility to seizures when exposed to a low dose (20mg/kg) pentylenetetrazol (PTZ) or moderate level of exercise via treadmill was investigated. EEG was recorded using Labchart 8.0 to characterize epileptiform activity. To examine the seizure mechanisms, mice with interneurons marked with Ai14-Td Tomato were generated. The vulnerability of inhibitory neurons to *Ndufs4* KO was assessed by confocal imaging. Changes in interneuron excitability were examined by patch-clamp recordings. Changes in hippocampal neuronal excitability were examined using Cfos immunohistology. When exposed the proconvulsant PTZ, *Gad2-Ndufs4-KO* mice showed increased susceptibility to PTZ-provoked seizures. All mutants (100%) (8/8) showed myoclonic (MC) seizures compared to 62% (5/8) of controls. In addition, 37.5% (3/8) of mutants exhibited generalized tonic-clonic (GTC) seizures in contrast to 0% (0/8) of controls. During moderate exercise, both young and old mutants were susceptible to exercise-induced GTCs, with 80% of young and old mutants showing GTC seizures by the recovery stage compared to 0% of controls. Histological assays revealed a 30% interneuron cell loss in the hippocampus and other key brain regions associated with epilepsy in mutants. Electrophysiological recordings showed that mutants did not show any abnormal EEG activities during the interictal period. However, a large portion (2/3) of mutant CA1 interneurons failed to fire with increased depolarization currents. These findings suggest that concurrent interneuron cell loss and impaired firing are part of the cellular mechanisms underlying epilepsy in these LS mice.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.06

Topic: C.01. Brain Wellness and Aging

Support: H2020-MSCA-IF-2016 749697 GaMePLAY
PRIN2017 2017HMH8FA
NIH grant GM123558

Title: Brain histone beta-hydroxy-butyrylation couples metabolism with gene expression

Authors: *S. CORNUTI¹, S. CHEN², L. LUPORI¹, F. FINAMORE³, M. SAMAD², F. RAIMONDI¹, M. CALDARELLI⁴, R. MAZZIOTTI⁵, C. MAGNAN², S. ROCCHICCIOLI³, P. BALDI², P. TOGNINI^{4,1};

¹BIO@SNS lab, Scuola Normale Superiore, Pisa, Italy; ²Inst. for Genomics and Bioinformatics, Sch. of Information and Computer Sciences., Univ. of California, Irvine, CA; ³Inst. of Clin. Physiol., Natl. Res. Council, Pisa, Italy; ⁴Dept. of Translational Res. and New Technologies in Med. and Surgery, Univ. of Pisa, Pisa, Italy; ⁵Dept. of Developmental Neurosci., IRCCS Stella Maris Fndn., Calambrone (Pisa), Italy

Abstract: Nutrition is a key regulator of the physiology and pathology of peripheral organs. However, mounting evidence shows that it also affects neuronal function, metabolism, and ultimately, cognitive processes and behavior. For instance, fasting and ketogenic diet (KD) can ameliorate epileptic seizures in kids, although the molecular mechanisms involved are still unknown. KD, fasting, and prolonged aerobic exercise promote the depletion of liver glycogen stores and the decrease of glycemia. Thus, to maintain metabolic homeostasis, our body switches to an alternative fuel: ketone bodies. Among them, beta-hydroxy-butyrate (BHB) is the most abundant circulating ketone body and the major energy fuel for metabolic active tissues such as the brain. Intriguingly, recent data obtained in the liver suggest that BHB also acts as a new epigenetic mark regulating gene transcription. Since little is known about the impact of fasting on brain epigenome and transcriptome, we adopted a ketogenic metabolic challenge, based on 48 hrs fasting, to assess the molecular and epigenetic adaptation of the cerebral cortex to enhanced levels of circulating BHB. Lysine beta-hydroxybutyrylation (K-bhb) was significantly increased in the cortex of fasted mice, suggesting that the boost in cortical BHB could be exploited as a chemical donor for this unexplored post-translational modification. We found that fasting enhanced K-bhb in a variety of proteins including histone H3. ChIP-seq experiments pointed out that K9 beta-hydroxybutyrylation of H3 (H3K9-bhb) was significantly enriched by fasting on more than 8000 DNA loci. The RNA-seq analysis showed that fasting caused a dramatic transcriptional change in the cortex. These changes were significantly correlated with the enrichment of H3K9-bhb on enhancers and promoters, suggesting brain H3K9-bhb is linked to active gene expression. One of the most enriched functional annotations both at the epigenetic and transcriptional level was "circadian rhythms". Indeed, we found that the diurnal oscillation of specific transcripts was modulated by fasting at distinct zeitgebers both in the cortex and suprachiasmatic nucleus. Moreover, specific changes in locomotor activity daily features were observed during the re-feeding phase after 48-hour of fasting. Our results suggest that fasting

impinges on the cerebral cortex transcriptional and epigenetic landscape, and BHB acts as a powerful epigenetic molecule in the brain through direct and specific histone marks remodeling in neural cells.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.07

Topic: C.01. Brain Wellness and Aging

Support: Grant Ministry of Economy and Competitiveness DIN2019-010902
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17-09409

Title: Characterization of the lipid environment of whole, raft and non-raft membranes isolated from human astrocytes exposed to pro-oxidant conditions

Authors: *L. SÁNCHEZ-SÁNCHEZ, R. FERNÁNDEZ, E. ASTIGARRAGA, G. BARREDA-GÓMEZ;

Dept. of Res. and Develop., IMG Pharma Biotech, Derio, Spain

Abstract: Neurodegenerative diseases related to age have been increasing due to an improvement of life socioeconomic conditions. During aging, cell changes in cell homeostasis can be produced, such as augmentation of oxidized products from lipids, sugars, or nucleic acids or lipid membrane composition variations. These lipid environment modifications could imply a loss of cell membrane properties or the deregulation of diverse signaling pathways. Specifically, changes in lipid ordered domains (lipid raft), a platform which involved in cellular signaling, may cause its disruption. Some of these alterations can be simulated using a pro-oxidant external agent in order to reproduce an oxidative environment. To study the influence of an external pro-oxidant agent, firstly cell membrane microarrays (CMMAs) were developed from human astrocytic cell line (1321N1) with and without Paraquat treatment as pro-oxidant agent, in addition to the presence or absence of an antioxidant compound pre-treatment with α -tocopherol (vitamin E). Moreover, in order to clarify if oxidation processes affect lipid ordered domains (LOD, or lipid rafts) in the same way as lipid disordered membranes (LDM, or non-raft membranes), we have developed CMMAs of isolated LOD and LDM from human astrocytic cell line with and without Paraquat treatment. For this purpose, MALDI mass spectrometry assays were performed in complete membranes, from Control and Paraquat treated cells with and without vitamin E pre-treatment, as well as, in raft, and non-raft membranes from control or Paraquat treated cells in positive and negative-ion mode. Significant differences between lipid

adducts present in raft and non-raft membranes were observed in both conditions as well as differences exist between control and paraquat non-raft membranes. The presence of this compound entails the oxidation of lipid membranes, obtaining lipid adducts with higher number of unsaturations or more oxygen molecules. Furthermore, these data are consistent with oxidative stress experiments performed previously. Our result points out that this technology is useful not only to analyze the lipidic environment but also to perform different assays employing very little amount of sample, allowing the possibility of developing assays in human samples.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.08

Topic: C.01. Brain Wellness and Aging

Support: R01 AG065428

Title: Functional glucose uptake and glycolysis are essential to neuronal metabolism

Authors: *Y. SEI¹, H. LI¹, N. BENNET¹, J. YANG¹, J. RATHMELL², M. KAMPMANN³, K. NAKAMURA¹;

¹Gladstone Inst. of Neurolog. Dis., Gladstone Inst., San Francisco, CA; ²Vanderbilt Ctr. for Immunobiology, Vanderbilt Univ. Med. Ctr., Nashville, TN; ³Inst. for Neurodegenerative Dis., UCSF, San Francisco, CA

Abstract: Neurons and glia are metabolically linked, and primary neuronal cultures invariably contain glia, making it difficult to distinguish the basal metabolism of neurons from the contributing metabolites derived from astrocytes and oligodendrocytes. As such, it remains unclear if neurons even directly metabolize glucose through glycolysis to fuel mitochondrial respiration. Moreover, patients with Alzheimer's Disease, have reduced glucose uptake and expression of the primary neuronal glucose transporter GLUT3 in the brain, raising the possibility that changes in neuronal glucose uptake contribute to neurodegeneration. In order to understand if neurons rely on direct glucose uptake and glycolysis, we cultured human induced pluripotent stem cells expressing a doxycycline-induced form of neurogenin 2 to yield a scalable population of pure neurons for metabolomic analysis. We compared neurons with CRISPRi-mediated knockdown of PKM (PKM KD), the last enzyme in glycolysis responsible for the conversion of phosphoenolpyruvate (PEP) to pyruvate, or GLUT3 (GLUT3 KD), to neurons with a non-targeting CRISPRi guide. Using targeted metabolomics with uniformly ¹³C-labeled glucose ([U-¹³C]glucose) we found that knocking down GLUT3 and PKM both markedly impacted their immediate downstream metabolites. Knocking down PKM in the human neurons resulted in a ≈1500% increase in the upstream metabolite PEP relative to non-targeting neurons,

providing strong evidence of glucose flux through glycolysis in neurons. Knocking down GLUT3 expression did not alter the relative amounts of ^{13}C -labeled citrate, indicating that the limited amount of glucose in the cells was used to fuel mitochondrial respiration via the tricarboxylic acid cycle. In addition to the unchanged citrate levels, only $\approx 10\%$ of ribose-5P (R5P) was labeled with ^{13}C in the GLUT3 KD neurons, whereas $\approx 70\%$ of R5P was labeled in non-targeting neurons, further illustrating that $[\text{U-}^{13}\text{C}]$ glucose was used for mitochondrial respiration rather than for the pentose phosphate pathway, a metabolic pathway peripheral to glycolysis. Taken together, these results for GLUT3 KD and PKM KD show that neurons rely on glucose uptake and glycolysis for their basal metabolism. Ongoing studies focus on understanding the mechanisms through which neurons respond to disrupted glucose uptake in vivo. Preliminary results using spatial transcriptomic comparisons of neurons in mice with postnatal deletion of GLUT3 hippocampal neurons identify galactose metabolism and mitochondrial genes as altered in CA1 neurons, suggesting potential compensatory pathways.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

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Topic: C.01. Brain Wellness and Aging

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German Research Foundation (SFB-TRR58, Project C09)

Title: Association between body mass index and cortical brain structure in bipolar disorders - An ENIGMA study in 2,832 individuals

Authors: *S. MCWHINNEY¹, M. ALDA⁴, F. BENEDETTI⁵, D. M. CANNON⁶, U. DANNLOWSKI⁷, L. DIETZE², T. ELVSÅSHAGEN⁸, B. HAARMAN⁹, F. M. HOWELLS¹⁰, T. T. KIRCHER¹¹, M. LANDÉN¹², C. LOPEZ-JARAMILLO¹³, C. MCDONALD⁶, S. MEIER³, P. MITCHELL¹⁴, E. POMAROL-CLOTET¹⁵, A. ROSS³, J. SAVITZ¹⁶, K. SIM¹⁷, N. VAN HAREN¹⁸, E. VIETA¹⁹, T. HAJEK³;

¹Dalhousie Univ., Middle Sackville, NS, Canada; ²Med. Neurosci., ³Dalhousie Univ., Halifax, NS, Canada; ⁴Dept. of Psychiatry, Dalhousie Univ., Halifax, NS, Canada; ⁵Vita-Salute San

Raffaello Univ., Milan, Italy; ⁶Natl. Univ. of Ireland Galway, Galway, Ireland; ⁷Univ. of Münster, Münster, Germany; ⁸Univ. of Oslo, Oslo, Norway; ⁹Univ. of Groningen, Groningen, Netherlands; ¹⁰Dept. of Psychiatry and Mental Hlth., Univ. of Cape Town, Cape Town, South Africa; ¹¹Philipps Univ. Marburg, Marburg, Germany; ¹²Sahlgrenska Acad. at Gothenburg Univ., Gothenburg, Sweden; ¹³Univ. de Antioquia, Medellin, Colombia; ¹⁴Univ. of New South Wales, Sydney, Australia; ¹⁵FIDMAG Germanes Hospitalàries Res. Fndn., Barcelona, Spain; ¹⁶Laureate Inst. for Brain Res., Tulsa, OK; ¹⁷Inst. of Mental Hlth., Singapore, Singapore; ¹⁸Univ. Med. Ctr. Utrecht, Utrecht, Netherlands; ¹⁹Inst. d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

Abstract: Obesity has reached epidemic proportions, especially amongst people with psychiatric disorders. While effects of obesity on the brain are of interest in medicine, they remain markedly under-researched in psychiatry. There are few large-scale, generalizable studies describing the brain correlates of obesity and how these map onto the cortical alterations in bipolar disorders (BD). We combined T1-weighted brain MRI data from 1,231 individuals with BD and 1,601 healthy controls (HC) in 13 countries from the ENIGMA BD Working Group. The ENIGMA-standardized FreeSurfer processing and quality control protocol was used to segment 34 regions per hemisphere. We tested cortical thickness (CT) and surface area (SA) for association with diagnosis (BD or HC), body mass index (BMI) and their interaction while adjusting for age and sex using linear mixed modeling. We also investigated the interplay between psychiatric (antipsychotic, anticonvulsant, or antidepressant) medications at the time of scanning, BMI and brain structure, and tested whether BMI mediated any effects of medication. BMI was negatively associated with CT in 10 regions. Except for a single ROI (entorhinal cortex), all regions that were negatively associated with BMI were also negatively associated with BD. In contrast, only a single ROI (isthmus of the cingulate gyrus) showed an association between BMI and SA, which was positive. Individuals with BD showed thinner cortex than HC in 32 regions, 9 of which were also associated with BMI. In those with BD, taking more medication classes was associated with lower CT in 65% of regions, even while adjusting for BMI. Lastly, BMI significantly mediated the association between medication and lower CT in the fusiform and superior frontal gyrus. We demonstrated associations between higher BMI and lower CT across the cerebral mantle, in regions that were also associated with BD. In some regions the association between medications and lower CT may be in part explained by obesogenic effects of such medications. Overall, BMI is important for understanding neuroanatomical changes in BD and the effects of psychiatric medications on the brain. We need prospective longitudinal studies to investigate whether such brain alterations are a cause or consequence of obesity.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.11

Topic: C.01. Brain Wellness and Aging

Title: Superoxide enters neurons and causes oxidative stress via the volume-sensitive organic anion channel

Authors: ***K. L. HARRIS**^{1,2}, G. URUK^{1,2}, S. WON^{1,2}, J. WANG^{1,2}, P. S. BAXTER^{1,2}, R. A. SWANSON^{1,2};

¹Neurol., U.C.S.F., San Francisco, CA; ²San Francisco Veterans Affairs Med. Ctr., San Francisco, CA

Abstract: Oxidative stress causes neuronal DNA damage and cell death in a variety of pathological conditions. The primary oxidant produced in the brain is superoxide, which is released into the extracellular space by glia, neurons, and inflammatory cells. It has been postulated that extracellular superoxide anions enter cells by first forming non-polar H₂O₂, which can pass through certain channels and passively cross lipid membranes. We show here instead that superoxide enters directly into neurons through LRRC8A containing volume sensitive organic anion channels. We measured intracellular superoxide (using dihydroethidium; DHE) in primary mouse cortical neurons in response to 20-minute incubations with potassium superoxide or N-methyl-D-aspartate (NMDA). The DHE signal induced by these treatments was suppressed by co-incubation with superoxide dismutase (SOD), thus confirming that signal production was attributable to superoxide entering cells from the medium. The DHE signal was likewise attenuated by the non-specific anion channel blocker DIDS and by the LRRC8A channel inhibitor 4-(2-butyl-6,7-dichlor-2-cyclopentylindan-1-on-5-yl) oxobutyric acid (DCPIB). Evaluations of neuronal DNA damage by quantification of gammaH2AX puncta showed a parallel result: DNA damage was reduced by co-treatment with SOD, DIDS, or DCPIB. We then replicated these findings *in vivo*, using mice treated with either intracortical injections of NMDA or with transient middle cerebral artery occlusion to stimulate superoxide production. The neuronal DHE signal and gammaH2AX formation induced by these treatments were both attenuated by DCPIB. Next, we achieved genetic knockdown of neuronal LRCC8A using LRRC8A-floxed mice injected intracortically with AAV9 containing cre-recombinase under the synapsin-1 promoter. We found that neurons with reduced LRCC8A expression showed less DHE signal and less DNA damage after either NMDA injection or transient ischemia. Together, these findings indicate that extracellular superoxide enters neurons primarily via the LRRC8A channel, and that blocking this channel can be neuroprotective.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.12

Topic: C.01. Brain Wellness and Aging

Support: Brain and Behavior Research Foundation NARSAD Young Investigator Award
AdamFest Research Fund
NIH Psychiatry INSPIRE T32 5T32MH019113-28

Title: An in vitro model of metabolic dysfunction in bipolar disorder

Authors: *K. KRUTH¹, A. J. WILLIAMS²;
²Psychiatry, ¹Univ. of Iowa, Iowa City, IA

Abstract: Though bipolar disorder (BD) is common, it remains one of the most severe, and least treatable, psychiatric illnesses. Poor understanding of disease etiology has led to a lack of treatment targets, greatly hindering progress toward new therapies. However, a number of studies using a variety of techniques—from MRS imaging to metabolomics to genetic analyses—have identified changes in cellular energy metabolism and hallmarks of mitochondrial dysfunction in patients with BD. We therefore hypothesize that mitochondrial dysfunction may be a key potential therapeutic target in BD. To test our hypothesis, we generated induced pluripotent stem cells from three patients with BD and three healthy controls, which we then differentiated into cortical glutamatergic neurons for phenotypic assessment. Surprisingly, we found that BD stem cells are more unstable than control stem cells, and neurons differentiated from BD stem cells develop more quickly, reliably producing neurites days before their control counterparts. In addition, after two months of culture, BD neurons developed a striking amount of neuritic beading compared to control neurons, a phenotype associated with mitochondrial dysfunction and ATP deficiency. Our results suggest that stem cell-derived neurons present with a disease-relevant phenotype, allowing us to investigate the molecular etiology of BD in vitro, as well as providing a means to screen potential treatments.

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Poster

480. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 480.13

Topic: C.01. Brain Wellness and Aging

Support: NINDS Grant to Mcmanus GRT-00000131

Title: Noninvasive imaging of oxidative stress as a biomarker of Parkinson's disease

Authors: *Y. ZHU^{1,2}, N. KOHLI², A. YOUNG¹, M. SHELDON², S. RILEY², J. JOSE², N. PATEL², R. DOOT¹, R. MACH¹, M. MCMANUS²;

¹Radiology, Univ. of Pennsylvania, Philadelphia, PA; ²Anesthesiol. and Critical Care Med., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: As the second most common neurodegenerative disease, Parkinson's disease (PD) has emerged to become a pressing public health crisis in modern society. In general, PD patients experience mild or unnoticed symptoms at the early stage of the disease and gradually descend into motor dysfunction (tremor, bradykinesia, impaired posture, etc.) due to the progressive death of dopaminergic (DA) neurons in the substantia nigra. Despite recent advancements in PD research, the precise molecular mechanisms responsible for neuronal death and motor dysfunction in late-onset PD are unknown. Evidence suggests that mitochondrial dysfunction and neuroinflammation occur early, leading to a collective increase in reactive oxygen species (ROS) production and oxidative stress that persists throughout the course of the disease. However, the lack of noninvasive methods for tracking oxidative stress in the living brain has precluded its use as a potential biomarker. The current study addresses this need through the evaluation of the first superoxide ($O_2^{\cdot-}$)-sensitive radioactive tracer, [^{18}F]ROStrace, in the MitoPark mouse model. MitoPark mice have a DA-specific deletion of *TFAM* (transcription factor A mitochondrial), which encodes a major mtDNA binding protein that regulates mtDNA stability. Loss of TFAM in DA neurons leads to mitochondrial dysfunction, the release of mtDNA, and constitutive activation of STING signaling, resulting in neuroinflammation and progressive nigrostriatal deterioration. The goal of this study was to determine if superoxide ($O_2^{\cdot-}$) is a key, trackable signal of mitochondria-induced neuroinflammation during the course of PD-like progression in MitoPark mice. To achieve this goal, MitoPark mice were imaged with [^{18}F]ROStrace from the prodromal phase (2mo.) to the end-stage of PD-like disease (6mo.). The micro-PET/CT results were correlated with behavior and metabolic measures at each time point and compared to age-matched controls (n =10). Our micro-PET/CT analysis demonstrated increased [^{18}F]ROStrace retention during the prodromal phase of MitoPark pathology which persisted to the end stage. [^{18}F]ROStrace is sensitive to oxidative stress caused by DA-specific mitochondrial dysfunction and neuroinflammation during the early stages of PD-like pathology in mice. Thus, [^{18}F]ROStrace may provide a method to identify patients at-risk of neurodegenerative disease before irreparable neurodegeneration occurs and enhance clinical trial design by identifying patients who are most likely to benefit from mitochondrial therapeutics. This research is supported by NINDS grant GRT-00000131.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 481.01

Topic: F.07. Biological Rhythms and Sleep

Title: Increased REM sleep without atonia in mice with neurotrauma: circuit mapping using functional ultrafast ultrasound (fUS)

Authors: C. TINSLEY^{1,2}, K. GUTOWSKY¹, P. WICKHAM¹, C. REYNOLDS², R. LERICHE¹, N. MILMAN^{1,2}, R. OPEL¹, D. AKINS¹, C. MESHUL^{1,2}, V. UNNI², *M. LIM^{1,2};
¹Veterans Affairs Portland Hlth. Care Syst., Portland, OR; ²Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Neurotrauma, including traumatic brain injury (TBI) and/or post-traumatic stress disorder (PTSD), often results in persistent sleep disturbances and impaired neurological function. Among US Veterans, emerging evidence suggests that exposure to neurotrauma increases rates of Rapid Eye Movement (REM) Sleep Behavior Disorder (RBD), a condition characterized by loss of muscle atonia during REM sleep. An estimated 50-90% of patients with RBD go on to develop Parkinson's disease or related synucleinopathies; however, the underlying mechanisms are unclear. Here, we aim to create a rodent model of neurotrauma and RBD in order to elucidate the behavioral and sleep profile of mice exposed to neurotrauma. Mice underwent either Single Prolonged Stress (SPS) and/or Controlled Cortical Impact (CCI). We then evaluated sleep EEG/EMG along with gait using a DigiGait treadmill and cognition using contextual fear conditioning. We also evaluated functional connectivity using functional ultrafast ultrasound (fUS) as a proxy for neuronal activation. Similar to human populations, we found that a subset of mice exposed to neurotrauma displayed altered gait patterns, reduced contextual fear recall, and had an increased frequency of muscle twitches during REM sleep, compared to controls. Results from these experiments will lead to a better understanding of the neural circuits underlying the links between neurotrauma, REM sleep without atonia, and ultimately neurodegenerative disease.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 481.02

Topic: F.07. Biological Rhythms and Sleep

Support: Intramural Research Program, National Institute of Neurological Disorders and Stroke

Title: Exploring the effect of region size on seed-based functional correlations during sleep fMRI

Authors: N. LAM, J. A. DE ZWART, H. MANDELKOW, Y. WANG, P. VAN GELDEREN, J. H. DUYN, *D. PICCHIONI;
NIH - Intramural Res. Program, Bethesda, MD

Abstract: The functional relationship between brain regions and its change across wakefulness and sleep can be gauged by computing Pearson correlation coefficients between fMRI signals from different brain regions. Previous studies recognized that differing region sizes can have implications for estimating these functional correlations (Hermundstad et al., 2013; Fallon et al., 2020). Researchers employed a uniform-sampling technique to address this issue without deeper investigation into the nuances of this technique. To further explore the impact of region size on the estimation of functional correlations, we replicated this sampling technique using three seed region masks of uniform size using randomly sampled voxels, and their whole-region counterparts. Functional correlations were computed and compared for three bilateral Regions Of Interest (ROIs) of varying mask sizes with voxel size of 1mm^3 - Hippocampus (Hipp): 10,803, Posterior Cingulate Cortex (PCC): 4,884, and Postcentral Gyrus (PCG): 45,234. Bilaterally uniform sampled regions contained 392 voxels for every region. Spatial means were calculated across the voxels in each ROI. Paired-samples t tests were generated for one run per subject for group-level analyses ($n=12$). Visible but minor qualitative variances appeared in the correlations for Hipp and PCC at the single-subject level, while even fewer differences appeared for the PCG. Regardless of mask type, the strongest correlations occurred in the region itself, indicating the general integrity of the technique. For the group-level analyses, whole-region PCC correlations with the whole-region ($M=0.462$, $SD=0.205$) and sampled Hipp ($M=0.302$, $SD=0.136$) demonstrated that the whole-region Hipp had a significantly stronger correlation than sampled Hipp: $t(11)=3.85$, $p=0.003$, $d=1.1$. None of the differences in the average correlation were statistically significant (all $p>0.10$, all $d<0.06$) for the remaining five permeations (e.g., whole-region versus sampled Hipp for PCG). The quantitative results were consistent with the qualitative results; the uniform-region mask showed similar correlations compared to its whole-region counterpart. The quantitative results revealed few significant differences when using either a whole or uniformly sampled region for analysis. Smaller region sizes may accurately represent functional correlations, even though maximizing the number of voxels that create the region means is more ideal. These results could have implications for fMRI analyses in general. Future application of this technique will include all regions in the brain, and all runs in this and other sleep fMRI datasets.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

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Program #/Poster #: 481.03

Topic: F.07. Biological Rhythms and Sleep

Support: Canada Research Chair (B.A.K)
SFU New faculty Start-up Grant (B.A.K)

Title: Sleep, mood, and performance on the Cambridge Neuropsychological Test Automated Battery (CANTAB) in healthy young adults

Authors: *D. GILL¹, A. ROENNINGEN¹, B. A. KENT^{1,2};
¹Psychology, Simon Fraser Univ., Burnaby, BC, Canada; ²Inst. for Neurosci. and Neurotechnology, Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Sleep disturbance is often associated with cognitive impairment and is considered a risk factor for neurodegenerative diseases and dementia. The current project includes two experiments designed to identify what cognitive tests are sensitive to sleep-dependent cognition in healthy young adults using the Alzheimer's disease test battery from the Cambridge Neuropsychological Test Automated Battery (CANTAB), the Psychomotor Vigilance Task (PVT), and the Mnemonic Similarity Task (MST) of pattern separation. The first study recruited healthy young adults (n = 40; aged 18-30 years) and monitored their sleep for seven consecutive days using Condor ActTrust 2 actigraphy watches and daily sleep diaries. At the end of the week, participants were brought into the lab to complete the cognitive testing. Participants also completed the Beck's Depression Inventory and the Beck's Anxiety Inventory. The second study examined the effects of one night of total sleep deprivation on performance on the same battery of cognitive tests. The sleep deprived group (n = 16; aged 18 - 40) was observed overnight in the laboratory to ensure compliance to the sleep deprivation protocol. The participants wore UVEX S1933X blue-wavelength blocking glasses to minimize phase-shifting effects of nighttime light exposure. The rested control group (n = 16; aged 18 - 40), was not required to stay in the laboratory overnight and was asked to sleep as they normally do at home. All participants wore actigraphy watches for the duration of the study and sleep diaries were completed each day. Preliminary findings show that shorter sleep latency is associated with less errors made on the Delayed Matching to Sample (DMS) task and longer total sleep time is associated with less errors made on the DMS task, faster average reaction time on the PVT, and greater accuracy on the MST in the first experiment. In the second experiment, the rested control group showed greater accuracy on the MST compared to the sleep deprived group, which indicates that sleep deprivation may impair pattern separation processes; however, data collection and analysis is ongoing for both studies. The goal for this project is to identify cognitive tests that are sensitive to sleep-dependent cognition, which can be used as clinical trial outcome measures for treatments targeting sleep.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

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Program #/Poster #: 481.04

Topic: F.07. Biological Rhythms and Sleep

Title: Modeling the impact of SARS-Cov-2 on sleep quality through the analysis of mental, behavioral, and physical states of adults before and after COVID-19 vaccination

Authors: *S. JAHANIKIA, A. RAVI, A. MANTRIPRAGADA, A. UPPALAPATI, D. PINTO, H. YOON, D. MELWANI, S. ABHIJIT;
The Aspiring Scholars Directed Res. Program, Fremont, CA

Abstract: COVID-19-related disturbances, such as the shelter-in-place order, have been linked with sleep disruption amongst the general population. Due to an increase of screen time and consumption of addictive substances correlated with the pandemic, sleep issues such as difficulty falling and/or staying asleep and imbalances in circadian rhythm have become increasingly common in adults. Such disturbances weaken the immune system, leading to an increased susceptibility to the COVID-19 virus, creating a further cycle of stress and loss of regular sleeping patterns. The goal of the study is to examine the relationship between sleep quality and COVID-19 vaccination status by computing a sleep quality index (SQI) for participants before and after vaccination. Over 50 research articles and papers measuring sleep quality were assessed to eliminate outlier populations, such as healthcare workers and those with preexisting sleep disorders; such populations may not accurately reflect the sleep trends of the general population. Previously developed sleep questionnaires, including the Insomnia Severity Index and the Pittsburgh Sleep Quality Index, were used to design the study's eligibility and sleep assessment questionnaires. Additionally, a demographic questionnaire was created to monitor the diversity of the participants. To calculate participants' SQI, they were asked questions about their sleep-related behavior and mental state, where a higher SQI signified poorer sleep quality. As of today, the study has had approximately 141 interested participants and 68 SQI scores from eligible participants, which include 35% male and 65% female with an average age of 35.5 years. The average sleep quality index of the participants before the vaccination date is 29.8 in comparison to their sleep quality after, which is 28.7. The correlation coefficient is 0.69, which shows a strong correlation between the independent and dependent variables. The null hypothesis stated there was no significant difference between sleep quality index scores before and after vaccination. The T-test analysis from the ongoing study shows a significant effect of the vaccine, indicating that the null hypothesis can be rejected. The results of this study will introduce the possible psychological benefits, specifically related to sleep, of the COVID-19 vaccine to reduce biological and mental harm caused by the ongoing pandemic. Further, the study will focus on causal modeling and the various factors of sleep index based on mental, environmental, and biological factors.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 481.05

Topic: F.07. Biological Rhythms and Sleep

Support: NSF Grant BCS-2001025

Title: Predictors of sleep's effect on emotional memory

Authors: ***K. E. G. SANDERS**¹, D. DENIS², E. A. KENSINGER³, J. D. PAYNE¹;

¹Univ. of Notre Dame, South Bend, IN; ²Univ. of York, York, United Kingdom; ³Psychology and Neurosci., Boston Col., Chestnut Hill, MA

Abstract: We do not remember everything that we encounter in our waking lives. Instead, information that is emotional, important, and of future relevance is selectively preserved. Sleep has been associated with the selective consolidation of emotional information compared to a similar period awake; however, recent meta-analyses suggest that the effect may not be as consistent as previously thought. One task that has shown a consistent effect of sleep on emotional memory is the emotional memory tradeoff task. In this task, participants incidentally encode complex scenes that consist of an emotional or neutral object on an always neutral background. When later tested for their memory of the objects and backgrounds separately, participants remember the emotional objects better than the neutral objects but at the cost of worse memory for the negative objects' associated backgrounds, known as the emotional memory tradeoff effect. If participants sleep between encoding and retrieval, this tradeoff effect is enhanced suggesting a role for sleep in selective memory consolidation. In a series of three experiments, we explored whether sleep's effect on the emotional memory tradeoff is preserved in larger more representative samples, in young adulthood through middle age, and in both negative and positive emotional stimuli; and we identify neurological and biological signals that predict the effect. In a sample of more than 250 participants aged 18-59 years old from across the United States, we found that sleep selectively enhanced the negative emotional memory tradeoff effect providing strong evidence for a sleep benefit in selectively consolidating negative information. The effect was not moderated by age or sleep quality suggesting that it was preserved into middle age. In a second study with a comparable population and sample size, we did not find any evidence of an effect of sleep on positive emotional memory, suggesting that the sleep benefit does not extend to positive information. Finally, in a third study we found measures of EEG, heart rate, and skin conductance during encoding and overnight PSG recording during consolidation predicted a benefit of sleep on the negative emotional memory tradeoff effect. In combination these studies demonstrate that sleep selectively preserves negative information in larger, more representative samples than have been used in previous research and identify neurological and biological predictors of the effect.

Disclosures: **K.E.G. Sanders:** None. **D. Denis:** None. **E.A. Kensinger:** None. **J.D. Payne:** None.

Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 481.06

Topic: F.07. Biological Rhythms and Sleep

Support: CSC fellowship

Title: The role of sleep in stimulus-response learning

Authors: X. MIAO¹, C. MÜLLER¹, Q. YANG², J. BORN¹, F. WASZAK², *K. RAUSS¹;
¹Univ. of Tübingen, Tübingen, Germany; ²Integrative Neurosci. and Cognition Ctr., Univ. Paris Cité & CNRS, Paris, France

Abstract: Performing a motor action in response to a sensory stimulus creates a memory trace whose behavioral correlates are classically investigated in terms of priming effects. Specifically, if the same stimulus is encountered again, behavioral performance is usually improved if the same motor action is required, but impaired if a different motor response must be given. There is clear evidence that such Stimulus-Response (S-R) learning entails at least two types of associations which are at least partly independent: first, an association between the stimulus and the motor response; and second, an association between the stimulus and the task context in which it was encountered. Recent research indicates that both types of associations are surprisingly long-lived, with behavioral effects persisting over several days. In the present experiment, we tested whether sleep in general supports such long-lasting S-R learning; and whether particular sleep stages are selectively correlated with stimulus-action or stimulus-task associations. We tested 48 healthy volunteers in a between-subjects design comparing daytime wakefulness against nighttime sleep. Preliminary behavioral results indicate successful replication of independent stimulus-action and stimulus-task associations. At the same time, there were no clear-cut differences between wake and sleep groups. This indicates that behavioral performance in such relatively simple tasks may be supported equally well by sleep-dependent and sleep-independent consolidation mechanisms. Ongoing analyses of polysomnographic recordings will allow us to test whether behavioral effects in the sleep group are selectively associated with specific sleep stages.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

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Program #/Poster #: 481.07

Topic: F.07. Biological Rhythms and Sleep

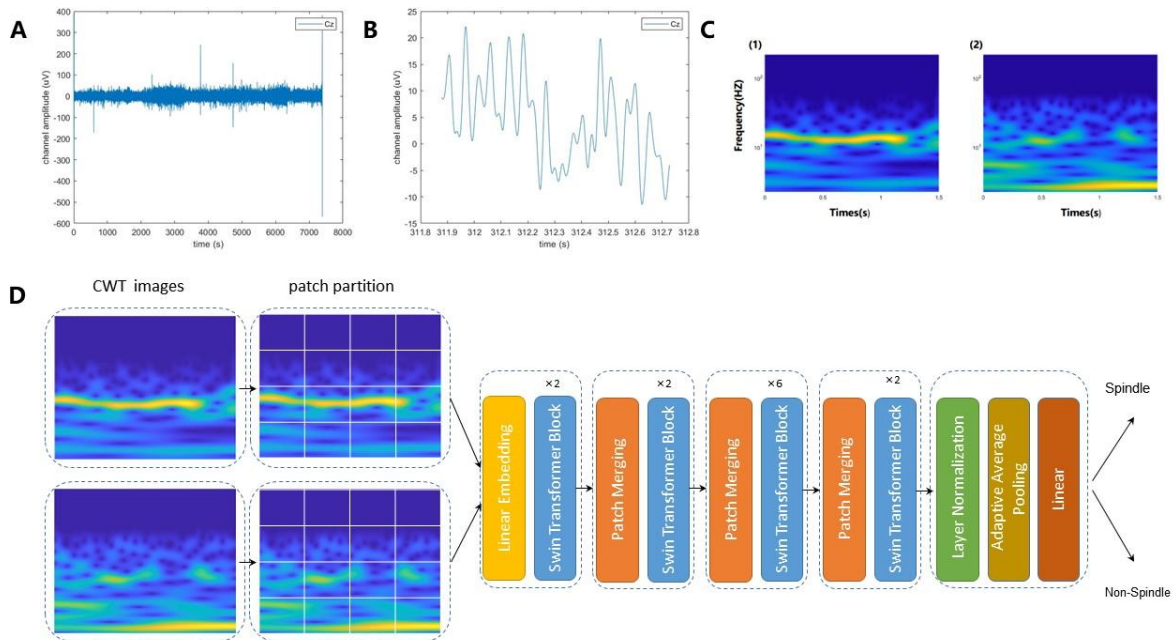
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Title: Swin-wavelet: a robust sleep spindle detection approach for simultaneous EEG-fMRI based on Wavelet and Swin-Transformer

Authors: *S. XIA^{1,2}, Y. SHAO³, G. ZOU⁴, X. GAO³, Q. HU⁴, Y.-S. SUN¹, H.-T. ZHU¹, H. SUN³, J.-H. GAO⁴, Q. ZOU⁴;

¹Peking Univ. Cancer Hosp. & Inst., Beijing, China; ²Inst. of Med. Technology, Peking Univ. Hlth. Sci. Ctr., Beijing, China; ³Peking Univ. Sixth Hospital, Peking Univ., Beijing, China; ⁴Ctr. for MRI Research, Acad. for Advanced Interdisciplinary Studies, Peking Univ., Beijing, China

Abstract: Introduction: Sleep spindle is an important physiological event which can be monitored by EEG that typically occurs in non-REM sleep. With advances in simultaneous EEG-fMRI, the detected spindle can be taken as a significant reference for fMRI modeling, which contributes to the neural mechanisms of cognition associated with sleep and pathophysiological underpinnings of sleep disorders. In this study, we proposed a spindle detection approach, i.e., Swin-Wavelet, on EEG data simultaneously acquired with fMRI recordings, combining wavelet transform and Swin-Transformer. **Methods:** Twenty-eight healthy subjects were scanned during sleep using EEG-fMRI. EEG data were preprocessed and sleep spindles were annotated by two technicians. A continuous wavelet filter bank was then built and 4047 time-frequency maps of all the labeled spindles (Fig. 1C-1) were generated(1.5s). Meanwhile, the preprocessed EEG signals of each subject were randomly sampled (1.5s) and 4047 non-spindle time-frequency maps (Fig. 1C-2) were produced. Then the 8094 time-frequency maps were put into the pretrained Swin-Transformer for classification (optimizer: AdamW, batchsize = 8, lr = 0.0001). **Results:** We tested the performance of Swin-Wavelet on independent training and validation datasets. Data from half of the subjects were treated as training group and data of the other subjects were used as validation. We repeated the independent validation procedure three times, the accuracy, recall, F1-score were (88.40%, 97.42%, 89.34%), (89.76%, 81.44%, 88.83%), and (86.91%, 82.00%, 86.23%), separately. **Conclusion:** Swin-Wavelet model showed robust performance in sleep spindle detection, including high accuracy and stable generalization based on EEG data acquired simultaneously with fMRI. This model could be used as an alternative for manual labeling of sleep spindles. Future studies will be conducted by validating this model on a larger sample and generalizing this model to clinical populations such as insomnia.



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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 481.08

Topic: F.07. Biological Rhythms and Sleep

Support: Slamen-Fast New Initiatives, University of Toronto

Title: Examining links between general fatigue in myasthenia gravis with objectively measured sleep

Authors: *J. YANG¹, H. KATZBERG², A. ABRAHAO³, C. D. KASSARDJIAN⁴, L. ZINMAN³, A. IZENBERG³, C. BARNETT-TAPIA², B. J. MURRAY³, M. I. BOULOS³; ¹Inst. of Med. Sci., Univ. of Toronto, Toronto, ON, Canada; ²Toronto Gen. Hospital, Univ. Hlth. Network, Toronto, ON, Canada; ³Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada; ⁴Med. (Neurology), St. Michael's Hosp., Toronto, ON, Canada

Abstract: Myasthenia gravis (MG) is an autoimmune disease that affects the neuromuscular system. Previous research demonstrates that there is a higher prevalence of general fatigue in MG patients, which can negatively impact their quality of life. The pathophysiology of general fatigue in MG remains poorly understood. Sleep disorders are important mediators of general

fatigue in other neurological conditions, but limited research has examined the association between sleep disorders and general fatigue in MG. The objective of this study is to assess correlates of general fatigue in MG by examining its association with objectively measured sleep apnea and sleep quality. Fifty MG and fifty control participants will be identified at three recruiting hospitals located in Toronto, Canada. Participants will use a home sleep apnea testing (HSAT) device for two nights, wear a wrist-actigraphy watch for seven days, and complete questionnaires assessing for sleep disorders, general fatigue, and depression. Linear regression models will be used to examine the association of objectively measured obstructive sleep apnea severity (as assessed by the apnea-hypopnea index generated from scored HSAT) with general fatigue in MG (scores on the NeuroQOL fatigue scale), while controlling for age, sex, depressive symptoms, and the interaction of sleep apnea severity with study group (MG vs. control). We will also examine the association of objectively measured sleep quality (assessed via wrist actigraphy) with general fatigue in MG. We expect that MG patients will have a higher prevalence of sleep apnea than controls, and sleep apnea severity will be correlated with the presence of general fatigue. Lastly, this study aims to examine the feasibility of using home sleep apnea testing in patients with MG. As of date, 25 participants were approached for the study, 12 (11 MG plus 1 control, mean age: 62 years, five females, seven males) consented to the study, and three successfully completed all assessments. Out of the two scored home sleep apnea tests, one control (48, female) participant was found to have no sleep apnea, and one MG participant (49, male) was found to have mild sleep apnea. Since sleep disorders are readily treatable, if obstructive sleep apnea and/or poor-quality sleep are found to significantly contribute to general fatigue in MG, this would provide a novel treatment target to address the common yet debilitating impact of general fatigue in MG.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 481.09

Topic: F.07. Biological Rhythms and Sleep

Support: ZIA NR000020-06

Title: Identifying approaches to process actigraphy data: a scoping review

Authors: S. GONSALVES¹, M. STEELE¹, T.-F. WANG¹, A. LIVINSKI¹, T. FUSS², A. ROSS¹, C. NGUYEN¹, C. KWIAT¹, *L. SALIGAN¹;

¹NIH, Bethesda, MD; ²Montgomery Col., Silver Spring, MD

Abstract: Numerous psychoneurological studies examine the link between behaviors and physical activity to understand etiology, establish preventive algorithms, or develop therapeutics. There are several actigraphy data processing methods, but there is no consensus to define activity values or cleaning guidelines that can be used to facilitate comparison across studies. This scoping review examined existing literature on processing of actigraphy data, as guided by Arksey and O'Malley (2005) and further refined by the Joanna Briggs Institute. Comprehensive review of electronic databases including PubMed (US National Library of Medicine), Scopus (Elsevier), and Web of Science: Core Collection (Clarivate Analytics) were searched by a biomedical librarian yielding 7,024 articles meeting the eligibility criteria for initial screening. Keywords and controlled vocabulary terms (e.g., MeSH) terms for each concept of interest (e.g., actigraphy, data cleaning) were used. The searches were limited to articles published in English from 2017-2022, considering the rapid commercialization and availability of physical activity devices. The unique records were exported into Covidence (Veritas Health Innovations) to conduct the title/abstract and full text screenings. So far, 52% of the studies have been screened, and only 3% of the publications were found relevant to the topic of data cleaning involving human physical activity measured by accelerometers, affirming the vast utility of actigraphy data across disciplines. Common theme areas noted from the reviewed articles included studies on sleep, gait, fall detection, heart monitoring, motion sensing, and sedentary behavior. The results will highlight current directions and gaps to improve rigor in future psychoneurological investigations involving physical activity data gathered through actigraphy. The dissemination of this work will advance the neuroscience of physical activity and its association with health-related variables.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

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Program #/Poster #: 481.10

Topic: F.07. Biological Rhythms and Sleep

Support: The ICT & Future Planning and Korea Health Technology R&D Project grant through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI21C0572) The Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF2020R1A6A3A0110041011)

Title: Adverse Impact of Impaired Sleep on Depressive Symptoms and Quality of Life among Socially Isolated Older Adults during COVID-19: Mediation by Arthritic Pain

Authors: *S. SONG, Y. KIM, H. YOO, J. KIM;
Korea Univ., Korea Univ., Seoul, Korea, Republic of

Abstract: Although recent studies showed the adverse impact of COVID-19 pandemic on mental health among older adults, less is known about the impacts of pandemic on socially vulnerable populations. This study aims to examine the adverse impact of impaired sleep on depressive symptoms and quality of life among socially isolated older adults and whether pain, stiffness, and functional capacity from arthritis may mediate those associations between sleep and depressive symptoms/quality of life. All participants were recipients of social welfare support due to poverty and lived alone during COVID-19 pandemic (mean age = 80.02 years old (SD = 4.64); 80.81% women; the average number of chronic disease conditions = 4.4 (SD = 2.28)). Participants were asked to participate in either focused group interview (n = 16) and/or survey (n = 99). Thematic analysis was conducted by two coders on interview scripts on major difficulties and health problems during the pandemic using the Braun & Clarke's 6 step approach. The survey questionnaires were asked on social exchanges with family, friends, neighbors, sleep quality (PSQI), depression (GDS), pain (WOMAC), and quality of life (SF-12). Structural equation modeling (SEM) was conducted on survey data. The inter-rater reliability of thematic analysis was acceptable (Agreement rate = 74.7%; Cohen's kappa = .80); disagreements were resolved by discussions between two coders. Thematic analysis results identified that fear of dying alone, chronic disease managements, and sleep disorders as the common health problems and feeling lonely and depressed and economic poverty as the major sources of suffering for older adults. Survey results showed that 74.7% of older adults reported extreme social isolation without any regular social contacts. Two third of them appeared to be at risk for sleep disorders (74.7% with PSQI>5) and depression (69.6% with GDS>10). The SEM results found that impaired sleep was significantly associated with both higher depressive symptoms and poorer quality of life among them after adjusting for age, gender, education, and the number of chronic disease (p 's < .001). In addition, pain related physical function, but not the levels of pain or stiffness, significantly mediated the associations between sleep quality and depression as well as the association between sleep quality and quality of life (p 's < .05). The findings reveal the alarming rates of social isolation, depression, and sleep disorders among older adults who lived alone under poverty during COVID-19. Results also suggest that developing online interventions to prevent sleep disorders and pain related functional declines may be beneficial for them.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 481.11

Topic: F.07. Biological Rhythms and Sleep

Support: U54GM133807

Title: Effect of the "Night-float" rotation on circadian oscillation of temperature and sleep/wake cycles in medical residents

Authors: ***J. MARRERO**¹, Y. DE JESÚS², A. SEGARRA², L. ORTA ANÉS², C. NAZARIO², F. RAMIREZ-MARRERO¹, J. L. AGOSTO¹;

¹UPR-RP, Rio Piedras, Puerto Rico; ²UPR-RCM, San Juan, Puerto Rico

Abstract: Introduction: Sleep disruption during medical residency is recognized as a significant risk factor for medical trainees' exhaustion. Burnout increases the risk of medical errors, leads to the development of anxiety and emotional health complications, which may end in physician suicide. Coaching intervention with physicians in-training revealed that some residents are struggling with sleep difficulties during the night-float rotation. Misalignment between residents' internal circadian synchrony and the work shift may be one possible reason. **Methods:** This is a longitudinal cohort study of repeated measures in sixteen medical residents before, during, and after the night-float rotation. Participants completed the Composite Scale of Morningness to determine their chronotype; and the Pittsburg Sleep Quality Index to measure perceived sleep quality during each rotation. To explore confounding variables a sociodemographic questionnaire and the Demand Control Support Questionnaire were completed by the participants. A dermal sensor was used to evaluate the oscillations in core body temperature to determine circadian oscillation. A wrist sensor monitored sleep and activity of the participants to assess the degree of synchrony between parameters measured. Saliva samples were collected to measure cortisol and melatonin levels, to analyze stress and explore circadian fluctuations. **Analysis:** The measure of outcome variation according to hazard exposures and other characteristics has been performed by collecting information on each participant. To increase power, we have taken repeated measurements on the same participants at various points in time. Due to the small sample size, the importance of study's results is not based solely on statistical significance, but also on clinical significance. We used specialized software to study the oscillation of measurements and assess the shift in phases and periodicity along the night float rotation. **Conclusions:** Preliminary evaluation of temperature data shows a disruption in the oscillation of temperature including shifts in phase and period during the night float. Further exploration of the effects of the night-float rotation in residents' sleeping pattern and its relationship to their chronotypes could reveal missed opportunities to prevent BOS.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 481.12

Topic: F.07. Biological Rhythms and Sleep

Support: SNSF Grant PCEFP1-181279
SNSF Grant P0ZHP1-178697
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Clinical Research Priority Program “Sleep and Health”
Foundation for Research in Science and the 17 Humanities STWF-17-008

Title: From bedtimes to the brain: Sleep neurophysiology links sleep behavior and developmental outcomes

Authors: *S. F. SCHOCH¹, V. JARAMILLO², A. MARKOVIC³, R. HUBER⁴, M. KOHLER³, O. G. JENNI⁵, C. LUSTENBERGER⁶, S. KURTH⁷;

¹Donders Inst., Nijmegen, Netherlands; ²Univ. Children's Hosp. Zurich, Zuerich, Switzerland; ³Univ. Hosp. Zurich, Zurich, Switzerland; ⁴Psychiatry, Univ. Zurich, Zurich, Switzerland; ⁵Univ. of Zurich, Zurich, Switzerland; ⁶ETH Zürich, Zurich, Switzerland; ⁷Univ. of Fribourg, Fribourg, Switzerland

Abstract: Observational research has reported that chronic sleep problems in young children are associated with reduced later cognitive, psychosocial, and somatic health outcomes. It is hypothesized that this is due to the critical role sleep has in the maturation of neurophysiological circuitries, as shown in animal models. However, studies in human infants that objectively measure sleep neurophysiology and associate it with sleep-wake behavior and later developmental outcomes are lacking. Here, we examined how sleep neurophysiology, including slow wave activity (0.75 - 4.25 Hz), theta activity (4.5 - 7.5 Hz), and spindle density, during infancy is linked to sleep-wake behavior and later developmental outcomes. In a sample of 32 healthy infants, we measured sleep-wake behaviors (actigraphy and sleep diaries) at 3, 6, and 12 months, high-density electroencephalography (hdEEG) for two hours of nighttime sleep at age 6 months, and developmental outcomes (parent-reported ages and stages questionnaire) at 3, 6, 12 and 24 months. Results reveal that sleep neurophysiology at 6 months is linked to sleep-wake behaviors at 6 months: daytime sleep is linked to slow wave activity, nighttime movement and awakenings relate to spindle density. Furthermore, sleep neurophysiology is associated with later developmental outcomes: spindle density at 6 months predicts developmental outcomes at 12 and 24 months, with the strongest associations with gross motor development. These novel findings widen our understanding of infants' sleep neurophysiology as the link between sleep-wake behaviors and later developmental outcomes. The crucial next step is to extend this concept to clinical groups to test if sleep neurophysiology is an early marker to detect neurodevelopmental disorders and if early sleep interventions may be effective in positively influencing brain and behavioral maturation.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 481.13

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant MH070886
NIH Grant AG18784
NIH Grant AG17824
the Office of Academic Affiliations, Advanced Fellowship Program in Mental Illness Research and Treatment, Department of Veterans Affairs

Title: Differential impact of fast vs. slow spindles on the consolidation of declarative memory in older adults

Authors: ***T. ISHII**^{1,2}, **M. KAWAI**^{1,3}, **C. CHICK**^{1,3}, **I. COTTO**¹, **R. O'HARA**¹;
¹Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA; ²Human Brain Res. Ctr., Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan; ³Sierra Pacific Mental Illness Res. Educ. and Clin. Centers, VA Palo Alto Hlth. Care Syst., Palo Alto, CA

Abstract: There is growing evidence supporting the importance of sleep spindle and slow oscillation (SO) coupling as a mechanism for efficiently transferring information from the hippocampus to the neocortex. Yet, the differential association of fast vs. slow sleep spindles with SO is controversial, especially in older adults. The extant studies showed the synchronization of fast-spindles (FS) to the up-state of SOs induces synaptic plasticity, which underlies the formation of longer-term memory. Meanwhile, other studies have emphasized the importance of SO-slow spindle (SS) coupling in the formation of declarative memory. This study aimed to examine the differential association of fast vs. slow spindles-SO coupling with overnight memory retention in healthy older adults. Participants were 65 community-dwelling older adults (31 females, age: 70.4 ± 7.5 years). All participants underwent ambulatory overnight polysomnography (PSG) for sleep assessment. List-learning tests were performed before and after PSG to measure the overnight memory retention. All EEG analyses were performed using the Luna C/C++ pipeline, and the quantification of SO-spindles coupling was based on the phase of SWA at spindle peaks. We targeted two classes of the spindle: fast (FC = 15 Hz) and slow (FC = 11 Hz), in each case detecting spindles within approximately ± 2 Hz of the target frequency. The main findings of this study include 1) both the fast/slow spindle activity and the frequency of SO are positively correlated with overnight memory retention (OMR) ($r=0.28$, $p=0.001$; $r=0.26$, $p=0.001$; $r=0.24$, $p=0.01$), 2) significant non-uniform distributions were found in both FS (Rayleigh test: $z = 26.11$, $p < 0.0001$) and SS (Rayleigh test: $z = 17.31$, $p < 0.0001$) against SO phase angle, which differed significantly (Watson-Williams test: $p < 0.0001$), 3) only SO-FS coupling showed a positive correlation with OMR ($r=0.24$, $p=0.01$), not SO-SS coupling. These results suggest that the FS plays the main role in the effect of spindle-SO coupling on memory retention in older adults. The SS may contribute to memory retention through another pathway, which can be crucial in older adults because the SO and spindle-SO coupling effect on memory is known to decrease with age.

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Poster

481. Autonomic Regulation of Sleep: Behavior I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 481.14

Topic: H.04. Executive Functions

Support: NIH Grant R00-MH111748
NIH Grant R01-AG070135
NIH Grant R01-EB019437
Brain and Behavior Research Foundation NARSAD Young Investigator Award
Searle Scholars Program

Title: A sequence of activation across subcortical structures locked to behavioral arousal

Authors: ***B. SETZER**¹, N. CICERO¹, R. ZHU¹, L. D. LEWIS^{1,2};
¹Boston Univ., Boston, MA; ²Martinos Ctr., Boston, MA

Abstract: Awakening from sleep reflects a profound transformation in neural activity and behavior. Multiple deep-brain structures such as thalamus, basal forebrain, and brainstem nuclei can control arousal state, but how activity is coordinated across this large-scale network at the moment of arousal state transitions is not known. We used fast, ultra-high field (7 Tesla) functional magnetic resonance imaging (fMRI) to measure sub-second activity across the brain, to delineate these dynamics during behavioral arousal. Behavioral arousal was identified as the first response to a self-paced task after at least 20 seconds of unresponsiveness. In a subset of subjects, we used simultaneous electrophysiology (EEG) and fMRI to link EEG sleep and arousal rhythms with behavior and fMRI dynamics. We first confirmed that occipital alpha power, a hallmark rhythm of wakefulness, increased during behavioral arousals, indicating a switch in cortical electrophysiological state. We then investigated key regions of interest and found that the thalamus, basal forebrain, and the brainstem activated before behavioral arousal, and cortex deactivated seconds after. To analyze simultaneous activity across the individual nuclei of the thalamus, we used individual-level anatomical segmentation to identify these small, deep brain structures in each participant. We found that a sequence of activation across thalamic nuclei, the basal forebrain, and the brainstem occurs at arousal. In particular, we found distinct timing properties in the activity patterns of the centromedian nucleus of the thalamus, the basal forebrain, and the brainstem. Furthermore, fMRI dynamics were linked to the subsequent duration of behavioral arousal, reflecting whether participants remained awake or fell back asleep. These results identify a temporal sequence of subcortical activity that underlies behavioral arousal state transitions, and a distinct pattern linked to subsequent stable maintenance of awake behavior.

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Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 482.01

Topic: F.08. Food and Water Intake and Energy Balance

Support: BRAINS'LABORATORY S.A.S B4D1
NIMES UNIVERSITY

Title: From breath to the brain with food disorders

Authors: *V. COMPAN¹, A. HACHACHE², S. CHOQUART³;

¹Sciences, BRAINS' LABORATORY, Nîmes Univ., Nîmes, France; ²Sci., Nîmes Univ., NIMES, France; ³Sci., BRAINS'LABORATORY S.A.S., NIMES, France

Abstract: Integrating nanobiology into small portable devices could introduce new pathways for extending actual noninvasive and nonintrusive monitoring of physiological states, treatment effects and pathogens or toxins impact. However, sophisticated procedures with the use of large equipment, and different technologies, generally make difficult real time detection of these nano-agents of exhaled air in freely moving individuals. Here, we describe our e-connected lightweight wearable device. As a proof-of-concept, we tested the diagnostic performance of our device for providing at room and outside temperature in the exhaled air of humans, the levels of SARS-CoV-2 within 3 min. It does not require no user intervention other than turn on the device and breath. Series of biomarkers are currently tested and related to the brain of different animal models of addiction and food disorders.

Disclosures: V. Compan: None. A. Hachache: None. S. Choquart: None.

Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 482.02

Topic: F.08. Food and Water Intake and Energy Balance

Title: D_{1R} antagonist sch 23390 or 5-HT_{1A} receptor agonist 8-oh-dpat administered into the hypothalamus promotes chocolate-intake but does not change monoamine contents collected from nucleus accumbens in rats

Authors: *X. AVELAR¹, D. A. SANTIAGO¹, A. SALVADOR¹, G. ARANKOWSKY-SANDOVAL², E. MURILLO-RODRÍGUEZ¹;

¹Lab. de Neurociencias Moleculares e Integrativas, Escuela de Medicina División Ciencias de la

Salud. Univ. Anáhuac Mayab, Mérida, Mexico; ²Ctr. De Investigaciones Regionales “Dr. Hideyo Noguchi”, Univ. Autónoma de Yucatán, Mérida, Mexico

Abstract: Obesity represents a public health problem around the world and has been related to several factors, including consumption of low nutritional value food, known as “junk food”. Moreover, behavioral, neurochemical, and molecular effects in animal models have been described after the intake of specific ultra-processed foods, as in the case of chocolate. Due to chocolate activates brain areas and neurobiological networks with potentially similar psychoactive effects as substances of abuse, such as dopaminergic or serotonergic neurotransmission, the current study aims to evaluate whether chocolate-intake would be blocked by injecting into the lateral hypothalamus of rats the food-intake suppressors, D₁-receptor antagonist (SCH23390), or 8-hydroxy-2-(di-n-propylamino), tetralin (8-OH-DPAT), a serotonin receptor agonist (5-HT_{1A}). Rats received an intrahypothalamic injection of SCH23390 or 8-OH-DPAT (10µg/1µL, each compound), and chocolate intake as well as levels of dopamine (DA), serotonin (5-HT), and adenosine (AD), were analyzed. We found that the administration of these compounds enhanced chocolate intake. In addition, the extracellular levels of monoamines as well as adenosine, collected from nucleus accumbens, showed significant changes. For example, Microinjections of SCH23390 or 8-OH-DPAT did not modify the contents of dopamine or 5-HT; however, treatments increased the levels of AD. Mechanistic studies are needed to elucidate the implication of dopamine or serotonin receptors on chocolate intake. The findings point to a previously unrecognized role for AD in the regulation of chocolate consumption.

Keywords: Adenosine, dopamine, microdialysis, rat, serotonin.

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Poster

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Title: The exposure of high fat diet plus sucrose solution increases the gene expression of dopamine receptors in lateral septum of Sprague Dawley female rats.

Authors: *R. OLIVARES-BARRAZA^{1,2}, V. COLLIO¹, J. MARTÍNEZ-PINTO¹, R. SOTOMAYOR-ZÁRATE¹;

¹Ctr. de Neurobiología y Fisiopatología Integrativa, Inst. de Fisiología, Facultad de Ciencias, Univ. de Valparaíso, Valparaíso, Chile, Valparaíso, Chile; ²Programa de Doctorado en Ciencias

mención Neurociencias, Facultad de Ciencias, Univ. de Valparaiso, Valparaiso, Chile, Valparaiso, Chile

Abstract: Currently, obesity is a pandemic that has been strongly associated with changes in the lifestyles, such as an increase in the consumption of hypercaloric foods and a decrease in physical activity. In addition, it has been observed dysregulations in the brain circuits responsible for food intake. In this context, the homeostatic (hypothalamic regulation) and hedonic (reward circuit) systems that to control food intake are directly and indirectly regulated by the lateral septum (LS). LS is a GABAergic relay nucleus whose efferents innervate lateral hypothalamus (LH), nucleus accumbens (NAcc) and ventral tegmental area (VTA), between others. On the other hand, LS neurons are regulated for several neurotransmitters and neuropeptides such as dopamine, serotonin, endorphins and corticotropin releasing factor (CRF), between others. The aim of this work was to study the effects of exposure to high fat diet (HFD) plus sucrose (S) solution (5 %) (HFD + S) in Sprague Dawley rats for 6 weeks (from weaning postnatal day [PND] 21 to PND 62) on the gene expression of dopaminergic, GABAergic, and CRFergic receptors in LS and NAcc. Parallel control groups fed chow diet were used in these experiments. Our results show that the exposure to HFD + S increase D₁ and D₂ gene expression in LS of female rats, without affect the GABA_A, GABA_B and CRF₁ gene expression in the same nucleus. On the other hand, HFD + S only decrease D₁ and GABA_B gene expression in NAcc. The gene expression of these receptors was not affected by HFD + S in male rats. The neurotransmission in both nuclei could be affected by the expression changes of the D₁ receptor, generating in LS an activation of the GABAergic projections towards LH and VTA. However, LS GABAergic interneurons could also be activated, which would reduce the activation of the LS projection neurons. Other studies are necessary to test this hypothesis. In NAcc, the D₁ expression is decreased possibly affecting the activation of the direct pathway and favoring food addiction.

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Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

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CMMC

Title: Dopamine-inhibited POMC^{Drd2+} neurons in the arcuate nucleus acutely regulate feeding and body temperature

Authors: *S. CORNELIUSSEN^{1,2}, I. GAZIANO^{3,4,2}, N. BIGLARI^{3,4,2}, R. NEUHAUS^{3,4,2}, L. SHEN^{3,4,2}, T. SOTELO HITSCHFELD^{3,4,2}, P. KLEMM^{3,4,2}, L. STEUERNAGEL^{3,4,2}, A. DE SOLIS^{3,4,2}, W. CHEN^{3,4,2}, T. WUNDERLICH^{3,4,2}, J. BRÜNING^{3,4,2,5}, P. KLOPPENBURG^{1,2}; ¹Univ. of Cologne, Cologne, Germany; ²Excellence Cluster on Cell. Stress Responses in Aging Associated Dis. (CECAD) and Ctr. of Mol. Med. Cologne (CMMC), Cologne, Germany; ³Max Planck Inst. for Metabolism Res., Cologne, Germany; ⁴Ctr. for Endocrinology, Diabetes and Preventive Med. (CEDP), Cologne, Germany; ⁵Natl. Ctr. for Diabetes Res. (DZD), Neuherberg, Germany

Abstract: Food intake and energy homeostasis are regulated by neuronal networks in different brain areas, including the arcuate nucleus of the hypothalamus (ARH). In the ARH, orexigenic agouti-related peptide (AgRP) and anorectic proopiomelanocortin (POMC) neurons are the key regulators of homeostatic feeding. These functionally antagonistic neuron populations sense and integrate multimodal endocrine and neuronal signals that reflect the body's energy status to regulate feeding behavior. While homeostatic feeding describes the feeding response when signals of energy depletion are sensed within the ARH, hedonic feeding refers to feeding beyond the body's nutritional need. Neuronal networks that control hedonic feeding are located in the mesocorticolimbic system, in which dopamine plays an essential role in the rewarding effects of food intake. The neuronal connection between the ARC and mesocorticolimbic systems has been studied intensively. However, less is known about the direct action of dopamine on ARH neurons. In this study, we used newly developed mouse models, where intersectional Cre/Dre-dependent recombination allows successful labeling, translational profiling, and functionally electrophysiological characterization to investigate the action of the neurotransmitter dopamine on POMC and AgRP neurons. We showed that a large proportion of food-promoting AgRP neurons express the excitatory Drd1 receptor (~15 %), while POMC neurons enrich the inhibitory Drd2 receptor (~30%). Perforated patch clamp recordings of POMC and AgRP neurons confirmed that most of the AgRP neurons are activated by dopamine in a dose-dependent manner. At the same time, a large amount of POMC neurons were inhibited by dopamine. Using intersectional targeting of Drd2-expressing POMC neurons, we could show that dopamine inhibition is Drd2-dependent and that POMC^{Drd2+} neurons express different neuropeptide signaling, like an enhanced somatostatin expression in the POMC^{Drd2+} compared to the global POMC population. Furthermore, selective chemogenetic activation of POMC^{Drd2+} neurons uncovers their ability to acutely suppress feeding and to preserve body temperature. Summarized, this work provided a comprehensive characterization of POMC^{Drd2+} neurons and gave new insight into the understanding of the dopamine-dependent control of homeostatic feeding.

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Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

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Topic: F.08. Food and Water Intake and Energy Balance

Support: UNAM-DGAPA grant IN 307720.

Title: Central blockade of dopamine D4 receptor decreases hedonic intake without induce locomotor impairments

Authors: ***D. DÍAZ-URBINA**¹, S. HERNANDEZ-CORREA², L. M. MONREAL-GONZALES³, R. E. ESCARTÍN-PÉREZ², F. CORTES-SALAZAR², J. M. MANCILLA-DIAZ², V. E. LÓPEZ-ALONSO²;

¹FES IZTACALA-UNAM, Tlalnepantla de Baz, Mexico; ²FES-IZTACALA- UNAM, Tlalnepantla de Baz, Mexico; ³Univ. Insurgentes, Tlalnepantla de Baz, Mexico

Abstract: Sugar-sweetened drinks are highly consumed in occidental countries and have been considered as one of the main causes of obesity and metabolic disorders. Moreover, dopamine rD4 receptor (rD4) is implicated in the preference for palatable diets and drug addiction. However, whether rD4 signaling is involved in for the hedonic intake of a liquid palatable solution has not been explored. **Aim:** we evaluated the effect of the central blocking of rD4 on the consumption of a 20% sucrose solution, drinking microstructure and locomotor activity in male rats. **Method:** in the experiment 1, rats were cannulated in the right lateral ventricle. After a recovery period, animals had one hour of access to a 20% sucrose solution at the onset of light phase. On day 10, animals received an intracerebroventricular injection of vehicle or L-745870 (rD4 selective antagonist; 1µg or 2 µg) and sucrose consumption and drinking microstructure were monitored for 1h. In the experiment 2, rats were maintained in the same experimental conditions described in the experiment 1 (cannulation, sucrose schedule access). On day 10, 60-min after the end of sucrose access, rats received vehicle or L-745870 (1 and 2 µg) and then were individually placed in an open field box for 10 minutes. The number of crossings through the squares and number of rearing were scored. **Results:** L-745870 decreased the sucrose consumption, increased bout frequency, but reduced bout duration, but size and inter-bout intervals. Moreover, L-745870 did not affect the levels of spontaneous locomotor activity measured such as number of crosses in the arena or number of rears. **Conclusion:** present results suggest that central blockade of rD4 decreased the appetite for the sugar solution, accelerating the satiation development without affect the spontaneous locomotor activity.

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Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

Location: SDCC Halls B-H

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Program #/Poster #: 482.06

Topic: F.08. Food and Water Intake and Energy Balance

Title: Ventrolateral medullary catecholamine/neuropeptide Y neuronal projections to the perifornical lateral hypothalamus mediate feeding during glucoprivation.

Authors: *P. CHOI, K. DILWORTH, Q. WANG, L. BRENNER, A. LI, R. RITTER, S. RITTER, S. APPELYARD;

Washington state university, Washington State Univ. Grad. IPN, Pullman, WA

Abstract: During acute glucose deficit (glucoprivation), the brain elicits several counter-regulatory responses (CRRs) such as increased food intake, corticosterone and epinephrine release that facilitate fatty acid metabolism and gluconeogenesis to restore euglycemia. However, repeated incidents of hypoglycemia, especially in diabetic patients, can cause development of Hypoglycemia Associated Autonomic Failure (HAAF), a loss of ability to detect blood glucose levels, and thus failure to elicit CRRs. This can result in seizures, irreversible brain damage, or even death, so it is imperative that we investigate the extended neural circuitry of the CRRs. Prior work from our lab established that hypothalamically projecting ventrolateral medullary (VLM) catecholamine (CA) neurons that co-express neuropeptide Y (NPY) are required to elicit glucoprivic feeding, and selective chemogenetic activation of these VLM CA/NPY neurons elicits increased feeding. However, the pathways and the mechanisms by which VLM CA/NPY neurons trigger increased feeding are not clear. Activation of VLM A1/C1 CA neurons results in increased activity of perifornical lateral hypothalamic (PeFLH) neurons. We therefore hypothesized that NPY from the VLM CA neurons activates the PeFLH neurons to elicit glucoprivic feeding response. To investigate this, we utilized the ribosomal toxin conjugate, NPY-saporin (NPY-SAP) to make targeted lesion of NPY receptor-expressing neurons in the PeFLH of male rats, and we tested its effect on glucoprivic feeding and other CRRs evoked by 2-Deoxy-D-glucose (2DG), an anti-metabolic glucose analogue that inhibits glycolysis. Our results show that NPY-SAP treatment lesioned a significant number of PeFLH orexin neurons, but not melanin-concentrating hormone neurons. The PeFLH NPY-SAP lesion attenuated 2DG-evoked feeding, but not 2DG-evoked sympathoadrenal hyperglycemia or serum corticosterone levels. NPY-SAP also attenuated 2DG-evoked feeding, but not sympathoadrenal hyperglycemia, in female rats, with no significant sex difference observed. Next, to examine the subtypes of NPY receptors involved in the VLM-to-PeFLH signaling, we injected NPY receptor antagonists into the PeFLH just prior to a clozapine-N-oxide-evoked chemogenetic activation of VLM A1/C1 CA neurons in transgenic TH-Cre⁺ rats injected with an AAV2-DIO-hSyn-hM3D(Gq)-mCherry virus. Antagonism of PeFLH NPY Y1, Y2, and Y5 receptors individually all attenuated feeding evoked by chemogenetic activation of VLM CA neurons. Together, we conclude that the VLM CA neuronal projection to the PeFLH is necessary for eliciting glucoprivic feeding, and it is mediated by NPY.

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Poster

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Program #/Poster #: 482.07

Topic: F.08. Food and Water Intake and Energy Balance

Title: Interaction between the lipid synthesis enzyme, DGAT1, and dopamine signaling

Authors: *C. A. GRUETER¹, T. W. MURRAY¹, L. A. LINQUIST¹, B. A. GRUETER²;
¹Vanderbilt Univ. Med. Ctr., Nashville, TN; ²Anesthesiol., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: The mesolimbic dopamine system is a target for energy-state related hormones and transmitters. Within this system, lipids are recognized as signaling molecules that have the capacity to trigger profound physiological responses to control whole-body energy balance. Imbalances in lipid signaling networks may contribute to the pathogenesis of multiple disease states including obesity and related neuropsychiatric (motivational) disorders. In the central nervous system (CNS), lipid signaling facilitated by intracellular triglyceride (TG) metabolism has been understudied. In fact, enzymes catalyzing TG synthesis, acyl-CoA:diacylglycerol acyltransferase-1 and -2 (DGAT-1 and -2), have yet to be studied in the CNS. We hypothesize that intracellular TG metabolism, mediated by DGAT, plays an important role in lipid-signaling, in the CNS, regulating 1) whole-body energy balance and 2) motivational state. Here we show that *Dgat1* and *Dgat2* mRNA are expressed in specific brain regions known to regulate whole-body energy balance, including the hypothalamus and dorsal striatum. Furthermore, we demonstrate *Dgat1* and *Dgat2* mRNA to be differentially expressed at various metabolic states. Viral-mediated knockdown of *Dgat1* in the CNS, results in decreased energy expenditure and increased body weight gain on a high-calorie diet. Additionally, anxiety- and depressive-like behaviors are dysregulated in global *Dgat1* knockout mice. Preliminary behavioral experiments suggest an interaction between *Dgat1* and the D2 dopamine receptor. We assess D2 dopamine receptor and DGAT interaction using acute brain slice of the nucleus accumbens using fast-scan cyclic voltammetry (FSCV). Collectively, our results implicate the importance of TG synthesis in shaping motivational state potentially through antagonism of the D2 receptor.

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Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

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Topic: F.08. Food and Water Intake and Energy Balance

Title: Systemic injection of oleoylethanolamide blocks food-intake in rats subjected to food selection in the cafeteria diet protocol but does not change dopamine levels collected from Nucleus Accumbens (NAc) in rats

Authors: *A. LÓPEZ MARTÍNEZ¹, F. M. CRUZ-ANCHEYTA¹, M. SOLIS-ENRIQUEZ¹, M. CRUZ HERNÁNDEZ², M. PERAZA HERRERA¹, V. LAVIE-VALLADARES², E. S. MURILLO RODRÍGUEZ², G. ARANKOWSKY-SANDOVAL³;

¹Biotech., ²Neurosciences, Anahuac Mayab Univ., Merida, Mexico; ³Ctr. De Investigaciones Regionales “Dr. Hideyo Noguchi”, Univ. Autónoma de Yucatán, Merida, Mexico

Abstract: Obesity is a world-wide health problem and requires different experimental perspectives to manage the control or treatment of this disease. From the pharmacological point of view, oleoylethanolamide (OEA), has anorexic properties and regulates diet and body weight by activating α -type receptor activated by peroxisome proliferator (PPAR- α); however, the link between behavioral and neurochemical properties of OEA on food selection remains unknown. Nevertheless, here we hypothesized that systemic injections of OEA decrease food selection in as well as extracellular levels of dopamine (DA) collected from nucleus accumbens. To achieve this goal, Male Wistar rats were implanted with a guide-cannula for microdialysis sampling. Later, they were fed with cafeteria diet items for 12 days and on day 13th, animals received an injection of OEA (5mg/Kg, i.p.) and they were exposed to all food items of cafeteria diet with free access to select a specific item. Once chosen, microdialysis samples were collected for analysis of DA extracellular levels using HPLC means. Under our experimental conditions, and compared to respective controls, rats that received OEA administration did not select any of the food items. Thus, OEA caused an anorexic effect; however, the extracellular levels of DA did not show significant changes in OEA-treated rats. Our results suggest that OEA regulates the behavioral component of feeding; notwithstanding, neurochemically it seems that the anorexic lipid did not cause significant changes on DA contents. Indeed, further studies are needed to explore the neurochemical relationship of the food-intake modulatory properties of OEA and its behavioral effect.

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Poster

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Topic: F.08. Food and Water Intake and Energy Balance

Title: Taar1 agonist ulotaront improves glycemic control and reduces body weight in rodent models of diabetes, obesity and iatrogenic weight gain

Authors: *N. DEDIC¹, E. HAJOS-KORCSOK¹, P. G. JONES¹, C. SYNAN¹, S. WU², C. ANACKER², S. P. VICKERS³, J. HECKSHER-SØRENSEN⁴, S. MILANOVIC¹, S. C. HOPKINS¹, L. J. BRISTOW¹, K. S. KOBLAN¹;

¹Sunovion Pharmaceuticals, Marlborough, MA; ²Dept. of Psychiatry, New York State Psychiatric Inst. (NYPSI), Columbia Univ., New York City, NY; ³SygnatureDiscovery, Nottingham, United Kingdom; ⁴Gubra ApS, Hørsholm, Denmark

Abstract: Ulotaront (SEP-363856) is a TAAR1 and 5-HT1A agonist currently in Phase 3 clinical trials for the treatment of schizophrenia (SCZ). Metabolic Syndrome (e.g. central obesity, dyslipidemia, hyperglycemia, etc.), which can be induced or exacerbated by antipsychotic drugs (APDs), is highly prevalent in SCZ patients. The need for novel treatments that lack APD class-specific metabolic side effects is therefore apparent. As a new pharmacological class, ulotaront has no significant activity at receptors commonly associated with APD-induced metabolic alterations (i.e. D2, 5-HT2C, H1 and M3). Recent preclinical evidence has identified TAAR1 as novel regulator of metabolic control and a promising target for obesity and type 2 diabetes. Here we evaluated the risk-benefit profile of ulotaront for the treatment of SCZ by assessing its effects on metabolic parameters in rodent models. Following 15-day oral administration of ulotaront, rats on HFD showed a dose-dependent reduction in body weight, food intake and liver triglyceride content compared to vehicle controls. In addition, a more rapid reversal of olanzapine-induced weight gain and food intake was observed in rats switched to ulotaront treatment compared to vehicle alone. Consistent with the body weight-lowering effects in rats, chronic treatment with ulotaront normalized corticosterone-induced body weight gain in mice. Assessment of oral glucose tolerance (oGTT) showed a dose-dependent reduction of glucose excursion in response to acute ulotaront administration in naive and diabetic *db/db* mice. Acute ulotaront treatment also delayed gastric emptying in mice, which is likely the main mechanism driving reductions in glucose excursion during the oGTT. 3D whole-brain c-fos imaging of ulotaront-treated mice revealed increased neuronal activity in several brain regions associated with the regulation of food intake and integration of peripheral metabolic signals (i.e., arcuate, and paraventricular nucleus of the hypothalamus, and dorsal vagal complex). Overall, the data indicate that ulotaront not only lacks APD-induced metabolic liabilities but can reduce body weight and improve glucose tolerance in rodent models. The underlying mechanisms may include TAAR1-mediated peripheral effects on glucose homeostasis and gastric emptying, and/or direct modulation of homeostatic and hedonic neurocircuits regulating energy balance. The beneficial metabolic effects of ulotaront suggest a substantially improved risk-benefit profile compared to established APDs. Thus, TAAR1 agonists may not only represent a novel therapeutic class for the treatment of SCZ, but potentially also for metabolic disorders.

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Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

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Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH Grant R01DK126740

Title: Dorsal Raphe Serotonergic Neurons Suppress Feeding Through Redundant Forebrain Circuits

Authors: ***I. AKLAN**¹, **N. SAYAR**¹, **F. DENG**², **Y. YAVUZ**³, **J. RYSTED**¹, **C. LAULE**¹, **D. DAVIS**¹, **Y. LI**², **D. ATASOY**¹;

¹Univ. of Iowa, Univ. of Iowa, Coralville, IA; ²Membrane Biol., Peking Univ. Sch. of Life Sci., Beijing, China; ³Physiol. Dept., Yeditepe Univ., Istanbul, Turkey

Abstract: Serotonin (5HT) is a well-known anorexigenic molecule and 5HT neurons of dorsal raphe nucleus (DRN) has been implicated in suppression of feeding; however, the downstream circuitry is poorly understood. Here, we found that selective activation of DRN^{5HT} axons in lateral hypothalamus (DRN^{5HT}→LH) and bed nucleus of stria terminalis (DRN^{5HT}→BNST) suppresses feeding whereas activating medial hypothalamic projections has no effect. Using *in vivo* imaging, we found that food access and satiety hormones activate DRN^{5HT} projections to LH where they also rapidly increase extracellular 5HT levels. Optogenetic mapping revealed that DRN^{5HT}→LH^{vGAT} and DRN^{5HT}→LH^{vGlut2} connections are primarily inhibitory and excitatory respectively. Further, in addition to its direct action on LH neurons, we found that 5HT suppresses GABA release from presynaptic terminals arriving from AgRP neurons. These findings define redundant functionally forebrain circuits through which DRN^{5HT} neurons suppress feeding and reveal that these projections are modulated by metabolic hormones.

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Poster

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Topic: F.08. Food and Water Intake and Energy Balance

Support: Florida State University startup funding

Title: Bidirectional modulation of serotonin on zona incerta neurons

Authors: *Q. YE, J. NUNEZ, X. ZHANG;

Dept. of Psychology, Florida State Univ. Program In Neurosci., Tallahassee, FL

Abstract: Zona incerta (ZI) is a subthalamic nucleus that is rich of GABA neurons with widespread projections to paraventricular nucleus of thalamus, cerebral cortex, and many other brain regions for the regulation of food intake, sleep, and emotion. Recent studies show that ZI plays an important role in integrating multisensory information, initiating predatory hunting, regulating binge-like eating, and mediating defensive behavior. However, it remains largely unknown how central neural signals such as serotonin (5-HT) modulate the activity of ZI neurons. Although several 5-HT receptor subtypes have been found in the ZI, little is known about the functional role of 5-HT signaling in ZI. In this study, we used slice patch-clamp recordings to functionally characterize the effects of 5-HT on ZI neurons. We found that 5-HT (50 μ M) produced inhibitory effect on 48.6%, excitatory effect on 34.3%, and short inhibitory followed by long-lasting excitatory effect on 17.1% of ZI neurons. The 5-HT-induced excitation was mimicked by the 5-HT₇ receptor agonist AS19. Also, 5-HT treatment increased the frequency, but not amplitude of excitatory postsynaptic currents (EPSCs) in some of 5-HT-excited ZI neurons. On contrast, the 5-HT-induced inhibitory effect was mimicked by both 8-OH-DPAT, a selective 5-HT_{1A} agonist, and WAY629, a selective 5-HT_{2C} agonist. The 5-HT-evoked outward currents were blocked by a cocktail of a 5-HT_{1A} antagonist WAY100635 and a 5-HT_{2C} antagonist SB242084, while 5-HT-induced inward currents were abolished by a 5-HT₇ antagonist SB269970. Together, these preliminary data suggest that 5-HT exerts a bidirectional modulation on ZI neurons through acting on 5-HT_{1A}, 5-HT_{2C}, and 5-HT₇ receptors. Based on these pilot data, we will examine whether internal 5-HT projections from raphe nuclei control feeding and emotion by targeting ZI in our future studies.

Disclosures: Q. Ye: None. J. Nunez: None. X. Zhang: None.

Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 482.12

Topic: F.08. Food and Water Intake and Energy Balance

Title: The Septohypothalamic nucleus: A brain region where GABA receptor antagonism elicits feeding

Authors: *I. GABRIELLA¹, A. MUKUNDAN², J. DANG², V. NAMBIAR², A. VENKATRAGHAVAN², A. TSENG³, B. STANLEY⁴;

¹Psychology, ²Molecular, Cell, and Systems Biol., ³Neurosci., Univ. of California, Riverside, Riverside, CA; ⁴Psychology, & Mol. Cell Systems Biol., Univ. California, Riverside, Riverside, CA

Abstract: The prevalence of obesity has been steadily increasing (Hales et al., 2020), as have the rates of eating disorders (Udo & Grilo, 2018), emphasizing the importance of understanding the neural circuits of eating behavior and body weight control. Toward that end, our study explored a potential role for the Septohypothalamic Nucleus (SHy) in feeding mechanisms. It has previously been shown that c-fos in SHy cell nuclei increases after feeding, suggesting neural activity associated with this behavior (Csikos et al., 2020; Nakahara et al., 2004). As the SHy is densely populated with GABAergic cells, we hypothesized that central injections of GABA_A and GABA_B receptor antagonists would elicit feeding. Adult male rats with chronic indwelling guide cannulas targeted towards the SHy were microinjected with artificial cerebrospinal fluid, or the GABA_A receptor antagonist picrotoxin (n=12), or the GABA_B receptor antagonist 2-OH Saclofen (n=6). Food and water intakes were measured 1, 2, 3, and 24 hours after injection, and repeated measures ANOVA was employed to assess the results. We found a significant increase in food intake 2 and 3 hours after picrotoxin injection (p=.02) and a trending increase in food intake after 2-OH Saclofen (p=.06) injection. In contrast, injection of either picrotoxin or 2-OH Saclofen in brain regions immediately surrounding the SHy were ineffective, suggesting that the elicited eating was due to effects within the SHy. To our knowledge, this is the first evidence for eating elicited by a manipulation within the Shy. More specifically, this evidence suggests that GABA_A and GABA_B receptor suppression within the Shy produces eating with a delay and may be elements of a neurocircuit that participates in the regulation of feeding. Our study may provide information for future research relating to obesity or eating disorders.

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Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 482.13

Topic: F.08. Food and Water Intake and Energy Balance

Support: UW-Eau Claire Summer Research Experiences for Undergraduates

Title: Effects of clozapine and ziprasidone in rats trained to discriminate between 22 and 2 hours food deprivation

Authors: S. T. TANGEN, E. K. CEDARBLOOM, S. K. WENZEL, G. PUZOVIC, E. K. REBAINE, S. J. JORDAN, *D. C. JEWETT;
Psychology, Univ. of Wisconsin, Eau Claire, Eau Claire, WI

Abstract: In humans, weight gain is a side effect of several atypical antipsychotics. Published reports indicate clozapine increases food intake in rats, but reduces food-related, operant behaviors. Reports indicate ziprasidone has smaller or no effects on food intake in rats. Given the short half-life of clozapine in rats, we tested the effects of acutely administered clozapine and ziprasidone on the discriminative stimulus effects of food deprivation, rates of lever pressing, and food intake. Male, Sprague-Dawley rats ($n = 11$, ~2 months old during initial training and 12-26 months old during drug testing) were trained to discriminate between 22 and 2 hrs of food deprivation in an operant task. Under 22 hrs deprivation, rats pressed the left lever 15 times to earn a 45 mg food pellet (FR 15 schedule). Right lever presses were punished with 8 seconds of darkness under the FR 15. Under 2 hrs deprivation conditions, the contingencies were reversed (right lever presses were reinforced with food delivery and left lever presses resulted in 8 seconds of darkness). Training sessions lasted until 10 reinforcers were earned or 15 minutes elapsed. Discrimination criteria were 80% or greater condition appropriate responding before the first reinforcer was earned and for the entire training session for 8 out of 10 consecutive sessions. After acquiring the discrimination ($M = 71$, $SEM = 6$ sessions), subjects were food deprived for 2 or 22 hrs and injected subcutaneously with either clozapine (1.0-5.6 mg/kg), ziprasidone (0.32-1.0 mg/kg) or vehicle (1.0 ml/kg, for clozapine: 30% DMSO; for ziprasidone: 85% acetic acid, Tween 80, distilled water, and sodium hydroxide to pH ~7). For the 5 minute or 5 reinforcer test sessions, responses toward either lever were reinforced under the FR 15. Test sessions were conducted every 15 min for 2 hrs. After test sessions, food intake was recorded for 1 hour. Statistical analyses via ANOVA and Dunnett t-tests indicated clozapine did not induce hunger-like responses under the 2 hrs deprivation. After 22 hrs food deprivation, clozapine (1.0-5.6 mg/kg) significantly reduced lever presses associated with 22 hr deprivation. Under both deprivation conditions, clozapine (3.2-5.6 mg/kg) decreased food intake. Under 22 hr deprivation conditions, ziprasidone did not affect the discriminative stimulus effects of food deprivation. Ziprasidone (1.0 mg/kg) reduced rates of lever pressing in most subjects and did not significantly affect food intake. These data further support for the notion that clozapine decreases feeding-related behaviors in rats and indicates atypical antipsychotic medications may have differential effects on food-related behaviors in rats.

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Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 482.14

Topic: F.08. Food and Water Intake and Energy Balance

Support: NSF GRFP

Title: A High Protein Diet can Increase Appetite through Interactions with the Gut Microbiota and Neurobiological Satiety Signaling

Authors: *E. SPILLMAN, M. KUANG, A. SHEPHERD, J. WANG;
Univ. Of California San Diego Neurosciences Grad. Program, La Jolla, CA

Abstract: The food choices we make affect the composition of our gut microbiota which may, in turn, affect brain responses to food and what we choose to eat. Yet, little is known about the underlying neurobiological mechanisms of this dynamic interaction. Gut microbiota not only receive energy from the host in the form of nutrients but also supply the host with energy and regulate host physiology by producing microbial metabolites using available nutrients in the gut. These microbial metabolites in turn can be usable nutrients for the host or signaling molecules that regulate host physiology. This study aims to test the hypothesis that diet interacts with and changes gut microbiota quantity and composition and that these changes subsequently affect host satiety signaling and appetite. We started by investigating the effects of dietary composition on feeding behavior and observed a significant increase in protein appetite compared to sucrose or the standard *Drosophila* diet. In a follow-up study, we found that flies fed with protein-rich foods gradually increased their appetite and that this increase in appetite is dependent on the duration of the protein diet. Given these results, we hypothesized that protein-rich diets interact with the flies' gut microbiome to increase appetite. We then examined the contribution of the gut microbes on food intake; we included antibiotics in the conditioning diet. Indeed, antibiotics eliminated the effect of the protein diet, reducing food intake to the same level as the control groups. Next, we asked whether a composition change in the gut microbiome can alter feeding. For this, we added metformin to the conditioning food. In mammals, metformin is known to increase populations of short chain fatty acid (SCFA) producing bacteria. Indeed, metformin further increased protein consumption. Importantly, antibiotics abolished the effect of the metformin diet, suggesting that metformin's effect on food intake is mediated by gut microbes. Together, these results suggest that protein-rich food increases food intake by promoting the growth of gut microbes, and SCFA-producing microbes in the gut increase host food intake. Understanding how the gut microbiome interacts with the enteroendocrine system and changes appetite can provide insight into the evolution of complex, adaptive behaviors. Despite approximately 800 million years of evolutionary divergence between *Drosophila* and humans, there are homologies in gut microbiome populations and mechanisms for regulating food intake.

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Poster

482. Monoamines, Amino Acids, and Other Regulators of Food Intake

Location: SDCC Halls B-H

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Program #/Poster #: 482.15

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH Grant R01DK124801
NIH Grant R01NS124844

Title: Exploration of species distinctions and cerebellar involvement in binge-like behavior

Authors: *R. E. PRESBY¹, A. Y. T. LOW², J. BETLEY², A. I. CHEN¹;
¹Scintillon Inst., San Diego, CA; ²Univ. of Pennsylvania, Philadelphia, PA

Abstract: Binge Eating Disorder (BED) is a category of disordered eating behavior distinct from overeating and is associated with feeling a lack of control in food consumption leading directly to an array of physical and mental health sequelae. Novel treatment options for BED include transcranial magnetic stimulation (TMS) which has been shown to be effective at reducing binge episodes in small patient cohorts. Our recent study in humans and mice led to the discovery that neurons in the deep cerebellar nuclei (DCN) are potent regulators of food intake through modulating dopamine (DA) efflux within the ventral striatum (Low et al. 2021). Disruption of this pathway may be involved in the development and persistence of disordered eating behaviors, such as BED, and thus represents a potential new target for TMS. A number of pre-clinical models developed over the years mimic the overconsumption of food during a binge episode in both rats and mice. In order to examine the involvement of the DCN-striatum pathway in regards to binge-like eating behavior (BLE), we tested whether a schedule of exposure to a highly palatable food to induce BLE in rats (Presby et al. 2020) could be used in mice. Results with ground chocolate (Cadbury's Milk Chocolate) as the highly palatable food for induction of BLE show non-food restricted rats have a steady increase over the course of the exposure schedule with a much higher rate of consumption at the end than observed in mice. In order to determine if it was the type of food limiting the amount consumed by mice, we replaced the ground chocolate with fat (Crisco). We found that intake over the course of the exposure schedule and at the end with fat was not significantly different from that seen with chocolate in mice. These results indicate that, in regards to behavioral output, rats may be a more suitable species to utilize due to their significant increase of intake over the course of exposure that remains high upon completion, which is not seen in mice. Future work aims to determine if this is limited to a species-specific difference or if this extends to biological sex differences. In ongoing studies, we have observed that manipulating the activity of neurons in superficial regions of the cerebellar Crus I influences food intake, supporting the feasibility of cerebellar TMS for treating eating disorders. Together, our studies revealed insight into how disruption of novel nodes of the neural network of feeding behavior leads to disordered eating and development of potential non-invasive treatment strategies, and the importance of carefully assessing species-specific behavioral distinctions.

Disclosures: R.E. Presby: None. A.Y.T. Low: None. J. Betley: None. A.I. Chen: None.

Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 483.01

Topic: G.03. Motivation

Support: NIDA P50 PPG grant P50 DA042012
NIMH Clinical Investigator Award (CIA) (K08) 5K08MH110610-02

Title: Brain-wide activity mapping reveals a required role for the dorsal endopiriform nucleus in MDMA-evoked prosocial behavior

Authors: *D. R. RIJSKETIC¹, J. SALGADO¹, N. R. WALL², P. LLORACH¹, R. C. LOPEZ, Jr.², A. B. CASEY¹, T. HIETAMIES¹, Z. RASTEGAR¹, D. A. N. BARBOSA³, K. BEIER¹, R. MALENKA², B. HEIFETS¹;

¹Dept. of Anesthesiology, Perioperative and Pain Med., Stanford Univ. Sch. of Med., Stanford, CA; ²Nancy Pritzker Laboratory, Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA; ³Dept. of Neurosurgery, Perelman Sch. of Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: MDMA- (ecstasy) assisted psychotherapy is a potentially effective treatment for PTSD. Its long-lasting therapeutic effects may relate to enhanced feelings of social connection, empathy, and trust during therapy. However, MDMA's abuse potential warrants understanding its mechanism to develop safer, scalable treatments. We previously discovered that MDMA-driven serotonin release in the nucleus accumbens (NAc) is a prerequisite to induce prosocial effects in mice. Here we aim to uncover the broader network of brain regions required to produce MDMA's prosocial effects. Brain-wide neuronal activity was mapped in wild-type (WT) mice following i.p. injection of MDMA (7.5 mg/kg) or saline in social (with littermates) and non-social (single housed) contexts. 2 hours post injection mice were perfused, brains were immunofluorescently labeled for cFos (a protein expressed in previously active neurons), made optically transparent (iDISCO+), and imaged via light sheet microscopy. Using open-source image analysis tools we detected active cells, registered brains to a modified version of the Allen Brain Atlas from Gubra, and made voxel-wise p-value maps. We validated hotspots with an *Ai14* reporter line crossed to *TRAP2* mice (Targeted Recombination in Active Populations 2), in which the *cFos* promoter drives CreER expression. 4-hydroxytamoxifen (co-administered with MDMA or saline) transiently binds CreER, triggering tdTomato expression. Active ensembles in *TRAP2* mice were chemogenetic silenced by injecting AAV8-DIO-hM4Di-mCherry or AAV8-DIO-mCherry, TRAPing the MDMA social ensemble, and silencing TRAPed regions of interest (CNO vs vehicle) before a 3-chamber sociability test with MDMA or saline. In both social and non-social contexts, MDMA evoked activity in the ventral lateral shell of the NAc and several prefrontal cortical regions (e.g., prelimbic and orbital areas). However, in the social context MDMA preferentially enhanced activity in the dorsal medial shell of the NAc and the dorsal endopiriform nucleus (dEN)/claustrum. We confirmed these findings twice (with brain slices and whole brains from *TRAP2;Ai14* mice). Wide-spread chemogenetic silencing of the mPFC MDMA social ensemble preserved the prosocial effect of MDMA, whereas silencing ensembles in the dorsal medial shell of the NAc or the dEN/claustrum blocked the prosocial effect of MDMA. Unbiased brain-wide activity mapping revealed focal MDMA social ensembles that are required for MDMA-elicited sociability. Future experiments will better characterize these neurons, revealing their connectivity and roles in social behavior and drug reward, which may lead to improved treatments for PTSD.

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 483.02

Topic: G.03. Motivation

Support: NIH Grant T32DA035165 to MBP
HHMI Gilliam Fellowship to DFCP
NSF GRFP Fellowship to DFCP
NIMH R08MH110610

Title: Modulation of 5-HT release by dynorphin mediates social deficits during opioid withdrawal

Authors: ***M. B. POMRENZE**¹, D. F. CARDOZO PINTO¹, P. A. NEUMANN¹, P. LLORACH², J. M. TUCCIARONE¹, N. ESHEL¹, B. D. HEIFETS², R. C. MALENKA¹;
¹Nancy Pritzker Laboratory, Psychiatry and Behavioral Sci., ²Anesthesiology, Perioperative and Pain Med., Stanford Univ., Stanford, CA

Abstract: Lethal overdoses from opioids have increased dramatically over the past decade, critically contributing to the “opioid crisis”. Despite the colossal economic and societal costs of opioid use disorder (OUD), prognosis remains poor because existing treatments are often inadequate in helping users maintain abstinence over the long-term. During the abstinent weeks, months, and years following opioid use (i.e. protracted withdrawal or abstinence), the risk of relapse is increased by emotional symptoms, such as social avoidance, depression, and opioid cravings. In particular, social avoidance and isolation during withdrawal appear to be major contributors to both relapse and lethal overdose. To model protracted opioid withdrawal, we designed a procedure in which mice are administered escalating doses of morphine in conditioned place preference (CPP) chambers and tested for social behaviors after three weeks of abstinence. We observed robust sociability deficits during protracted withdrawal that correlated with long-term morphine place preference. The sociability deficits required activation of kappa opioid receptors (KORs) in the nucleus accumbens (NAc) medial shell. Blockade of transmitter release from dynorphin (*Pdyn*) expressing dorsal raphe neurons (DR^{*Pdyn*}), but not from NAc^{*Pdyn*} neurons, with tetanus toxin prevented these deficits in prosocial behaviors and reduced morphine place preference during withdrawal. Conversely, optogenetic activation of DR^{*Pdyn*} neurons or their inputs in NAc medial shell reproduced NAc KOR-dependent decreases in sociability.

Deletion of KORs from serotonin (5-HT) neurons, but not from NAc neurons or dopamine neurons, prevented sociability deficits and morphine place preference. Finally, fiber photometry recordings with the genetically encoded GRAB_{5-HT} sensor revealed that during withdrawal, KORs reduce the 5-HT release into the NAc that normally occurs during social interactions. These results define a novel neuromodulatory mechanism that is engaged during protracted opioid withdrawal to induce maladaptive deficits in prosocial behaviors, which in humans afflicted with OUD contribute to relapse.

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 483.03

Topic: G.03. Motivation

Support: NIDA P50 PPG Grant (P50DA042012)

Title: The central amygdala mediates ketamine's opioid-receptor dependent behavioral effects in mice.

Authors: ***P. LLORACH**, D. RIJSKETIC, M. B. POMRENZE, A. B. CASEY, J. S. SALGADO, T. M. HIETAMIES, R. C. MALENKA, B. D. HEIFETS; Stanford Univ., Palo Alto, CA

Abstract: Treatments for major depressive disorder often take several weeks to show therapeutic benefits, and many patients continue to be unresponsive. The dissociative anesthetic ketamine (KET) has gained popularity as a fast-acting, but short-lasting antidepressant. While most research focuses on KET's interaction with NMDA receptors, a recent clinical trial showed that naltrexone (NTX), a nonselective opioid antagonist, blocked KET's antidepressant effect (Williams et al., 2019). Here we investigated KET's interaction with the opioid system in C57/BL6 male mice (8-12 weeks old, n=10-12 per experimental group). We aimed to identify behaviors sensitive to KET's (10 mg/kg) acute effect that could be blocked by administering NTX (5 mg/kg) 30 min prior. NTX did not block KET's effects on analgesia (Von Frey), immobility (forced swim test), and affective pain responses (hot plate). However, KET-induced acute hyperlocomotion was significantly reduced by NTX in a 30 min open field test (OFT). To determine which opioid receptor subtypes mediate the locomotor effects of KET, we administered irreversible selective opioid antagonists 24 hrs prior to KET. The κ opioid receptor antagonist Nor-BNI (10 mg/kg) did not block KET hyperlocomotion, while the μ opioid receptor

(MOR) antagonist β -FNA significantly reduced KET hyperlocomotion when administered at 15 mg/kg. We used iDISCO+ and light sheet microscopy to image brain-wide activity with immunofluorescence of the immediate early gene *cFos*. We identified the central amygdala (CeA) as a region with significantly higher *cFos*+ cells in the KET+NTX group compared to KET. We implanted bilateral drug infusion cannulae in the CeA to test the role of CeA MORs in KET's locomotor effect. Vehicle or the MOR antagonist CTAP (300ng/500nl) was infused bilaterally into CeA prior to systemic KET injection. We found that intra-CeA administration of CTAP significantly decreased KET-induced hyperlocomotion compared to KET + VEH. Conversely, CTAP alone did not alter locomotor activity. Additional studies are underway to investigate the role of central amygdalar MORs in locomotor stimulating effects of KET using DREADDs and a conditional MOR knockout line. These results suggest that some of KET's behavioral effects are mediated via MORs, and that the CeA is a brain region involved in this interaction. Further investigation of KET's interaction with the opioid system will expand our understanding of KET's complex pharmacology with the goal of maximizing the therapeutic effects while reducing negative side effects.

Disclosures: **P. Llorach:** None. **D. Rijsketic:** None. **M.B. Pomrenze:** None. **A.B. Casey:** None. **J.S. Salgado:** None. **T.M. Hietamies:** None. **R.C. Malenka:** F. Consulting Fees (e.g., advisory boards); MapLight Therapeutics, MindMed, Bright Minds Biosciences, AZ Therapies, Cycleron. **B.D. Heifets:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Vine Ventures, MindMed, Clairvoyant Therapeutics. F. Consulting Fees (e.g., advisory boards); Osmind Mental Health and Journey Clinical.

Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 483.04

Topic: G.03. Motivation

Support: National Science Foundation Graduate Research Fellowship to DCP
Howard Hughes Medical Institute Gilliam Fellowship for Advanced Study to DCP and RCM

Title: Integration of convergent dopamine and serotonin signals in the Nucleus Accumbens gates Pavlovian learning

Authors: ***D. F. CARDOZO PINTO**, M. B. POMRENZE, J. VISWANATHAN, M. GUO, B. S. BENTZLEY, N. ESHEL, R. C. MALENKA;
Stanford Univ., Stanford Univ., Stanford, CA

Abstract: Survival depends on an organism's ability to seek rewards and learn about environmental cues that predict them. Two major neuromodulatory systems - dopamine (DA) and serotonin (5-hydroxytryptamine; 5HT) - are thought to play a critical role in this Pavlovian (i.e. cue-outcome) learning process because they respond to rewards and project to downstream targets involved in shaping motivated behaviors. For decades, these similarities in activity and connectivity have inspired conceptual frameworks and computational models about how DA and 5HT may act in concert to drive learning, arriving at two primary ideas. The opponency hypothesis proposes that DA and 5HT drive appetitive and aversive learning respectively, while the synergy hypothesis posits that both DA and 5HT are required for appetitive learning but function on different timescales. To the best of our knowledge however, these hypotheses have never been tested directly; in large part because it has been difficult or impossible to precisely manipulate multiple neuromodulator systems in a single animal. Here, we use a double transgenic DAT-Cre/SERT-Flp mouse line to enable simultaneous genetic access to the brain's DA and 5HT systems. Anterograde axon tracing revealed that midbrain DA and 5HT neurons innervate limbic targets with subregion specificity to create putative hotspots for the integration of DA and 5HT release. Focusing on the site with the densest convergence of DA and 5HT axons - the Nucleus Accumbens (NAc) medial shell - we applied fiber photometry to simultaneously record DA and 5HT axon calcium activity during Pavlovian conditioning and find that DA axons are excited during reward consumption while 5HT axons are inhibited. Optogenetically reproducing this coincident increase in DA and decrease in 5HT, but not either manipulation alone, was sufficient to drive simple forms of associative learning. Finally, we find that 5HT receptor expression is highly organized across subregions and D1 and D2- receptor expressing cell-types in the NAc. Altogether our results provide a blueprint for the organization of convergent mesolimbic DA and 5HT circuits and suggest that the integration of these teaching signals is a crucial mechanism underlying Pavlovian learning.

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 483.05

Topic: G.03. Motivation

Support: P50DA042012
K08MH110610

Title: Differential 5-HT receptor activation regulates the prosocial and rewarding effects of MDMA

Authors: M. B. POMRENZE¹, J. M. SALGADO⁷, P. LLORACH², Z. RASTEGAR³, G. RAMIREZ OVALLE⁴, A. B. CASEY⁵, D. R. RIJSKETIC⁶, R. C. MALENKA³, ***B. HEIFETS**³; ¹Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA; ²Anesthesia, Stanford Univ., Palo Alto, CA; ³Stanford Univ., Stanford, CA; ⁴Stanford Univ., Palo Alto, CA; ⁵Anesthesiol., Stanford Univ., Stanford, CA; ⁶Anesthesiol., Stanford Univ., Palo Alto, CA; ⁷FMUSP, Sao Paulo, Brazil

Abstract: Introduction: 3,4-methylenedioxy-methamphetamine (MDMA, also known as “ecstasy”) can induce acute prosocial and rewarding states that may underly its clinical efficacy towards post-traumatic stress disorder and its abuse potential, respectively. Recent work from our lab suggest MDMA may drive these prosocial and rewarding effects by promoting neurotransmitter efflux via serotonin and dopamine transporters (SERT and DAT, respectively) in the nucleus accumbens (NAc; Heifets et al., 2019). Interestingly, MDMA has markedly less abuse liability compared to other amphetamines, and is able to facilitate social reward learning. We hypothesized that MDMA-stimulated serotonergic and dopaminergic circuits interact in the NAc to elicit these unique properties. Understanding these processes in detail may facilitate development of prosocial therapeutics with minimal abuse liability. **Methods:** Behavioral assays: conditioned place preference (CPP; nonsocial drug reward); social CPP (social reward learning); 3-chamber test (3CT; social preference). Mouse lines: C57/Bl6 (wild-type;WT); tamoxifen-induced DAT and SERT-KO. NAc 5-HT receptors, including SERT, 5-HT_{1B} receptor, and 5-HT_{2C} receptor were blocked by intra-NAc infusions of (*S*)-citalopram (*S*-CIT), 0.5 μg), Nas-181 (0.5 μg) or SB242084 (1 μM) respectively. DA release in NAc was measured by viral fluorescent reporter (AAV-hSyn-GRAB-DA-4). **Results:** MDMA-evoked social CPP requires both SERT and DAT function, while reward-related CPP and social preference-related 3CT behaviors required only normal DAT and SERT function, respectively. In SERT-KO mice, or in WT mice after intra-NAc infusion of *S*-CIT, MDMA failed to elicit acute social preference while enhancing the rewarding properties of MDMA. Selective antagonism of 5-HT_{2C}, but not 5-HT_{1B}, receptors in the NAc also enhanced the rewarding properties of MDMA in WT mice. DA fluorescence experiments indicated that 5-HT_{2C} receptors regulate MDMA-mediated dopamine release. **Discussion:** MDMA engages distinct NAc circuitry to produce social preference, social reward learning and nonsocial drug reward. We find that social reward learning, which may be clinically relevant, requires both serotonergic and dopaminergic mechanisms. We have also identified 5-HT_{2C} receptors as a key mechanism to regulate MDMA abuse liability, consistent with literature evidence suggesting that 5-HT_{2C} receptors are promising target for the treatment of substance use disorder. This research was supported in part by a NIDA P50 PPG Grant (P50DA042012) and NIMH K08 (K08MH110610).

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 483.06

Topic: G.03. Motivation

Support: 5R01DA011289-21
5T32DA035165-08
1F32NS123012-01
Stanford Neurochoice

Title: Excitatory periaqueductal grey afferents to ventral tegmental area GABA neurons exhibited altered NMDA currents following inflammatory injury

Authors: *C. MANNING, J. A. KAUER;
Stanford Univ., Stanford, CA

Abstract: Many ventral tegmental area dopamine neurons (VTA_{DA}) exhibit reduced firing rates in response to injury and disinhibition of VTA_{DA} neurons results in analgesia, indicating that dopamine cell firing contributes to behavioral responses to noxious events. However, VTA_{DA} synaptic physiology in response to injury has not been extensively characterized. The VTA is innervated by several regions implicated in the control of pain. To characterize one of these circuits after injury we isolated glutamatergic afferents from the periaqueductal gray (PAG) to identified VTA dopamine or GABAergic neurons (VTA_{DA}:PITX3-GFP, VTA_{GABA}:vGat-CrexAi14), and compared their function in a carrageenan model of inflammatory pain. To achieve afferent specificity, we injected mice with a cre-dependent ChR2 virus, and 3 weeks later recorded optically-evoked EPSCs. We first pursued the PAG-VTA_{DA} afferent, as PAG has a known role in opioid analgesia and its excitatory afferents to the VTA can drive aversion. 24 hours after inflammatory hindpaw injection of carrageenan, no significant changes were seen in paired pulse ratio (50ms interstimulus interval) nor in $1/(CV^2)$ (N=36 cells, 16 animals, $p>.05$), consistent with no presynaptic changes at these synapses. We also characterized postsynaptic responses at PAG-VTA_{DA} synapses. Intriguingly, many of these VTA_{DA} neurons displayed excitatory currents at -70 mV that were not blocked by NBQX (10uM), as well as excitatory currents at +40mV not blocked by d-AP5 (50uM). We therefore tested the GluA2-lacking AMPAR antagonist, NASPM, as well as the NR2C/D receptor antagonist, UBP141. Many optically-evoked PAG-VTA_{DA} EPSCs were decreased by UBP141(24uM) and NASPM (20uM), indicating the presence of both NR2C/D NMDARs and GluA2-lacking AMPA receptors at these synapses. However, acute inflammation did not alter AMPA/NMDA ratios nor sensitivity to NASPM and UBP141 (N=15-25 cells, 13-17 animals, $p>0.05$). Our data show that presynaptic and postsynaptic function at PAG-VTA_{DA} synapses appears unchanged 24 hours after carrageenan injection. However, we did observe a significant reduction in UBP141 sensitive current at excitatory PAG synapses on VTA GABAergic neurons following acute injury (N=15 cells, 9 animals, $p=0.04$), although no other changes in presynaptic nor postsynaptic responses (N=22-28 cells, 15-16 animals, $p>0.05$), nor in NASPM sensitivity (N=13 cells, 7 animals, $p>0.05$) were observed. Taken together this indicates that acute inflammatory injury may subtly

alter the resting Ca^{2+} levels in VTA_{GABA} neurons, possibly altering their ability to acquire and express plastic changes.

Disclosures: C. Manning: None. J.A. Kauer: None.

Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 483.07

Topic: G.03. Motivation

Support: NIDA Grant DA042012

Title: Brain-wide neural correlates of psilocybin's context-dependent and -independent effects in mice

Authors: *A. B. CASEY¹, D. A. RYSKAMP¹, D. BARBOSA³, G. R. OVALLE¹, T. HIETAMIES¹, D. F. CARDOZO PINTO², M. B. POMRENZE², R. C. MALENKA², B. D. HEIFETS¹;

¹Anesthesiology, Perioperative and Pain Med., ²Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA; ³Neurosurg., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Clinical evidence suggests psilocybin, a prodrug of the serotonergic psychedelic psilocin, may promote rapid and persistent antidepressant effects in humans. The therapeutic efficacy of psilocybin may depend on both the patient's mindset and the clinical context, but how context alters the neurobiological effects of psilocybin is unknown. Here, we aimed to identify neural populations in mice that are reliably modulated by psilocybin independent of context, as well as those selectively modulated in a single context. Adult mice were injected with saline or psilocybin (i.p., $2 \text{ mg} \cdot \text{kg}^{-1}$) and placed in their home cage or an enriched environment for 2 hours. Following sacrifice and perfusion, brains were permeabilized, bleached, immunolabeled (rabbit anti-cFos and donkey anti-rabbit Alexa647), and cleared using an iDISCO+ protocol. Image volumes of $\sim 3.5 \mu\text{m}$ isotropic resolution were acquired at 488 nm (autofluorescence channel) and 638 nm (cFos channel) wavelengths using a Zeiss Lightsheet 7 microscope. Anatomical registration of autofluorescence data to the Gubra brain atlas was performed with MIRACL; cFos image volumes underwent rolling ball background subtraction before being warped to standard space ($25 \mu\text{m}$), and z-scored. The randomise function in FSL was used to performed voxel-wise between group comparisons with GLM-based ANOVA and post hoc tests. Group differences in cFos fluorescent labeling, a marker of neural activity, were validated with cell counts in full resolution. Independent of context, psilocybin increased the density of layer 5 cortical cFos-labeled cells, as well as the density of cFos+ cells in the parasubthalamic nucleus and the lateral portion of the central amygdala. Cell counts were decreased in several subcortical regions including numerous hypothalamic nuclei. In the enriched environment condition, psilocybin selectively enhanced cFos labeling in the posterior caudoputamen and decreased

labeling in the zona incerta. In the home cage context, psilocybin selectively enhanced activity in a somatosensory subregion and decreased activity in the piriform area. Brain-wide imaging of cFos expression across environmental contexts coupled with voxel-wise statistics is an unbiased method of identifying pharmacologically relevant neural populations involved in drug mechanisms of action. Our results suggest that a behaviorally active dose of psilocybin elicits context-dependent and context-independent changes in cFos expression across the mouse brain. These findings shed new light on brain regions potentially involved in the therapeutic efficacy of psilocybin.

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 483.08

Topic: G.03. Motivation

Support: R15MH-114026-02

Title: The role of the substantia nigra to dorsal lateral striatum circuit in the maintenance of voluntary physical activity is sex-dependent

Authors: *N. M. BROWN, M. K. TANNER, A. A. HOHORST, E. C. LOETZ, B. N. GREENWOOD;

Univ. of Colorado Denver, Univ. of Colorado Denver, Denver, CO

Abstract: Understanding the brain circuits that contribute to the maintenance of exercise, particularly rapid escalation of exercise behavior in female rats, could help maximize the benefits of exercise. When allowed voluntary access to running wheels, rats escalate running distance daily during the acquisition phase, until reaching a plateau in daily running distance during the maintenance phase. Female rats escalate more readily and reach greater absolute daily running distances than do males. Substantia nigra (SN) projections to the dorsal lateral striatum (DLS) have been implicated in motor activity and habit behavior, but the role of the SN-to-DLS circuit in acquisition and maintenance of voluntary exercise has not been investigated. The goal of the current experiment is to determine the role of the SN-to-DLS circuit in the acquisition and maintenance of voluntary exercise and whether sex differences exist. We hypothesize that the SN-to-DLS circuit is responsible for the maintenance of voluntary exercise behavior. Moreover, given that females can develop DLS-dependent habitual responding during operant training more

readily than males, we also predict that the SN-to-DLS circuit drives voluntary wheel running earlier during the acquisition phase in females compared to males. An intersectional chemogenetic approach was used to silence the SN-DLS circuit during daily voluntary wheel running in male and female rats. This is the first time a circuit-specific approach has been used to investigate neural circuits underlying exercise behavior. Inactivating the SN-to-DLS circuit reduced wheel running in both sexes while not impacting locomotor activity, per se. Circuit silencing reduced nightly distance run in females starting from the first day of wheel access. Interestingly, silencing had no effect on daily distance run in males until several weeks after the start of wheel access. These data suggest that there are sex differences in the role of the SN-to-DLS circuit in the acquisition of voluntary exercise.

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 483.09

Topic: G.03. Motivation

Support: Intramural Research Program of National Institute on Drug Abuse, National Institutes of Health
Center on Compulsive Behavior, National Institution of Health

Title: Supramammillary neurons projecting to the lateral preoptic area modulate reward-seeking behavior

Authors: *Y. ARIMA, S. IKEMOTO;
Neurocircuitry of Motivation Section, NIDA/NIH, Baltimore, MD

Abstract: Midbrain dopamine neurons are known to be critical in reward-seeking behavior. However, it is not well understood how other neural systems interact with dopamine neurons in reward-seeking behavior. Previous studies suggest that the supramammillary nucleus (SuM), particularly SuM glutamatergic neurons (GluN) projecting to the medial septum (MS), are important in reward-seeking behavior. In addition, SuM neurons appear to regulate reward-seeking behavior by coordinating the activities of multiple brain regions. To further understand such coordination role of the SuM and its mechanisms, first, we examined the hypothesis that SuM-MS neurons send collateral projections to multiple brain regions. We confirmed that about 95% of SuM-MS neurons were GluN using retrograde tracing and mRNA in situ hybridization procedures. Then, we examined collateral projections of SuM-MS GluN by injecting a retrograde AAV-Cre into the MS and an AAV-FLEX-mGFP-2A-SYP-mRuby. We found that SuM-MS neurons densely project to the dorsal tenia tecta, vertical and horizontal limb of the diagonal band, MS, substantia innominata, lateral preoptic area (LPO), lateral hypothalamus, and

hippocampal CA1/CA2. Because the LPO is known to modulate reward-seeking behavior, we focused on the SuM-LPO pathway. Using the anterograde transsynaptic property of AAV serotype 1, we injected an AAV1-Cre into the SuM and found LPO neurons expressed Cre, confirming that SuM neurons have synaptic contacts with LPO neurons. We then examined whether optogenetic stimulation of SuM-LPO GluN instigates motivated behavior using an intracranial optogenetic self-stimulation test. We expressed channelrhodopsin-2 in SuM GluN-LPO of Vglut2-Cre mice. Mice quickly learned to press the lever that activated SuM GluN-LPO, suggesting that the stimulation of SuM-LPO GluN instigates and reinforces reward-seeking behavior. Finally, we tested SuM-LPO neuronal activity during reward-seeking behavior using a water-seeking test with GCaMP7s recording. We found that when mice nose-poked into water reward-port to drink water, SuM-LPO neuronal activity decreased, suggesting that SuM-LPO neurons are active during the reward-seeking phase, but not consummatory phase. In summary, our research suggests that SuM-MS GluN have collateral projections to multiple regions, including the LPO, and that SuM-LPO GluN instigate and reinforce reward-seeking behavior. Future studies will examine how SuM-LPO GluN interact with midbrain dopamine neurons in reward-seeking behavior.

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

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Topic: G.03. Motivation

Support: K99/R00 Brain Initiative Advanced Postdoctoral Career Transition Award to Promote Diversity (K99 MH126429, R00 MH126429, to S.B.)
NIMH IRP (1ZIAMH002950, to M.A.P.)

Title: Two parallel thalamo-striatal pathways oppositely encode food-seeking behavior.

Authors: *S. BEAS¹, I. KHAN², C. GAO³, E. MCDONNALD², A. BASHFORD², S. RODRIGUEZ², M. A. PENZO³;

¹Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL; ³Natl. Inst. of Mental Hlth.,
²Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Motivated behaviors enable individuals to pursue goals that are essential for survival. However, the mechanisms that underlie these behaviors are still not fully understood. Previous research has mainly centered on investigating the contribution of the dopaminergic mesolimbic system to these processes. Yet, the role that glutamatergic inputs to the NAc play in the control of motivation is far less clear. The paraventricular nucleus of the thalamus (PVT), a brain region that integrates bottom-up interoceptive signals with top-down cortical information, sends robust glutamatergic projections to the NAc. Recently, we identified two major distinct subpopulations

of neurons in the PVT (Type1^{PVT} and Type2^{PVT}) that differ in their genetic identity, connectational features, and functionality. These subpopulations send divergent projections to the NAc, which raises the possibility that Type1^{PVT} and Type2^{PVT} neurons are likely to enable different but complementary aspects of motivated behavior. However, very little is known about the involvement of thalamic inputs to the NAc in the mediation of motivational processes. Here, using fiber photometry, we investigated the *in vivo* dynamics of these two parallel thalamo-striatal pathways in mice performing a reward foraging task. Our findings revealed that activity in the Type1^{PVT}-NAc pathway increases during the reward approach and varies with aspects of motivation like behavioral vigor and level of satiety. In contrast, Type2^{PVT}-NAc neurons showed opposing *in vivo* dynamics and were not sensitive to motivational variables, suggesting that this pathway may participate in regulating other aspects of goal-oriented behavior. Collectively, the results gathered from this investigation demonstrate a novel dissociation between the Type1^{PVT}-NAc and Type2^{PVT}-NAc pathways and identify a specific neuronal subpopulation of the PVT that signals motivational states.

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 483.11

Topic: D.02. Somatosensation – Pain

Support: National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2018R1A5A2025272, 2019R1A2C1002555, and 2019R1A6A3A13090969)
Korea Institute of Oriental Medicine (KIOM) (KSN1812181, KSN2013210).

Title: Nociceptive stimuli activate the hypothalamus-habenula circuit to inhibit the mesolimbic reward system and cocaine seeking behaviors

Authors: *H.-Y. KIM¹, S. LEE¹, K. BILLS², S. C. STEFFENSEN³, B. LEE¹, S. KIM¹;
¹Daegu Haany Univ., Daegu, Korea, Republic of; ²Dept. of Biomed. Sci., Noorda Col. of Osteo. Med., Springville, UT; ³Dept of Psych and Neuro, Brigham Young Univ., Provo, UT

Abstract: Nociceptive signals interact with various regions of the brain, including those involved in physical sensation, reward, cognition, and emotion. Emerging evidence points to a role of nociception in the modulation of the mesolimbic reward system. The mechanism by which nociception affects dopamine (DA) signaling and reward is unclear. The lateral hypothalamus (LH) and the lateral habenula (LHb) receive somatosensory inputs and are structurally connected with the mesolimbic DA system. Here we show that the LH-LHb pathway is necessary for nociceptive modulation of this system using male Sprague-Dawley rats. Our

extracellular single-unit recordings and head-mounted microendoscopic calcium imaging revealed that nociceptive stimulation by tail-pinch excited LHb and LH neurons, which was inhibited by chemical lesion of the LH. Tail-pinch decreased extracellular DA release in the nucleus accumbens ventrolateral shell, which was blocked by disruption of the LH. Furthermore, tail-pinch attenuated cocaine-induced locomotor activity, 50-kHz ultrasonic vocalizations and reinstatement of cocaine-seeking behavior, which was inhibited by chemogenetic silencing of the LH-LHb pathway. Our findings suggest that nociceptive stimulation recruits the LH-LHb pathway to inhibit mesolimbic DA system and drug reinstatement.

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

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Program #/Poster #: 483.12

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA047102
NIH Grant T32 DA007287

Title: Final Validation of Barpress Durations as a Measure of Frustration-Related Behavior

Authors: *T. A. GREEN, P. SHAH, Y. MARMOL-CONTRERAS, T. E. S. VASQUEZ;
UT Med. Br., Galveston, TX

Abstract: Inappropriate response to frustrating situations has relevance to a number of neuropsychiatric conditions, including substance use disorders, mood disorders, conduct disorders, etc. Our previous work offered preliminary evidence for barpress durations as a real-time surrogate measure of frustration-related behavior in rat operant tasks. The current abstract is the final validation of this measure. We have set and satisfied 10 criteria for validating barpress durations as a measure of frustration, criteria that A) first describe the frustration effect, B) distinguish the effect from other measures such as seeking/taking, C) eliminate performance variables as confounds, and D) demonstrate the effect is tied to motivation for fentanyl, cocaine, or sucrose reinforcement. We further offer preliminary evidence for neurocircuitry underlying this frustration effect, specifically the nucleus accumbens shell. These results offer an easy, real-time quantification of the behavioral responses to frustrative nonreward in rat operant tasks, with relevance to a number of neuropsychiatric conditions.

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

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Program #/Poster #: 483.13

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA036657

Title: Offline Modification of Reward Value During Hippocampal Sharp-Wave Ripple Replay Events

Authors: *A. FRYC¹, K. CLEMENZA², L. SJULSON²;

¹Albert Einstein Col. of Med., New York, NY; ²Albert Einstein Col. of Med., Bronx, NY

Abstract: The opioid epidemic is a long standing public health crisis recently exacerbated by COVID-19, and has killed over 100,000 people in the United States in the past 12 months. An obstacle to overcoming the epidemic are high relapse rates associated with opioid use disorder. Drug-context associations act as triggers for relapse, and are thought to be reconsolidated, or maintained, with every re-exposure. However, the memory of the drug-context association is considered to be labile in the period directly after reexposure, and hence can be disrupted. Here, we aim to take advantage of the lability present in this offline reconsolidation period that is present upon reexposure of a drug-context association and alter it with optogenetic manipulation and chemogenetic approaches. Specifically, we will target hippocampal “replay” events of previously-experienced drug associated contexts, and manipulate the coordination of D1 and D2 medium spiny neurons in the nucleus accumbens, which are known to update and encode the reward value associated with a context, during these replay events. The results of this study have the potential to inform relapse interventions that aim to disrupt drug-context associations that can act as triggers for relapse.

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Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

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Program #/Poster #: 483.14

Topic: G.09. Drugs of Abuse and Addiction

Support: DA037207
Ancora Innovation, LLC
5T32MH065215-17

Title: Selective inhibition of M₅ muscarinic acetylcholine receptors attenuates dopamine release in the mesolimbic dopaminergic circuitry

Authors: *S. INGRAM^{1,2}, L. B. TEAL^{1,2}, A. K. YU¹, C. HAN^{1,2}, C. W. LINDSLEY^{1,3,2}, C. K. JONES^{1,2};

¹Dept. of Pharmacol., ²Warren Ctr. for Neurosci. Drug Discovery, ³Dept. of Chem., Vanderbilt Univ., Nashville, TN

Abstract: The mesolimbic dopamine (DA) circuitry plays critical roles in motivation, affect and reward functions. Alterations in signaling within the mesolimbic DA circuitry, i.e., the ventral tegmental area (VTA) and its projections to the nucleus accumbens (NAc), are associated with various symptoms observed in psychiatric disorders, including substance use disorders and depression. To date, there remains an unmet for novel therapeutic strategies for the pharmacologic treatment of mesolimbic DA dysfunction observed in these disorders. One novel approach is the modulation of mesolimbic DA circuitry by selectively targeting the M₅ muscarinic acetylcholine receptor (mAChR) subtype, which is located on VTA DA neurons. Previous studies have shown that increases VTA dopamine cell firing and extracellular DA release following electrical stimulation of brainstem cholinergic projections to the VTA are absent in M₅ knockout (KO) mice. Our group and others also have demonstrated that selective inhibition of M₅ mAChRs using either M₅ negative allosteric modulators (NAM) an M₅ orthosteric antagonist attenuates cocaine, alcohol and opioid drug seeking behaviors in rodents. Here we conducted studies to understand the effects of selective M₅ inhibition on changes in DA release in the mesolimbic DA circuitry in mice. Male DAT-Cre and DAT-Cre/M₅ KO mice were injected with a floxed/reversed Cre-dependent adeno-associated virus (AAV) driving expression of ChrimsonR fused with tdTomato protein under the synapsin (Syn) promoter into the VTA (further referred to as ChR) for optogenetic stimulation. In addition, AAV-mediated expression of dLight1.2 under the Syn promoter, fused with a circularly permuted green fluorescent protein (further referred to as dLight) was injected into the NAc to detect DA. The recording cannula was inserted at the injection site for dLight. Basal DA signaling was detected by dLight at 465 nm. Evoked DA release in the NAc was elicited by presynaptic optogenetic stimulation of ChR at 705 nm and DA was detected by dLight. After a one hour baseline, the dose-related acute effects of either the M₅ NAM VU6008667 or the M₅ orthosteric antagonist VU6019650 on DA release in the NAc were determined. Changes in basal and evoked dLight signaling were assessed relative to baseline for each experimental condition in 8-10 male DAT-Cre and DAT-Cre/M₅ KO mice. Our findings demonstrated that antagonism of the M₅ mAChRs attenuates basal and evoked DA signaling within the mesolimbic DA circuitry. Ongoing dLight studies are now examining the effects of these M₅ mAChRs inhibitors on opioid-induced alterations in DA signaling within the mesolimbic DA circuitry.

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options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); composition of matter patents protecting allosteric modulators of GPCRs..

Poster

483. Mechanisms Underlying Reward and Reinforcing Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 483.15

Topic: G.09. Drugs of Abuse and Addiction

Support: F32 DA054709
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Scan Design Foundation Innovative Pain Research Grant
UW Addictions, Drug and Alcohol Institute Research Grant

Title: Endocannabinoid tuning of behavioral engagement via a PVT-NAc circuit

Authors: *D. J. MARCUS^{1,2}, A. E. ENGLISH^{3,2}, S. HWANG^{1,2}, E. F. SETH^{1,2}, C. E. PEDERSEN¹, Y. LI⁶, L. S. ZWEIFEL^{4,2}, N. STELLA^{4,2}, M. R. BRUCHAS^{1,2,4,5};

¹Anesthesiol., ²Ctr. for Neurobio. of Addiction, Pain, and Emotion, Univ. of Washington, Seattle, WA; ³Pharmacol., Univ. of Washington, SEATTLE, WA; ⁴Pharmacol., ⁵Bioengineering, Univ. of Washington, Seattle, WA; ⁶Sch. of Life Sci., BEIJING, China

Abstract: A key clinical feature of addiction is compulsive engagement with rewarding stimuli, such as drugs of abuse, despite negative outcomes. However, we lack a detailed understanding of the neural mechanisms that regulate compulsive behavioral engagement. The Nucleus Accumbens (NAc) represents an integral functional component of the mesocorticolimbic pathway, canonically known as the “reward” pathway. The NAc receives dense projections from the Paraventricular Thalamus (PVT), and these projections have been shown to regulate the behavioral effects of opiate withdrawal, sucrose seeking/consumption, and behavioral responses to painful stimuli. The PVT is a highly heterogenous structure, and recent studies examining the PVT-NAc circuit have generated contradictory results, partially driven by a lack of genetic and anatomical targeting within the PVT. Here, we identify the neuropeptide neurotensin (NTS) as a novel marker for a select population of anterior PVT neurons. Furthermore, we demonstrate that these NTS neurons send strong excitatory projections to the NAc, and that this input is biased toward Dopamine Receptor 1 (D1R) expressing medium-spiny neurons. This input bias is mediated through tonic endocannabinoid (eCB) suppression of excitatory input onto Dopamine Receptor 2 (D2R) expressing neurons. Using fiber photometry in mice expressing DIO-GCaMP in PVT NTS neurons, we observed that PVT NTS-NAc projections are inhibited during engagement in both sucrose seeking/consumption and conditioned freezing behaviors, and activated upon behavioral disengagement. Using GRAB-eCB, a fluorescent sensor for eCB release, as well as CRISPR deletion of the CB1 receptor from PVT NTS neurons, we demonstrate that eCBs are released in the NAc and inhibit impinging PVT NTS terminals to

drive behavioral engagement. Disruption of this inhibition using photo-stimulation of this circuit is sufficient to drive behavioral disengagement. Furthermore, these manipulations are sensitive to eCBs, as exogenously augmenting eCB signaling attenuates the behavioral effects of photo-stimulation. Taken together, these results implicate a novel eCB neuromodulatory mechanism for regulation of behavioral engagement through a PVT-NAc circuit.

Disclosures: **D.J. Marcus:** None. **A.E. English:** None. **S. Hwang:** None. **E.F. Seth:** None. **C.E. Pedersen:** None. **Y. Li:** None. **L.S. Zweifel:** None. **N. Stella:** None. **M.R. Bruchas:** None.

Poster

484. Central Actions of Psychoactive Substances

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 484.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Baszucki Brain Research Fund and Milken Institute
Natural Sciences and Engineering Research Council of Canada

Title: Amphetamine induces a biphasic response in mouse striatal cells - the role of MANF

Authors: ***F. ABU-HIJLEH**, W. D. GWYNNE, N. RIGG, J. SHAH, B. FREY, R. MISHRA;
McMaster Univ., McMaster Univ., Hamilton, ON, Canada

Abstract: Background: Amphetamine-based molecules such as amphetamine, methamphetamine and 3,4-Methylenedioxymethamphetamine (MDMA) are potent psychostimulants that are widely abused. Their long-term use is associated with altered brain structures, including gray matter loss and increased intracellular calcium levels. Endoplasmic reticulum (ER) stress is a cellular state that can occur due to the depletion of ER calcium stores or an accumulation of unfolded/misfolded proteins. Prolonged ER stress activates pro-apoptotic proteins, which ultimately initiate cell death. This study aimed to investigate whether amphetamine-induced neurotoxicity is mediated by ER stress in a calcium-dependent manner.

Methods: Immortalized mouse striatal neurons (sthdhq7/q7) were treated with various concentrations of d-amphetamine for 24 hours. MTT assay was used to measure cell viability, and the Fluo-4 calcium assay kit was used to measure intracellular cytosolic calcium levels. mRNA gene and protein expression were quantified using RT-qPCR and western blots, respectively. MANF knockout cell lines were established using the CRISPR-Cas9 system.

Results: MTT assay indicates amphetamine-induced neuronal cell death is dose-dependent and directly correlated with increased intracellular calcium levels. A significant increase in the mRNA expression of ER stress proteins was observed (CHOP, GRP78, MANF, sXBP1, ATF6, AT4, Caspase-3). A significant increase in protein levels was observed for both CHOP and GRP78. MANF KO cells showed reduced cell proliferation when treated with 1mM d-amphetamine in serum-containing media. Lastly, MANF KO cells displayed increased

vulnerability to cell death when treated with 4mM d-amphetamine in serum-free media.

Conclusion: These results demonstrate that amphetamine induces neuronal apoptosis through the modulation of ER stress proteins. **Future Directions:** MANF has been identified as a neuroprotective protein against various ER stressors, including thapsigargin and tunicamycin. Future studies will determine whether the overexpression of MANF can alleviate amphetamine-induced ER stress.

Disclosures: **F. Abu-Hijleh:** None. **W.D. Gwynne:** None. **N. Rigg:** None. **J. Shah:** None. **B. Frey:** None. **R. Mishra:** None.

Poster

484. Central Actions of Psychoactive Substances

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 484.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Natural Sciences and Engineering Research Council (NSERC)

Title: Ketamine mediated neurotoxicity involves ER stress: Investigating the role of MANF

Authors: ***N. RIGG**, F. A. ABU-HIJLEH, W. D. GWYNNE, R. K. MISHRA;
McMaster Univ., Hamilton, ON, Canada

Abstract: Background: Ketamine is a dissociative anesthetic used in veterinary and clinical medicine and, more recently, as a therapy for treatment-resistant depression. It is also a popular drug of abuse worldwide due to its hallucinogenic and sensory-altering effects. Long-term ketamine use is associated with neurodegeneration in humans, rodents, and non-human primates, including loss of gray matter that correlates with severity and duration of use. Accumulating evidence suggests that ketamine cytotoxicity activates endoplasmic reticulum (ER) stress proteins, however, the mechanism of ER stress has not been investigated in neurons. Prolonged ER stress can trigger the upregulation of pro-apoptotic proteins such as C/EBP homologous protein (CHOP), ultimately initiating cell death. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an ER-resident protein that plays a critical role in maintaining and restoring ER homeostasis by attenuating ER stress in response to cellular insult. Therefore, this study aimed to investigate the role of ER stress and MANF regulation in ketamine-induced neurotoxicity. **Methods:** Mouse striatal STHdh^{Q7/Q7} cells were treated with ketamine (10uM, 100uM, 1mM) or DMEM for 24 hrs. Cell viability and gene expression were quantified using MTT assays and RT-qPCR. To investigate the role of MANF in ketamine neurotoxicity, we generated MANF-knockout (KO) mouse striatal cells using CRISPR/Cas9 with sgRNAs designed using CHOPCHOP and examined the effects of MANF deficiency on ketamine-induced neuronal apoptosis and ER stress. Validation of knockout was determined using Sanger sequencing and western blot. **Results:** (1) MTT results revealed that ketamine-induced cell death is dose-dependent and associated with a significant increase in mRNA expression of ER stress

markers (GRP78, CHOP, sXBP1, ATF6, ATF4). (2) The effect of ketamine on ER stress was robust, with transcriptional activation of all three ER stress pathways observed, demonstrated by increased sXBP1, ATF6, and ATF4 expression. (3) Ketamine non-significantly downregulated pro-survival MANF mRNA expression at 24 hrs alongside an increase in pro-apoptotic markers. (4) MANF deficiency significantly exacerbated ketamine-induced apoptosis and ER stress in a dose-dependent manner, with increased vulnerability observed at 10uM and 100uM in MANF-KO cells compared to controls. **Conclusions:** Taken together, these results indicate that ketamine-induced neurotoxicity is mediated through ER stress-dependent cell death and suggests that ER stress inhibitors such as MANF may serve as a novel therapeutic target for attenuating ketamine's neurotoxic effects.

Disclosures: N. Rigg: None. F.A. Abu-Hijleh: None. W.D. Gwynne: None. R.K. Mishra: None.

Poster

484. Central Actions of Psychoactive Substances

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 484.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant 1R01GM120519

Title: Isoflurane Exposure During Brain Development Alters long-term learning ability and ASD-like manifestations in genetically susceptible mice

Authors: *J. XU, R. P. MATHENA, J. WEN, C. D. MINTZ;
Dept. of Anesthesiol. and Critical Care Med., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Introduction: Autism spectrum disorder (ASD) is a neurodevelopmental condition that manifests as impairment in social interactions and repetitive behavior patterns [1]. There are considerable data from animal models and human epidemiologic studies suggesting that exposure to general anesthetic agents can have harmful consequences on brain development which result in lasting cognitive impairment [2]. Developmental exposure to isoflurane upregulates signaling in the mTOR pathway, a signaling system that is aberrantly activated in human ASD and mouse models of ASD [3]. We hypothesize that anesthetics will have neurotoxic effects that disrupt brain development among the autism genetically susceptible mice and alter their ASD-like manifestations and affect their long-term learning ability. Methods: For in vivo experiments, *FMR-1* knock-out and C57BL/6 Wild type mice at P7 were exposed to 1.5% isoflurane for 4 hours, and the control groups consisted of naïve littermates. Behavior tests were done when the mice turned P60. Y maze test and fear conditioning test were performed to assess learning behavior, while social interaction test, shredding test, and marble burying test were performed to assess ASD-like manifestations. Results: The isoflurane-treated *Fmr-1* KO

mice showed the same substantial deficits trend in the Y maze test and fear conditioning test as the wild-type animals. For the social interaction abilities, the genetically susceptible mice showed improvement in social communication after the early age of isoflurane exposure compared to the KO control ones, while the aggressive behavior appeared to be more frequent. The shredding test showed increasing anxiety among the wild-type animals after isoflurane exposure, while the ASD-like mice showed no change in the intensity. Conclusion: Early age isoflurane causes long-term learning deficits among both wild-type and ASD- genetically susceptible mice. ASD-like manifestations were altered after the exposure, and further mechanism needs to be discussed. References 1. Tchaconas, A. & Adesman, A. Autism spectrum disorders: a pediatric overview and update. *Current opinion in pediatrics* **25**, 130-144 (2013). 2. FDA Drug Safety Communication: FDA review results in new warnings about using general anesthetics and sedation drugs in young children and pregnant women. <http://www.fda.gov/Drugs/DrugSafety/ucm532356.htm> 3. Crino, P.B. The mTOR signalling cascade: paving new roads to cure neurological disease. *Nature reviews. Neurology* **12**, 379-392 (2016).

Disclosures: J. Xu: None. R.P. Mathena: None. J. Wen: None. C.D. Mintz: None.

Poster

484. Central Actions of Psychoactive Substances

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 484.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NCI Grant R01CA242158
Regenerative Medicine Minnesota RMM 091718 DS 005
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Rutgers Cancer Institute of New Jersey Pediatric Cancer-Blood Disorders Pilot
Program

Title: Caffeine prevents cisplatin-induced cognitive dysfunction in mice

Authors: *A. OLIVEROS AMAYA^{1,2}, M. MOSTAFA¹, I. LONCAR¹, K. YOO^{1,2}, A. CORUJO-RAMIREZ², M.-H. JANG¹;
¹Rutgers Univ., Piscataway, NJ; ²Mayo Clin., Rochester, MN

Abstract: Chemotherapy-induced cognitive impairments (CICI or chemobrain) affects approximately 14 million cancer survivors in the United States. There is no meaningful treatment due to a lack of pathophysiological mechanisms mediating CICI. Using cisplatin, a platinum-based compound that is widely used for various cancers to model CICI, we investigated differential gene expression from the hippocampus of mice administered cisplatin. Our RNA-sequencing analysis revealed that cisplatin profoundly increases gene expression of the

adenosine A_{2A} receptor A_{2A}R (> 20 fold), which is known to be involved in learning and memory dysfunction in neurodegenerative conditions, including Alzheimer's disease. Importantly, we demonstrated that the non-selective A_{2A}R antagonist caffeine prevents cisplatin-induced impairments in neurogenesis, spine density as well as memory (Y-maze) and emotive behaviors (elevated plus maze, nesting), suggesting a neuroprotective role of caffeine in cisplatin-induced neurotoxicity. We further show that cisplatin impairs neurogenesis and dendrite spine formation in the adult hippocampus, a brain region critical for learning and memory. Given that A_{2A}R antagonists show promising neuroprotection in neurodegenerative conditions and caffeine is one of the most used psychoactive substances worldwide, inhibiting A_{2A}R by caffeine during cancer treatment may provide a translational therapeutic option to prevent cognitive dysfunction associated with cisplatin administration, thus improving quality of life in cancer survivors.

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Poster

484. Central Actions of Psychoactive Substances

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 484.05

Topic: C.08. Ischemia

Support: Florida Department of Health # 20K09

Title: Effects of electronic cigarette vaping on histamine metabolism in rats of both sexes

Authors: *H. PRADHYUMNAN¹, B. MANKALIYE¹, S. H. PATEL¹, H. BRAMLETT^{2,3}, A. P. RAVAL^{1,3};

¹Peritz Scheinberg Cerebral Vascular Dis. Res. Labs. (CVDRL), Dept. of Neurol., ²Dept. of Neurolog. Surgery, Leonard M. Miller Sch. of Medicine, Univ. of Miami, Miami, FL; ³Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Battery-powered nicotine delivery devices, commonly known as electronic cigarettes (EC), have rapidly increased in use amongst adolescents globally. ECs aerosolize a solution containing nicotine, flavoring compounds, propylene glycol, and vegetable glycerin to allow for inhalation of the vapor. Though posed as an alternative to conventional cigarettes, EC vapor has been found to contain several harmful chemical agents such as formaldehyde hemiacetal in its aerosols with nicotine being the most toxic and addictive agent in the solution [1]. Our published studies demonstrated that chronic nicotine exposure attenuates paired-pulse facilitation, an indicator of short-term synaptic plasticity, and histamine metabolism thus causing severe ischemic stroke outcomes in female rats [2]. Here, we hypothesize that chronic EC exposure alters histamine metabolism and synaptic proteins, thus altering cognition in both male and female adolescent rats. Sprague-Dawley rats (2-3 months old) of both sexes were randomly

assorted into groups (n = 6/group) and exposed to either air or EC vapor (5% nicotine Juul pods) using the EcigAero-TM Aerosol Exposure Apparatus (between 7pm-7am; the active phase of circadian cycle in rats) for 16 nights. Per night, rats were exposed to 16 episodes of EC. Each episode consisted of 2 seconds of Juul puffs followed by 8 seconds of air over the period of 8 minutes. Rats were tested for learning and memory using a water maze for the last 7 days of EC exposure. Following behavioral testing, brain tissue was harvested for western blotting of synaptic proteins and metabolomic analysis (performed by Metabolon Inc.). The results of western blot analysis showed a significant decrease in the vesicular proteins munc-18 and synaptotagmin in the EC-exposed rats as compared to the control rats. The metabolomics data revealed a significant decrease in histamine metabolism. We observed significantly increased latency in finding the water maze hidden platform in EC-exposed rats, suggesting deficits in spatial learning. The observed changes may be responsible for cognitive decline and could contribute to an increased severity of stroke after nicotine exposure. References:1. Siegel J, Patel SH, Mankaliye B, Raval AP. Impact of Electronic Cigarette Vaping on Cerebral Ischemia: What We Know So Far. *Transl Stroke Res.* 2022 Apr 18.2. d'Adesky N, Diaz F, Zhao W, Bramlett HM, Perez-Pinzon MA, Dave KR, Raval AP. Nicotine Exposure Along with Oral Contraceptive Treatment in Female Rats Exacerbates Post-cerebral Ischemic Hypoperfusion Potentially via Altered Histamine Metabolism. *Transl Stroke Res.* 2021 Oct;12(5):817-828

Disclosures: **H. Pradhyumnan:** None. **B. Mankaliye:** None. **S.H. Patel:** None. **H. Bramlett:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; VA IO1 RX003506, Florida Department of Health # 21K06. **A.P. Raval:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; VA IO1 RX003506, Florida Department of Health # 20K09, Florida Department of Health # 21K06.

Poster

484. Central Actions of Psychoactive Substances

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 484.06

Topic: C.08. Ischemia

Support: Florida Department of Health #20K09
VA IO1 RX003506

Title: Nicotine withdrawal and stroke outcome in female rats

Authors: ***S. H. PATEL**¹, I. SAUL¹, K. DAVE¹, M. A. PEREZ-PINZON¹, A. P. RAVAL^{1,2};
¹Peritz Scheinberg Cerebral Vascular Dis. Res. Labs. (CVDRL), Dept. of Neurol., Leonard M. Miller Sch. of Medicine, Univ. of Miami, Miami, FL; ²Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Stroke disproportionately kills more women than men and is one of the leading causes of death and disability worldwide. Women have an increased lifetime risk for stroke, a higher mortality rate than men, and recent epidemiological data shows that young women suffer more strokes than young men. The major preventable risk factor for stroke is cigarette smoking and in the era of electronic cigarettes, it is a top concern. The common agent between the two types of cigarettes is nicotine (N). In a study using a rat model of ischemia, we demonstrated that N induces changes in histamine metabolism, leading to severe hypo-perfusion thus exacerbating ischemic brain damage in young female rats (1). The goal of the current study is to investigate how long this N toxicity persists in the brain after N withdrawal (NW). Additionally, post-ischemic cognitive decline is a significant consequence, and we aim to test how ischemic injury after N exposure or NW affects cognitive decline. In the current study, adult female Sprague-Dawley rats (n=8/group) were randomly exposed to either saline or N (4.5 mg/kg) for 16-21 days. Followed by the withdrawal of N exposure, the rats were allowed to recover for 0, 15, or 30 days. After completion of the assigned withdrawal period, the rats were randomly assigned to receive either a transient middle-cerebral artery occlusion (tMCAO; 90 min), sham surgery, or have their brain tissue collected for global metabolomic (Metabolon Inc) and western blot analysis. One-month post-surgery, the rats were tested for hippocampal-dependent contextual fear conditioning where % freeze time was measured. Following this behavioral testing, brain tissue was harvested for quantification of infarction. Upon completion of the treatment and subsequent withdrawal period, the infarct volume was quantified to be 26% (p<0.05), 25% (p<0.05), and 16% (p<0.05) higher in the 0, 15, and 30 day NW groups respectively, compared to the saline group. The recorded fear conditioning data demonstrated significantly lower freezing in all three NW groups as compared to the saline treated group, suggesting that there is a persistence in spatial memory deficits, even after 30 days of withdrawal. Metabolomics data analysis revealed a significant increase in histamine metabolites in the 30-day NW group as compared to the saline group. Confirmatory western blot analyses of histamine pathway supported these metabolomic findings. N-induced global metabolomic changes in the brain may persist after NW and could be responsible for exacerbating ischemic brain damage and cognitive deficits in female rats. References: 1. d'Adesky, N., et al., (2021). Translational stroke research, 12(5), 817-828.

Disclosures: **S.H. Patel:** None. **I. Saul:** None. **K. Dave:** None. **M.A. Perez-Pinzon:** None. **A.P. Raval:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Florida Department of Health #20K09, VA I01 RX003506.

Poster

484. Central Actions of Psychoactive Substances

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 484.07

Topic: C.08. Ischemia

Support: Florida Department of Health # 20K09

Title: Sex difference in the cerebral metabolism of amino acids in rats after electronic cigarette derived nicotine exposure

Authors: *N. D'ADESKY¹, S. H. PATEL¹, N. MANELIS¹, A. P. RAVAL^{1,2};

¹Peritz Scheinberg Cerebral Vascular Dis. Res. Labs. (CVDRL), Dept. of Neurol., Leonard M. Miller Sch. of Medicine, Univ. of Miami, Miami, FL; ²Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Electronic cigarettes are a growing contributor to nicotine use in the world. Nicotine is an addictive substance that is known to effect mood and behavior through regulation of neurotransmitters in the brain. However, the effects of chronic nicotine use in relation to neurochemistry and disease is still being investigated. Our previously published study demonstrated that chronic nicotine use alters the metabolism of histamine and exacerbates post-ischemic cerebral blood flow in the brain of female rats [1]. To further investigate the role of chronic nicotine use on neurotransmitter metabolism in the brain, we analyzed the amino acid metabolomic profile of male and female rats exposed to electronic cigarette derived nicotine. In this study, adolescent female and male Sprague-Dawley rats were randomly (n = 6/group) exposed to either air or electronic cigarette derived nicotine. To replicate human electronic cigarette use, rats were exposed to a 5% nicotine electronic cigarette Juul pods using the EcigAero™ Aerosol Exposure Apparatus for 16 episodes a night for 16 days. The controls subjects were exposed in a similar fashion to that of electronic cigarettes, however, exposed to air instead of Juul pods. Following this exposure, the rats were sacrificed, brain tissue was collected, and Metabolon Inc performed an unbiased metabolomic analysis of amino acid metabolites. Results from this study showed altered metabolism of several amino acid metabolites, interestingly results differed among male and female rats. Results found significantly increased histidine, glutamate, phenylalanine, and tyrosine metabolites in males and females (p<0.05). Tryptophan metabolites were only found to be significantly increased in males (p<0.05). Based on these results we conclude that chronic nicotine use alters levels of amino acid metabolites in the brains of rats in a sex dependent manner. The amino acids histidine, glutamate, phenylalanine, and tyrosine are essential in the production of the neurotransmitters histamine, glutamate, gamma-aminobutyric acid, serotonin, dopamine, norepinephrine, and epinephrine. Furthermore, alterations in neurotransmitter levels in the brain may have important implications in neurological disease such a stroke, epilepsy, movement disorders, and mood disorders. Therefore, further investigation is warranted on the effects of chronic nicotine use on neurochemistry and neurological disease. [1] d'Adesky, N.D., et al., Int J Mol Sci, 2018. 19(5).

Disclosures: N. d'Adesky: None. S.H. Patel: None. N. Manelis: None. A.P. Raval: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; VA IO1 RX003506, Florida Department of Health # 20K09, Florida Department of Health # 21K06.

Poster

484. Central Actions of Psychoactive Substances

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 484.08

Topic: A.01. Neurogenesis and Gliogenesis

Support: NCTR/FDA # E0767501

Title: Effect of the pediatric anesthetic, ketamine, on gene expression in zebrafish

Authors: ***J. KANUNGO**¹, Q. GU², B. ROBINSON³;

¹Natl. Ctr. For Toxicological Research/Food and Drug Admin., Natl. Ctr. For Toxicological Research/Food and Drug Admin., Jefferson, AR; ²FDA Natl. Ctr. for Toxicological Res., FDA Natl. Ctr. for Toxicological Res., Jefferson, AR; ³Natl. Ctr. For Toxicological Res., Natl. Ctr. For Toxicological Res., Jefferson, AR

Abstract: Ketamine is an N-methyl-D-aspartate (NMDA) receptor antagonist and a pediatric anesthetic. Potential neurotoxic and cardiotoxic effects of ketamine in animal models have been reported but the underlying mechanisms of ketamine-induced toxicity are not clear. The zebrafish is an ideal model for toxicity assays because of its predictive capability in chemical testing, which compares well with that of mammalian models. To gain insight into potential mechanisms of ketamine effects, we performed real-time quantitative polymerase chain reaction (qPCR)-based gene expression array analyses of RNA isolated from wild type zebrafish embryos exposed to a physiologically relevant concentration of ketamine. Gene expression analysis was conducted for multiple genes (a total of 84) related to ten major signaling pathways including TGF-beta, Wnt, NF-kappaB, JAK/STAT, p53, Notch, Hedgehog, PPAR, oxidative stress, and hypoxia pathways. Results indicated that ketamine altered the expression of specific genes related to p53, hypoxia, Notch, Wnt, TGF-beta, PPAR, and oxidative stress pathways. Although ketamine inhibited Notch thus showing a neurogenic potential, there were reduced number of motor neurons due to downregulation of NeuroD, that is expressed in mature neurons. Ketamine's effect on these specific pathways can help elucidate the mechanisms by which ketamine affects the nervous system.

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Poster

484. Central Actions of Psychoactive Substances

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 484.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01HL139712
R01HL128546

Children's Heart Foundation
Foglia and Hills families

Title: Primary cilia loss renders pyramidal neurons susceptible to perinatal ketamine-induced dendritic degeneration and learning deficits

Authors: *N. SARIC, F. SOMAA, M. STRAUSS, C. FOSTER SADE, G. RIVERO BALLON, L. WANG, K. HASHIMOTO-TORII, N. ISHIBASHI;
Children's Natl. Med. Ctr., Washington, DC

Abstract: Recent clinical studies have demonstrated a strong link between anesthetic exposure and neurodevelopmental outcomes in children with congenital heart disease (CHD). Protein-damaging *de novo* gene mutations have been demonstrated to be strong predictors of neurodevelopmental anomalies in CHD, and at least 50% of these genes have been found to be associated with primary cilia structure and/or function. Therefore, studying the combined effect of anesthesia and primary cilia gene mutations may shed light on the neurodevelopmental abnormalities observed in CHD children. Using *Emx1cre; Ift88 f/f*, in which primary cilia are lost specifically in cortical excitatory neurons, we administered either PBS or Ketamine at postnatal day 7 (P7). We examined markers of cytoskeletal degeneration at P8 in the medial prefrontal cortex as well as behavioral testing at P30 using water T-maze to assess their spatial memory performance and cognitive flexibility. Separate cohorts were also tested for gross and fine motor deficits using the accelerated rotarod and pellet reach task, respectively. During the reversal learning paradigm, the *Ifi88* knockout mice that were exposed to ketamine (cKO+Ket) showed a strongly reduced ability to learn compared to other groups, indicative of a cognitive flexibility deficit. This same group demonstrated significant motor deficits, with a reduced rate of motor learning in both gross and fine motor tasks. To assess pyramidal neuron morphology, we used two other inducible Cre/lox mice to specifically label layer II (*Nes-Cre/ERT2*) and layer V (*ER81-Cre/ERT2*) neurons. We found significant enhancement in immunoreactivity of cytoskeletal degeneration markers in the cKO+Ket mice compared to the other three groups (cHET+PBS, cHET+Ket, and cKO+PBS). The effects of ketamine on dendritic morphology were also specific to this group. To examine if the observed learning-related deficits were related to altered dendritic spine dynamics we then performed two-photon imaging on *Ifi88* cHET+Ket and cKO+Ket *Thy1-GFPM* animals and compared spine density and turnover in the motor cortex. We also utilized human induced pluripotent stem cells (hiPSCs) to examine the impact of identified *de novo* CHD mutations (CHD7, WDR5, and KDM5B) on cilia length/expression and caspase-3 activation in response to ketamine in both neural progenitor cells (NPCs) and differentiated neurons. Our findings indicate that primary cilia deficiency, due to a common genetic predisposition with CHD, exacerbates the toxicity of neonatal anesthesia, suggesting a novel therapeutic treatment strategy for the prevention of neurobehavioral abnormalities in this population.

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Poster

484. Central Actions of Psychoactive Substances

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 484.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01 DA050540

Title: Involvement of GluN2B signaling in methamphetamine potentiation of HIV-1 gp120-associated neurotoxicity

Authors: W. XIONG¹, J. LIU², *H. XIONG²;

¹Dept. of Pharmacol., Sichuan Univ., Chengdu, China; ²Dept. of Pharmacol. and Exptl. Neurosci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Methamphetamine (Meth) abuse and human immunodeficiency virus type 1 (HIV-1) infection are two global public health issues. Being frequently comorbid with HIV-1 infection, Meth abuse exacerbates HIV-1-associated neurocognitive disorders (HAND) seen clinically in HIV-1-infected individuals even in the era of combined antiretroviral therapy. While a large body of research has studied the individual effects of Meth and HIV-1 envelope glycoprotein 120 (gp120) in the brain, far less has focused on their synergistic influence. To uncover the mechanisms underlying Meth exacerbation of HAND, we investigated neurotoxic effects of Meth and gp120 on primary cortical neuronal cultures and hippocampal slices prepared from SD rats via MTT assay, TUNEL staining, western blotting and immunocytochemistry. After establishment of dose-dependent effects Meth (10 μ M) and gp120 (200pM) were adopted for the study. Application of Meth and gp120 in combination produced significant reduction on neuronal viability, triggered neuronal apoptosis and induced neuronal mitochondria membrane depolarization, in contrast to no apparent effects when applied each alone. Application of Meth and gp120 in combination was also found to enhance the expression levels of GluN2B and p-CREB and decreased the level of BDNF expression. Further examination revealed Meth and gp120 synergistically increased CaMKII and STEP expression and decreased p-ERK expression in rat cortical neuronal cultures. The synergic effects of Meth and gp120 were blocked or attenuated by ifenprodil (3 μ M) or memantine (5 μ M), indicating an involvement of the glutamate receptor subtype GluN2B in Meth- and gp120-associated neurotoxicity. These results demonstrated that Meth potentiation of gp120-mediated neurotoxicity via GluN2B signaling.

Disclosures: W. Xiong: None. J. Liu: None. H. Xiong: None.

Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 485.01

Topic: G.09. Drugs of Abuse and Addiction

Support: R21 DA049044 to LCD

Title: Organic Cation Transporter 3 is a Key Modulator of Amphetamine Reward

Authors: *L. E. HONAN, N. J. CLAUSS, W. A. OWENS, R. E. HORTON, W. KOEK, L. C. DAWS;

Cell. and Integrative Physiol., UT Hlth. San Antonio, San Antonio, TX

Abstract: Amphetamine-like stimulants are among the most commonly abused classes of drugs worldwide, posing a significant public health burden. Abuse of these drugs is on the rise globally, and overdose deaths resulting from psychostimulant abuse have increased nearly 76-fold in the United States in the past 10 years. Moreover, there are currently no effective treatments for stimulant use disorders. Thus, a better understanding of the precise mechanisms by which amphetamine-like stimulants elicit abuse-related effects is of fundamental importance. It has long been known that amphetamine acts as a competitive substrate for high affinity, low-capacity ‘uptake-1’ monoamine transporters, in particular the dopamine transporter. Recent work suggests that organic cation transporter 3 (OCT3), a low affinity, high-capacity ‘uptake-2’ transporter plays a significant role in regulating monoamine neurotransmission, making it a possible target for the effects of amphetamine as well. Indeed, our published studies using constitutive OCT3 knockout mice provide strong support for this premise. Here, using *in vivo* high-speed chronoamperometry coupled with behavioral assays, we provide further support that OCT3 is a key player in amphetamine reward circuitry in both male and female mice. Our results demonstrate that amphetamine-evoked dopamine release in dorsal striatum is reduced by pharmacological blockade of OCT3 in control mice, but not in mice with conditional (tamoxifen-inducible) global or local viral OCT3 knockout. Furthermore, we show that rewarding properties of amphetamine, measured via conditioned place preference, are attenuated in animals with conditional OCT3 knockout. These results suggest that OCT3 may be an attractive target for novel therapeutic development for amphetamine-stimulant use disorders.

Disclosures: L.E. Honan: None. N.J. Clauss: None. W.A. Owens: None. R.E. Horton: None. W. Koek: None. L.C. Daws: None.

Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: rant from Ministry of Food and Drug safety (20182MFDS425)

Title: Amphetamine-like drugs induced reward behavior is associated with cytosolic Ca²⁺ levels increase by Per2 down regulation

Authors: *H. EUNCHONG, K. AESEUL, K. HAECHAN, Z. YONG-QING, Y. JAESUK;
Chungbuk Natl. Univ., Chungbuk, Korea, Republic of

Abstract: Based on the basic chemical structure of psychoactive drugs, new psychoactive substances (NPS) are produced, leading to abuse. Therefore, the possibility of dependence of NPS such as amphetamine is being studied. methamphetamine induced dopamine release into the synaptic cleft. Dopamine release triggered by influx of Ca²⁺. The relationship between Ca²⁺ regulation of Period circadian regulator 2 (PER2) gene and reward behavior is not well known. To compare the drug reward behavior, intracranial self-stimulation (ICSS) induced by amphetamine-like substances (methamphetamine, PMMA) were studied in wild type (WT) and PER2 knockout (PER2KO) mice. We also measured Ca²⁺ fluorescence evoked by treatment of amphetamine-like substances using PC12 cells. Methamphetamine (2 mg/kg, i.p.) and PMMA (3.2 mg/kg, i.p.)-induced ICSS threshold of PER2KO mice is lower than those of WT mice. Ca²⁺ fluorescence induced by methamphetamine (10 μM) and PMMA(10 μM) in PER2 knockdown (PER2KD) PC12 cells was increased in comparison with naive PC12 cells. These results showed that amphetamine-like drugs induced reward behavior is associated with cytosolic Ca²⁺ levels regulated by PER2.

Disclosures: H. Eunchong: None. K. Aeseul: None. K. Haechan: None. Z. Yong-Qing: None. Y. Jaesuk: None.

Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA046794

Title: Effects of SSRI vilazodone on methylphenidate-induced gene regulation in the rat striatum: Role of 5-HT_{1A} receptor

Authors: M. HRABAK, C. MOON, *H. STEINER;
ROSALIND FRANKLIN UNIV OF MED AND SCIENCE, NORTH CHICAGO, IL

Abstract: The psychostimulant methylphenidate (MP, Ritalin) is used in the treatment of attention-deficit hyperactivity disorder (ADHD) and as a cognitive enhancer in the healthy. MP, like cocaine, acts by blocking the reuptake of dopamine. However, unlike cocaine, MP does not affect serotonin. Serotonin (5-HT) contributes to addiction-related gene regulation by cocaine. Consistent with this effect, our previous findings showed that enhancing serotonin action by adding a selective serotonin reuptake inhibitor (SSRI), fluoxetine (FLX) or citalopram, to MP indeed potentiates MP-induced gene regulation in the striatum and subsequent cocaine self-administration in rats. These SSRIs may thus increase MP's addiction liability. In the present study, we assessed the effects of a novel SSRI, vilazodone (VIL), on MP-induced gene

regulation and behavior. VIL differs from other SSRIs such as FLX in that, in addition to producing 5-HT reuptake inhibition, it also has 5-HT_{1A} partial agonist properties. Studies showed that 5-HT_{1A} receptor activation tempers serotonin input to the striatum. We compared striatal gene regulation (zif268, substance P) induced by MP+VIL vs. MP+FLX, by in situ hybridization histochemistry. Our results show that acute treatment with VIL (3-20 mg/kg) together with MP (5 mg/kg) increased MP-induced locomotion. However, in contrast to FLX, VIL did not potentiate MP-induced gene regulation in the striatum. Further studies showed that blocking 5-HT_{1A} receptors by the selective 5-HT_{1A} receptor antagonist WAY100635 (0.5-3 mg/kg) given 15 min before MP+VIL treatment did produce greater gene induction compared to MP+VIL treatment alone, confirming an inhibitory role for 5-HT_{1A} receptors. MP+SSRI concomitant therapies are indicated in ADHD/depression comorbidity and other disorders, and co-exposure also occurs with MP misuse as a cognitive enhancer by patients on SSRIs. Our findings indicate that, in contrast to MP+FLX combinations, MP+VIL combinations do not affect addiction-related gene regulation. VIL may thus serve as an alternative SSRI with diminished addiction facilitating properties.

Disclosures: M. Hrabak: None. C. Moon: None. H. Steiner: None.

Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

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Program #/Poster #: 485.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Intramural Program

Title: Footshock-induced decreases in hippocampal CRF mRNA expression are reversed by methamphetamine self-administration.

Authors: *J. L. CADET, B. LADENHEIM, M. T. MCCOY, S. JAYANTHI;
Natl. Inst. On Drug Abuse/ NIH, Natl. Inst. On Drug Abuse/ NIH, Baltimore, MD

Abstract: Methamphetamine use disorder (MUD) is an illicit substance used worldwide. Our laboratory has been investigating the basic mechanisms involved in punishment induced cessation of and compulsive drug taking behaviors in the rat. Towards that end, we employ a preclinical model in which rats are trained to self-administer methamphetamine for several days before lever presses for methamphetamine are punished by mild footshocks. We have also controlled for the effects of footshocks by using saline rats that are connected to methamphetamine rats to receive shocks at the same time that the methamphetamine rats get a shock. We have used this model to measure the expression of CRF and its receptors in the hippocampus because of its involvement in responses to stressful events. Rats that self-administered methamphetamine increased their intake over the first few days of exposure to the self-administration box. Punishment with footshocks separated these rats into two groups,

namely rats that continued to press the lever for methamphetamine (resistant) and those that decreased their methamphetamine intake (sensitive) as the shock intensity increased. Quantitative PCR revealed that saline rats yoked to resistant rats showed marked decreases in the expression of CRF, CRFR1, and CRFR2 mRNAs. Rats yoked to sensitive rats showed less decreases in the expression of these mRNAs. Resistant rats showed reversal of the effects of shock alone while sensitive rats showed significant increases in the expression of these mRNAs in comparison to all other groups. These findings suggest that chronic stress via footshock punishment is associated with perturbation in the CRF stress system in the hippocampus. Data on the epigenetic bases of these changes will be discussed.

Disclosures: **J.L. Cadet:** None. **B. Ladenheim:** None. **M.T. McCoy:** None. **S. Jayanthi:** None.

Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

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Topic: G.09. Drugs of Abuse and Addiction

Support: Cornerstone Award- Histochemical Society
NIDA Grant DA035435

Title: Inefficacy of N-Acetylcysteine in Mitigating Cue-Induced Relapse to Amphetamine

Authors: ***T. D. FORT**, D. A. LAUX, M. E. CAIN;
Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: Relapse poses a significant barrier to the development of effective treatments for substance use disorder (SUD). Glutamatergic imbalances are characteristic of SUDs. Glutamate is maintained by a combination of astrocytic and neuronal transporters within the nucleus accumbens (ACb) and medial prefrontal cortex (mPFC) and disruptions in this homeostasis engender SUD. One transporter, the cysteine-glutamate transporter (xCT), is primarily localized on astrocytes and helps maintain glutamate concentrations. This process is disrupted by cocaine use, and the therapeutic *N*-acetylcysteine (NAC) lowers cue-induced relapse to cocaine by restoring xCT function. However, little research has shown how these effects extend to other psychostimulants, such as amphetamine (AMP). In the present study, we assessed the disruption of astrocytes and xCT expression following AMP-induced cue seeking and the degree to which NAC can attenuate relapse via changes to astrocyte and xCT expression.

78 male Sprague-Dawley rats were surgically implanted with a jugular catheter to allow for intravenous self-administration of AMP (0.1 mg/kg/infusion) or saline during 2-hour sessions. After 14 SA sessions, all rats entered a forced abstinence period where they were not exposed to the drug or drug-related cues. During abstinence, rats received daily injections of NAC (100 mg/mL/ip) or the saline vehicle. Cue-induced relapse tests were conducted at the cessation of the

abstinence period. One subset of rats underwent relapse testing after one withdrawal day (WD) 1 and one subset underwent testing on WD 14. Following testing, all rats were perfused and brains were extracted for immunofluorescence (IF) analysis of astrocyte and xCT transporter expression within regions of the mPFC and ACb using simultaneous immunofluorescence (IF).

During the cue-induced relapse test, cue-induced responding was significantly higher in AMP-treated rats compared to SAL-treated rats. Importantly, NAC failed to attenuate relapse at either WD 1 or WD 14. Results from IF analyses indicated that rats exposed to AMP during SA increased astrocyte expression in the mPFC and ACb when compared to rats exposed to SAL. Repeated injection with NAC decreased xCT expression within regions of the mPFC and ACb only in animals with AMP exposure. Overall, our results suggest that NAC may be an ineffective treatment option for lowering cue-induced relapse to AMP. Further, the results suggest that stimulating xCT via NAC may not be an effective therapeutic approach for decreasing cue-seeking for AMP.

Disclosures: T.D. Fort: None. D.A. Laux: None. M.E. Cain: None.

Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

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Topic: G.09. Drugs of Abuse and Addiction

Support: Wayne State University Bridge Grant
Yale/NIDA Proteomics Center Pilot Research Project Grant

Title: Energy Metabolism-Parkin Link in Methamphetamine Use Disorder

Authors: A. SHARMA¹, B. L. SCHNEIDER², T. T. LAM³, R. GARCIA MILIAN³, *A. MOSZCZYNSKA¹;

¹Wayne State Univ., Detroit, MI; ²Swiss Federal Inst. of Technol., Geneve, Switzerland; ³Yale Univ., New Haven, CT

Abstract: Despite numerous preclinical and clinical research efforts, there is no FDA-approved medication for methamphetamine (METH) use disorder. New drug targets are needed to develop new medications for this disorder, especially for people who use METH heavily and, therefore, are at high risk for an overdose. We previously determined that protein parkin was a novel potential drug target for decreasing heavy use of METH. The goal of the present study was to elucidate molecular mechanisms underlying parkin-mediated regulation of METH taking and to assess whether parkin downregulates METH seeking during a relapse. We found that parkin deficit increased METH seeking whereas parkin overexpression decreased METH seeking during the drug-primed relapse. Comparison of proteomes from the ventral and dorsal striatum of parkin knockout (*Parkin*^{-/-}) and parkin-overexpressing young adult male Long Evans rats revealed that the biological process changed in opposite directions in these groups was aerobic respiration.

Krebs cycle function was altered in the ventral striatum whereas oxidative phosphorylation was altered in the dorsal striatum by parkin deficit or excess. The proteomic data suggests that changes in mitochondrial energy metabolism in striatal subregions underlie parkin-mediated regulation of METH taking and seeking during METH-induced relapse.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: DA042110
NIDA Intramural Research Program

Title: Reduced methamphetamine self-administration following single or dual hypocretin-receptor blockade or viral vector hypocretin-knockdown in adult male rats

Authors: *T. A. ZARIN¹, A. J. BRANDNER², G. F. KOOB², B. E. SCHMEICHEL^{1,2};
¹Biomed. Sci., East Tennessee State Univ., Johnson City, TN; ²Integrative Neurosci. Branch, Neurobio. of Addiction Section, Natl. Inst. on Drug Abuse, Intramural Res. Program, Baltimore, MD

Abstract: The hypocretin/orexin (HCRT) system is associated with compulsive stimulant drug use, involving both HCRT-receptor 1 (-R1) and HCRT-receptor 2 (-R2). Few studies, however, have examined the role of HCRT-R2 or combined HCRT-R1/2 on compulsive methamphetamine (METH) taking behavior. In this study, we examined the effects of HCRT-R1, -R2, and -R1/2 antagonists on compulsive METH self-administration, as modeled by escalated intake in adult male Wistar rats allowed extended access to METH. Three cohorts of rats were allowed either short (1h; ShA; n=7-10/cohort) or long (6h; LgA; n=7-9/cohort) access to METH intravenous self-administration for 14 sessions (fixed ratio 1 schedule). Each cohort was then systemically administered a single- or dual-HCRT-R antagonist 30 min prior to METH self-administration testing: cohort 1, selective HCRT-R1 antagonist (RTIOX-276; RTI-R1; 0, 10, and 20 mg/kg); cohort 2, selective HCRT-R2 antagonist (JNJ-10397049; JNJ-R2; 0, 10, and 20 mg/kg); and cohort 3, dual HCRT-R1/2 antagonist (Suvorexant; SUV-R1/2; 0, 30, and 60 mg/kg). RTI-R1 elicited a dose-dependent reduction in METH intake in LgA, but not ShA, in the first hour. Administration of JNJ-R2 had no effect on METH intake in the first hour in neither ShA nor LgA rats, but reduced METH intake during the full 6 h session at the lowest dose. SUV-R1/2 administration had no effect on METH intake in ShA rats, but showed significant attenuation of METH-taking at the highest dose in both the first hour and full 6h session for LgA rats. Locomotor activity was significantly reduced following RTI-R1 and SUV-R1/2 in ShA rats

only. To further explore the role that HCRT plays in METH dependence after a period of abstinence, we used a shRNA-encoding adeno-associated viral vector (AAV) to silence Hcrt in a separate cohort of previously-escalated METH-dependent rats. Following an initial escalation phase, and prior to a 3-week period of drug abstinence, rats were injected with either a control scramble-RNA AAV (AAV-Scram; n= 4) or a Hcrt-knockdown AAV (AAV-HCRT-KD; n= 5). AAV-Scram rats showed a significant decrease in METH self-administration post-abstinence, and a subsequent increase in METH-taking following a re-escalation period. In contrast, AAV-HCRT-KD rats showed a significant attenuation of METH self-administration following the re-escalation period. Combined, these results suggest HCRT neurotransmission at both HCRT-R1 and -R2 may contribute to compulsive METH-taking behavior.

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Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 1R01DA043172

Title: Methamphetamine-induced neuroinflammation in the prefrontal cortex corresponds with impairment of cognitive flexibility: effects of an anti-inflammatory medication

Authors: *A. M. ACUNA, E. K. NAGY, P. F. OVERBY, S. BICKLEY, L. FRANCIS, E. PEACOCK, C. SORENSON, C. SWAINE, M. F. OLIVE;
Arizona State Univ., Arizona State Univ., Tempe, AZ

Abstract: Methamphetamine (METH) overdose deaths continue to rise and about 60% of those seeking treatment for METH use disorder (MUD) relapse within one year of attempting to abstain. Recent work has revealed that neuroinflammatory responses to METH include increases in immune signaling, glial activation, neurotoxicity, and synaptic remodeling. Additionally, neuroinflammation may cause changes in cognition, which could convey a vulnerability to addictive behaviors and relapse in patients with MUD. The current study sought to investigate the effects of METH-induced neuroinflammation on cognitive flexibility following abstinence. Rats were given binge-like access to METH (96 consecutive hrs/week for 3 weeks) via intravenous self-administration (IVSA) at 0.05 mg/kg/infusion. Control animals were allowed to self-administer saline under the same conditions. Following 3 weeks of abstinence, neuroinflammatory markers in the prefrontal cortex (PFC) were assessed via multiplex ELISA. In separate animals, both prior to and after drug access, cognitive flexibility was assessed using a PFC-dependent task, the Attentional Set Shifting Task (ASST), in which rats retrieved a food reward by digging in bowls using odor or digging media as discrimination dimensions. During

testing, the dimension that predicted the reward was shifted and the number of trials needed to meet criterion (6 consecutive correct digging choices) was quantified. The effects of the non-steroidal anti-inflammatory drug, parecoxib, on post-drug ASST performance was also observed. We found that rats who self-administered METH showed an elevation in IL-6, IL-18, CX3CL1 (Fractalkine), INF-gamma, CCL2, and leptin in the PFC compared to saline controls (n=4-6 / group). Following IVSA, rats who self-administered METH followed by treatment with saline (n=12) required more trials to reach criterion in the extradimensional shift part of the ASST than they did prior to METH, whereas saline-administering controls treated with saline (n=13) required fewer trials to meet criterion after IVSA. However, rats treated with parecoxib (n=13) in the last week of abstinence following METH IVSA showed no difference in set shifting between pre- and post-METH assessments. These results show that neuroimmune activation in the PFC persists several weeks into abstinence from METH and that PFC-mediated cognitive flexibility is impaired at the same timepoint. Further, it appears that anti-inflammatory agents attenuate METH-induced cognitive impairments. These results reinforce the argument that pharmacologically targeting the neuroimmune response to METH might facilitate recovery in treatment of MUD.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Role of brain stem projections to anterior intralaminar nucleus of thalamus in incubation of methamphetamine craving

Authors: *H. LIN¹, A. OLANIRAN², X. LI³;

¹Univ. of Maryland, Univ. of Maryland, College Park, MD; ²Univ. of Maryland, Col. Park Neurosci. and Cognitive Sci. Program, Univ. of Maryland, Col. Park Neurosci. and Cognitive Sci. Program, lanham, MD; ³Univ. of Maryland Col. Park, Natl. Inst. of Drug Abuse, College Park, MD

Abstract: Relapse is a major obstacle to addiction treatment across various drugs of abuse, including methamphetamine (Meth). Meth craving progressively increases during abstinence and maintains for an extended period of time in both human and rodents, a phenomenon named incubation of Meth craving. We previously found that the projections from the lateral portion of anterior intralaminar nucleus of thalamus (AIT-L) to dorsomedial striatum (DMS) play a causal role in this incubation. Here, we aimed to identify critical upstream regions of AIT-L in

incubation of Meth craving by focusing on brainstem projections. In Exp.1, we first trained male Sprague-Dawley rats to self-administer Meth (0.1 mg/kg/infusion, 6 h/d) for 10 days. Next, we either tested (Seeking-test) or did not test (No-test) rats for Meth seeking on abstinence day 28. Immediately after the test, we perfused the rats for immunohistochemistry to label Fos (a neural activity marker) in four candidate brain stem nuclei, including pedunclopontine nucleus (PPN), dorsal raphe (DR), laterodorsal tegmental area (LDT), and locus coeruleus (LC). We found that Fos expression in PPN, DR, LDT, but not LC, significantly increased in the Seeking-test group compared with No-test group on abstinence day 28. In Exp.2, we combined the anterograde transsynaptic adeno-associated virus (AAV) expressing Cre (AAV1-cre, injected into brain stem nuclei) and the retrograde AAV expressing Cre-dependent tdTomato (retroAAV-FLEX-tdTomato, injected into the ipsilateral DMS) to examine if PPN, DR and/or LDT provide monosynaptic inputs to AIT-L \rightarrow DMS projection neurons. We observed tdTomato-expressing neurons in AIT-L of rats with AAV1-Cre injected into of PPN and DR, but not LDT. Taken together, our results showed that neuronal activation of PPN, DR and LDT were associated with incubated Meth seeking, and both PPN and DR provided monosynaptic inputs to AIT-L \rightarrow DMS projections. Ongoing studies are focusing on using chemogenetics to examine the causal roles of PPN, DR, and their projections to AIT-L in incubation of Meth craving.

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Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

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Topic: G.09. Drugs of Abuse and Addiction

Support: DA009621 to MEW

Title: Cell-type and pathway-specific synaptic adaptations in the rat nucleus accumbens core after incubation of methamphetamine craving

Authors: *E.-K. HWANG, A. L. MOUTIER, M. E. WOLF;
Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Use of methamphetamine (meth) is highly prevalent and the high rate of relapse even after prolonged abstinence is a major problem in treating meth use disorder. In a rat model of drug craving and relapse, cue-induced drug seeking progressively intensifies after withdrawal from drug self-administration (SA) (incubation of craving), which is associated with long-term synaptic adaptations of glutamatergic transmission in nucleus accumbens core (NAcc) medium spiny neurons (MSN). Our lab previously demonstrated that incubation of meth craving is associated with synaptic calcium-permeable AMPA receptor (CP-AMPA) upregulation in the NAcc and that CP-AMPA activation is required for expression of incubated meth craving (Scheyer et al., 2016). However, it is not known whether meth incubation is associated with cell-

type specific upregulation of CP-AMPA in MSN that express either the dopamine D1 or D2 receptor and which specific glutamatergic inputs are involved in CP-AMPA-mediated synaptic alterations in NAcc. Here, we examined CP-AMPA plasticity after meth incubation in a cell-type and pathway-specific manner in the NAcc. Male and female rats self-administered saline or meth under extended access conditions (6 h/day for 10 days; 0.1 mg/kg/infusion; infusions paired with light cue). We measured cue-induced meth seeking after 1 and 21 days of abstinence. On withdrawal day (WD) 25-50, whole-cell patch clamp recordings were performed to characterize AMPA-mediated synaptic transmission by measuring the rectification index and NASPM (CP-AMPA selective antagonist) sensitivity. To enable identification of MSN subtypes, we used D1-Cre or A2a-Cre rats (the A2aR colocalizes with the D2R) crossed with ZsGreen or TdTomato reporter rats. During seeking tests, significantly higher cue-induced seeking was observed on WD21 than on WD1. We found upregulation of CP-AMPA in D1+ but not D1- or A2a+ MSN after withdrawal from meth SA compared to saline controls. Furthermore, combining electrophysiology and optogenetics, we examined input-specific plasticity in the NAcc synapses projecting from the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), and paraventricular thalamus (PVT). We found potentiated glutamatergic synaptic transmission through upregulation of CP-AMPA in mPFC and PVT to NAcc pathways. There was considerable variability among cells for the BLA to NAcc pathway, so additional studies are in progress. This study is the first to demonstrate cell-type and pathway-specific plasticity of excitatory synaptic transmission in the NAc after incubation of meth craving.

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Poster

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Title: Optogenetic photoinhibition of the anterior insula and its projection to amygdala nuclei reduces abstinence-exacerbated expression of conditioned place preference

Authors: *A. AGOITIA, I. BALDERAS, F. BERMÚDEZ-RATTONI;
Inst. de Fisiología Celular, Univ. Nacional Autónoma de México, Ciudad de México, Mexico

Abstract: Substance use disorders are defined by a chronic and relapsing pattern of drug intake that persists despite experiencing adverse consequences. It has been established that re-exposure to drug-related cues may trigger drug-seeking behaviors on an abstinence-dependent magnitude,

an associative phenomenon termed incubation of drug craving (Grimm et al., 2001). The exacerbated expression of drug-seeking during extended abstinence periods may reflect an intensified motivational state (craving) for which the neurobiological basis is not yet fully understood. However, there is evidence for the critical involvement of several brain regions, such as the central amygdala and the nucleus accumbens. Clinical and preclinical studies indicate that the insular cortex has a crucial role in expressing drug-seeking responses. However, in most cases, the effect of abstinence on such behaviors has not been adequately accounted for. In the present study, we assessed the role of the anterior insular cortex (aIC) and its excitatory projection to amygdala nuclei on the expression of amphetamine-induced conditioned place preference (CPP) of mice subjected to brief or moderated periods of abstinence. First, we generated a CPP procedure on which we observed mice to spend a significantly greater amount of time on the amphetamine-paired chamber after 14 days of abstinence compared to just one day. Photoinhibition of either aIC or aIC→AMY projection reduced the abstinence-exacerbated CPP expression after 14 days of abstinence, but did not after one day. Furthermore, aIC NMDA receptors antagonism reduced CPP expression after 14 days of abstinence, but not after one day. Overall, these results suggest that aIC and its anatomical projection pathway to amygdala nuclei are critical neurobiological substrates related to the motivational process that occur during the expression of the incubation of drug craving. Additionally, glutamatergic signaling mediated by aIC NMDA receptors is necessary for the abstinence-exacerbated expression of conditioned place preference.

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Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Sex-specific alterations in DNMTs and TETS following methamphetamine self-administration in the rat hippocampus and prefrontal cortex

Authors: *M. T. MCCOY, A. P. DIAWILE, S. JAYANTHI, B. LADENHEIM, J. CADET; DHHS/NIH/NIDA/IRP, DHHS/NIH/NIDA/IRP, Baltimore, MD

Abstract: Methamphetamine use disorder (MUD) is a neuropsychiatric disorder that imposes significant health liabilities throughout the world. Significant sex differences in use and relapse rates have been found among human METH users. Sex differences in behaviors and biochemical changes have also been observed in preclinical models of MUD. Yet, the epigenetic bases of sex-dependent METH-induced molecular differences identified in male and female rats remain to be elucidated.

Herein we have used a METH self-administration model in an attempt to identify potential molecular substrates of behavioral differences between female and male rats. Rats were trained to self-administer METH (0.1 mg/kg/injection, i.v.) using two 3-hours daily sessions (separated by a 30-minute off interval) for 20 days. Rats were then assessed for cue-induced drug seeking at 3 and 30 withdrawal days (WD). Rats were euthanized 24 hours after WD30. Hippocampi and prefrontal cortex (PFC) were dissected and interrogated for the mRNA expression of DNA methyltransferases (DNMTs) and ten-eleven translocation (TETs). We observed that both female and male rats increased their METH intake during the 4 weeks of the experiment, with some rats (High Takers), but not others (Low Takers), escalating their intake of the drug. Both male and female rats showed incubation of METH seeking during withdrawal.

Quantitative PCR revealed that female control rats had higher basal RNA levels of Dnmt1, Dnmt3B, Tet1, and Tet3 in their PFC in comparison to control male rats. All male METH SA rats showed higher Dnmt1 expression than their controls in the PFC, with females showing no such effects.

These results identify additional evidence for sexual dimorphic responses to METH. These observations support the notion that sex-specific therapeutic approaches to the treatment of METH use disorder may be necessary.

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Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 485.13

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute on Drug Abuse (NIDA), NIH, and DHHS (grant #-DA000552 (2021))

Title: Sex- and brain region dependent alterations in enzymes involved in dopamine synthesis and its breakdown consequent to methamphetamine self-administration and abstinence

Authors: *A. P. DAIWILE¹, A. MILLER², J. L. CADET³;

¹NIH-NIDA, ²NIH, NIDA IRP, Baltimore, MD; ³Natl. Inst. On Drug Abuse/ NIH, Natl. Inst. On Drug Abuse/ NIH, Baltimore, MD

Abstract: Sex differences have been reported in methamphetamine (METH) use disorder. These include differences in METH intake, relapses, and adverse psychiatric outcomes in both clinical and preclinical studies. Therefore, it is important to identify the neurobiological bases for these sex-dependent phenomena. In the present study, we sought to determine if there were differential

expression in the mRNAs that encode dopamine metabolizing enzymes in rats that had undergone and withdrawn from METH self-administration (SA). We trained rats to self-administer METH for 20 days (6h/day) followed by a withdrawal period of 30 days. During the withdrawal period, rats were assessed for METH seeking behaviors on withdrawal days (WD) 3 and 30. Tissue samples from different brain regions were collected within 24h of the test on WD 30. Male rats had higher total METH infusions compared than females. Both male and female METH rats showed increased METH seeking behaviors on WD30 when compared to WD3. After we euthanized the animals, we measured mRNA expression of enzymes involved in dopamine synthesis and its breakdown in the prefrontal cortex (PFC), nucleus accumbens (NAc), dorsal striatum (dSTR), and hippocampus (HIP). Sex differences were observed in control rats, with males exhibited higher basal levels of *Th* in the PFC and dSTR, *Ddc* in the NAc, and *MaoB* in the HIP, whereas female control rats showed higher basal levels of *Comt* in the HIP. In addition, female METH rats displayed increased expression of *Ddc* and *MaoB* while male METH rats showed higher levels of *Comt* mRNA in the PFC compared to their respective controls. In the NAc, male METH rats showed decreased *Th* and *Ddc* mRNA levels. In contrast, female METH rats showed reduction in *Aldh1a1* mRNA levels in the dSTR and HIP. Together, our results show sex and brain region dependent alterations in the mRNA expression of enzymes involved in dopamine metabolism after METH SA and abstinence, with prominent changes in the mesocorticolimbic dopaminergic system. Our observations provide support for including sex as an important variable when evaluating therapeutic interventions against METH use disorder. **Acknowledgement:** This work is supported Department of Health and Human Services/NIH/NIDA/IRP.

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Poster

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Location: SDCC Halls B-H

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Program #/Poster #: 485.14

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex differences in the ability of exercise to attenuate methamphetamine-induced monoaminergic neurotoxicity

Authors: J. M. PELLETTIER, A. A. FINEBERG, I. C. DE LEON, K. A. DE LEON, L. J. HERRERA, E. SAURINI, Y. K. VILLAGOMEZ, *A. N. FRICKS-GLEASON;
Dept. of Psychology & Neurosci., Regis Univ., Denver, CO

Abstract: Methamphetamine (METH) use continues to be a major public health concern. Upwards of 14.7 million people in the U.S. report having tried METH. The use of METH is highly problematic, not only due to the acute effects of the drug which can include psychosis and aggressive behavior, but also due to the long-term consequences including neurotoxicity, cognitive deficits, and addiction. METH-induced monoaminergic neurotoxicity has been

modeled in numerous species. One well-known rodent model of METH use utilizes binge administration, where repeated doses of METH are given in a single day. This dosing regimen has been shown to cause long-lasting damage to monoaminergic nerve terminals in the striatum and prefrontal cortex similar to that seen in human METH users. In fact, individuals who use METH are more likely to develop Parkinson's disease, suggesting enduring and possibly progressive dopamine loss as a consequence of METH use. Exercise is well known for its beneficial physiological effects and cognition-enhancing properties and has long been investigated in the context of neurodegenerative disease; only recently has exercise gained traction in the treatment of drug use and addiction. Here we demonstrate a critical sex difference in the ability of exercise to attenuate METH-induced neurotoxicity. Previously, we've shown that 3 weeks of voluntary running after a METH binge protects against METH-induced dopaminergic neurotoxicity. Critically, delaying the start of exercise for 7 or 30 days also results in attenuated neurotoxicity, suggesting that post-METH exercise isn't simply disrupting the mechanisms that lead to neurotoxicity, but is reversing the neurotoxic effects post-hoc. While METH-induced neurotoxicity has been modeled in many species, these studies have largely excluded female subjects. The goal of this project was to replicate our previous work in females. Female Sprague Dawley rats were dosed with the same neurotoxic regimen of (+)-METH-HCl (4 x 4 mg/kg, s.c. at 2-hr intervals) or saline (4 x 1 ml/kg, s.c. at 2-hr intervals) used in preclinical studies of male rats. Beginning 1, 7, or 30 days after injections, animals were then subdivided into one of two exercise conditions, voluntary exercise (rats were given continuous access to a running wheel for 3 weeks) or sedentary control (rats were housed with a locked wheel for the same duration). Surprisingly, contrary to what was seen in the males, exercise was found to have no impact on METH-induced neurotoxicity in females. The lack of exercise effect in this female cohort highlights the necessity of including sex as a biological variable in methamphetamine neurotoxicity studies going forward.

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Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 485.15

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex difference in acute withdrawal following low doses of psychostimulant-opioid combination (speedball)

Authors: *I. WHITE¹, W. WHITE²;
¹Psychology, ²Morehead State Univ., Morehead, KY

Abstract: Previously, we reported that an indicator of acute withdrawal was elicited when male rats were given low doses of psychostimulant and opioid together, but not separately, and that acute withdrawal was blocked by D1 antagonist (SfN abstract, 2021). Though such “speedballs” (psychostimulant and opioid combinations) may enhance desired experiences immediately after administration, they may also increase the intensity and duration of acute withdrawal, posing significant health risks. The purpose of the research was to compare the responses of female and male rats to low psychostimulant-opioid combinations, and to assess the dependence of effects obtained on dopamine D1 and mu opioid receptor activation. Female and male Wistar rats received a series of six-day tests during which locomotor activity was monitored in an open-field for 24 hours after each treatment. Near light onset of Test Day 1, each animal received saline. In subsequent tests, on Test Day 4, each animal received amphetamine (0.5 mg/kg), morphine (1.25 mg/kg), or a combination of the two. Acute withdrawal was defined as reduced activity 13 to 24 hours post-drug, relative to saline control. Compared to males, females showed more locomotor activation to amphetamine alone, to morphine alone, and to the combination during the first several hours following administration. Females did not show acute withdrawal following amphetamine or morphine alone, but they did show acute withdrawal following the combination. Males did not show acute withdrawal following any of the drug treatments. Acute withdrawal was attenuated by D1 antagonist or mu antagonist given 15 minutes after the psychostimulant-opioid combination. Our data suggest that female rats are more acutely reactive to low doses of amphetamine or morphine and to their combination, and that females show evidence of acute withdrawal following lower-dose combinations. These sex differences suggest that psychostimulant-opioid combinations may pose greater health risks for females. The present findings demonstrate that the methods we have used to assess drug-induced changes in 24-hour activity patterns, including acute withdrawal, can be validly used with both female and male rats.

Disclosures: I. White: None. W. White: None.

Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

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Topic: G.09. Drugs of Abuse and Addiction

Support: Psychology Department
College of Science

Title: Contribution of mu receptor activation to acute withdrawal following administration of psychostimulant-opioid combination in rats

Authors: *W. WHITE, I. M. WHITE;
Morehead State Univ., Morehead, KY

Abstract: Short-term effects of combined psychostimulant and opioid administration have been studied. However, acute withdrawal produced by such combinations has not been studied systematically. Previously, we reported that when male rats were given low doses of psychostimulant and opioid together, but not separately, an indicator of acute withdrawal--reduced locomotor activity 13 to 24 hours post-drug administration--was observed, and that the withdrawal was blocked by D1 receptor antagonist. The purpose of the current research was to replicate the previous research using an alternative sequence of tests, and to assess the involvement of mu receptor in acute withdrawal from psychostimulant-opioid combination. Twelve male adult Wistar rats received a series of six-day tests during which locomotor activity was monitored in an open-field for 24 hours after each treatment. Near light onset of Test Day 1, animals were treated with saline. On Test Day 4, each animal received a drug treatment. Effects produced by saline and drug treatments were compared. During early tests, 0.5 mg/kg amphetamine and 1.25 mg/kg morphine were given alone and in combination, with the intention to adjust doses upward to identify a combination that produced acute withdrawal: Previous research used descending doses across tests, from higher to lower. During later tests, receptor antagonist was given 15 minutes after the combination, to see if acute withdrawal could be attenuated by receptor blockade. D1 antagonist completely blocked acute withdrawal, whereas mu antagonist produced partial blockade. Activation of mu and D1 receptors both contribute to the intensity of withdrawal following psychostimulant-opioid combination. The differential effects of receptor blockade are consistent with the view that morphine contributes to withdrawal by augmenting D1 receptor activation via activation of mu receptors.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIGMS Grant P20GM103423

Title: Mother's little helper: Choline levels in the maternal diet alter the locomotor response to amphetamine in adult offspring in a sex-dependent manner

Authors: L. AUGUSTIN, E. DONIGAN, M. MACOMBER, S. XUE, C. EVANGELISTA, *M. J. GLENN;

Psychology, Colby Col., Waterville, ME

Abstract: Choline is an essential dietary nutrient vital to the development and functioning of the central nervous system. Among its many biological roles, it is an integral component of cell membranes, a primary source of methyl groups for methylation reactions, and the precursor to acetylcholine. The availability of choline during developmental sensitive periods exerts profound

and persistent impacts on neural systems and behavior. Findings from rat studies show that dietary choline levels during prenatal and adolescent developmental periods affect cell signaling, metabolic pathways, and neural plasticity, mainly in hippocampal-cortical regions, with accompanying changes to cognition and emotion. In general, supplemental choline enhances functioning and confers neuroprotection; choline deficiency increases vulnerabilities to neural insults and behavioral challenges. We previously sought to extend these findings to the reward system with a focus on the response of rats to stimulant drugs that are primarily dopamine agonists. We found more locomotion to a low dose of amphetamine with choline supplementation in adolescence, but only in female rats. Here, we shifted our focus to the prenatal period; a wealth of data point to a heightened sensitivity to choline availability in the prenatal period. We hypothesized that prenatal choline supplementation would affect both females and males and that prenatal choline deficiency may have effects, despite being minimal in our previous study. To test this, Sprague Dawley dams were fed diets containing either 0, 1.1, or 5 mg/kg of choline chloride (DEF, STD, and SUP) during the second half of pregnancy. Female and male pups were reared to adulthood and locomotor responses to each of 3 doses of amphetamine (0, 0.5, and 1 mg/kg) was assessed using a modified Latin square design with rats receiving 1 dose per week for 3 weeks. Rats were given amphetamine and placed in a 50-cm diameter circular arena for 90 min to track activity. The findings were that, overall, female rats had significantly higher locomotor responses to amphetamine than males. Female SUP rats, unlike female DEF and STD rats, exhibited similar and high levels of locomotion when given 0.5 and 1 mg/kg of amphetamine. In males, there were no significant differences between the locomotor responses to 0.5 or 1 mg/kg doses in any choline condition but a new finding was that male SUP rats had higher overall locomotion in response to amphetamine than DEF and STD males. Taken together, these findings add to our existing work in pointing to marked differences in the sensitivity of neural systems that respond to dopamine agonists with biological sex and early life choline availability.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Methamphetamine self-administration in mice alter mitochondrial respiration and decreases dopamine neuron firing activity

Authors: *S. DOMINGUEZ-LOPEZ¹, B. AHN², K. SATARANATARAJAN², R. HOWELL¹, H. VAN REMMEN², M. J. BECKSTEAD², M. LOBO¹;

¹Anat. & Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; ²Aging and metabolism, Oklahoma Med. Res. Fndn., Oklahoma City, OK

Abstract: The effects of methamphetamine (METH) induced oxidative stress on mitochondrial respiratory metabolism along the mesolimbic pathway have not been systematically evaluated in the context of self-administration models of drug use. Here we implanted adult mice with a jugular catheter and trained them to nose poke for METH infusions (0.1 mg/kg/infusion). After training, mice were placed in a fixed ratio 3 (FR3) schedule to complete 20-40 days of total METH self-administration exposure. Tissue from the ventral striatum (vSTR) and the ventral midbrain (vMB) was collected at the end of METH self-administration exposure. We used HPLC to determine that in naïve mice, the levels of the reactive oxygen species (ROS) scavenger reduced glutathione (GSH) were higher in vSTR than in vMB samples. In addition, the levels of GSH in vSTR decreased with an inverse correlation to METH total intake. No significant changes were detected in oxidized glutathione (GSSG) levels in either brain region of mice with or without METH self-administration. Also, the GSH/GSSG ratio was higher in vSTR compared with vMB samples. Using high-resolution respirometry, we observed that basal mitochondrial oxygen consumption rate (mOCR) in both brain regions was higher in samples from METH-exposed mice. Respiration in Complex I substrate was higher in vMB versus vSTR in METH-exposed mice. Maximum respiration under Complex IV substrates was increased in METH-exposed animals independently of the brain region analyzed. Using in vivo single-unit extracellular recordings, we observed decreased firing and burst activity of dopamine neurons recorded in mice's ventral tegmental area (VTA) with METH self-administration exposure. However, dopamine neuron population activity, assessed as the number of neurons spontaneously active recorded per track, was increased with METH self-administration. Decreased dopamine neuron firing and increased cell excitability was also observed in whole-cell recordings in VTA slices from METH self-administer mice. Our results suggest that METH self-administration produces a progressive and specific decrease in ROS scavenger capabilities of mesolimbic dopamine terminal regions. Mitochondrial respiration in the mesolimbic dopamine regions seems to be enhanced with methamphetamine self-administration. However, the vMB showed more functional changes in the mitochondrial electron transport complexes. This last observation could be the origin of dopamine firing deficiencies observed after methamphetamine self-administration.

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Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Determining the distinct acute effects of methamphetamine stereoisomers on limbic norepinephrine and dopamine transmission

Authors: *R. PAULY, R. BHIMANI, J. PARK;

Biotechnical and Clin. Lab. Sci., State Univ. of New York, Univ. at Buffalo, Buffalo, NY

Abstract: The psychostimulant methamphetamine (METH) exists as two enantiomers, dextrorotary (d) METH and levorotary (l) METH. In contrast to d-METH, a commonly abused psychostimulant, l-METH can be purchased over the counter to produce nasal vasoconstriction and relieve congestion as a therapeutic due to its reduced addictive potential. Currently, l-METH is highlighted in treating stimulant use disorder as a potential agonist replacement. However, little is known regarding l-METH's effects on central catecholamine (dopamine (DA) and norepinephrine (NE)) transmission and their related behaviors. Here, we demonstrate distinct dose dependent d-/l-METH effects on catecholamine transmission in two limbic structures important in substance abuse, the bed nucleus of the stria terminalis (BNST) and nucleus accumbens (NAc). In this study, we used fast-scan cyclic voltammetry coupled with carbon-fiber microelectrodes to elucidate how METH isomers impact NE and DA regulation (release and clearance) in the BNST and NAc respectively, of anesthetized rats. d-METH with accumulating doses (0.5, 2.0, 5.0 mg/kg IP) increased both electrically evoked NE and DA in limbic structures. Additionally, high dose (5.0mg/kg) d-METH enhanced both basal NE and DA concentrations. In contrast, l-METH with accumulating doses enhanced electrically evoked NE concentrations in the BNST with similar potency to d-METH while no significant effect on NAc DA was observed. Furthermore, under α -2 adrenergic auto receptor antagonism both d-/l- METH produce elevated NE basal level concentrations at intermediate (2.0 mg/kg) and high (5.0 mg/kg) doses with similar potency. The differential effects of methamphetamine stereoisomers on catecholamine basal concentrations in the absence of α -2 adrenergic auto receptor antagonism suggest different mechanisms of action between d-/l-METH. Due to l-METH's asymmetric regulation of NE relative to DA, l-METH may have serious implications in drug-seeking and addiction. Which may be a framework for future studies examining l-METH as a potential agonist replacement for substance use disorder.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: DA038058-08

Title: Syntaxin1 Ser14 Phosphorylation is Required for Non-Vesicular Dopamine Release

Authors: *S. J. MABRY¹, A. SHEKAR¹, M. H. CHENG³, D. ZANELLA¹, J. I. AGUILAR¹, S. PATEL¹, D. P. SALEEBY¹, A. CARTER¹, I. BAHAR⁴, H. J. MATTHIES², A. GALLI¹;
¹Univ. of Alabama Birmingham, ²Surgery, Univ. of Alabama Birmingham, Birmingham, AL;
⁴Univ. of Pittsburgh, ³Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Amphetamine (AMPH), a psychostimulant that is commonly prescribed for the treatment of neuropsychiatric and neurological disorders, has a high liability for abuse. The abuse and psychomotor stimulant properties of AMPH are primarily associated with its ability to increase dopamine (DA) neurotransmission. This increase is mediated, in large part, by non-vesicular DA release (DA efflux). DA efflux is the result of reversal of the DA transporter (DAT) promoted by AMPH. Syntaxin 1 (Stx1) is a SNARE protein that plays a pivotal role in vesicular release. Previously, we have shown that Stx1 also interacts with the distal DAT N-terminus, an event promoted by AMPH. Stx1 is phosphorylated at Ser14 by casein kinase II (CK2). Using *Drosophila Melanogaster* as an animal model, we show that this phosphorylation event is critical for non-vesicular DA release and regulates the expression of AMPH preference as well as the ability of AMPH to promote mating drive. We also show that reverse transport of DA mediated by DAT underlies these complex behaviors promoted by AMPH. Our molecular dynamics (MD) simulations of the phosphorylated DAT/Stx1 complex demonstrate that the phosphorylation state of these proteins plays a key role in allowing DAT to dwell in an efflux-willing state. This state also supports constitutive DA efflux (CDE), an event that occurs in the absence of AMPH. The DAT-Stx1 phosphorylated complex is characterized by the breakdown of two key salt bridges in DAT, K66-D345 and E428-R445, which are critical for the formation of the intracellular (IC) gate and for transport function. The breaking of these salt bridges leads to an opening and hydration of the DAT intracellular vestibule, allowing DA to bind from the cytosol, a mechanism that we hypothesized leads to CDE. We further determine the importance of Stx1 phosphorylation in CDE by pharmacologically inhibiting CK2 with CX-4945, a molecule currently in phase II clinical trials for cancer treatment. CX-4945 treatment prevented the expression of CDE in isolated *Drosophila Melanogaster* brains as well as behaviors associated with CDE. Thus, our results suggest that Stx1 phosphorylation is a possible pharmacological target for the treatment of AMPH abuse.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: 5R01DA035263-10

Title: A network of phosphatidylinositol-4,5-biphosphate binding sites on the dopamine transporter regulates AMPH-induced behavior in *Drosophila melanogaster*

Authors: *Y. ZHU¹, J. AGUILAR², S. J. MABRY³, H. J. MATTHIES¹, A. GALLI³;
¹Surgery, Univ. of Alabama at Birmingham, Birmingham, AL; ²Vandebilt Univ., Vandebilt Univ., Nashville, TN; ³Surgery, Univ. of Alabama Birmingham, Birmingham, AL

Abstract: Dopamine (DA) is a neurotransmitter that modulates various behaviors such as learning and motor coordination. The dopamine transporter (DAT) is responsible for the clearance of DA from the synaptic cleft back into presynaptic neuron, thus regulating DA signaling and homeostasis. Amphetamine (AMPH) is a psychostimulant commonly prescribed for neuropsychiatric disorders such as ADHD, but also has a high liability for abuse. AMPH enhances DA neurotransmission by causing a reverse transport of DA, a phenomenon referred to as DA efflux. However, the underlying mechanisms of how AMPH elicits DA efflux remains elusive. Previous results have shown that the AMPH-induced DA efflux depends on the phosphorylation of DAT N-terminus and is modulated by the membrane lipid phosphatidylinositol-4,5-biphosphate (PIP₂). In cells expressing the human dopamine transporter (hDAT), we characterized multiple PIP₂ binding sites in the intracellular loop 4 (IL4) of hDAT including H442, R443 and H444. We found that single charge neutralizing substitution with alanine at these 3 residues reduced AMPH-induced DA efflux, possibly due to an impairment of PIP₂ binding at these residues. Furthermore, using *Drosophila* that expresses the hDAT transgene R443A specifically in DA neurons, we found that in addition to hDAT N-terminus phosphorylation, the interaction between PIP₂ and hDAT R443 is also necessary for AMPH-induced efflux recorded in excised fly brains. Additionally, these flies demonstrated impaired behavioral response to AMPH, including locomotion, food preference and sensitization. Our results show that PIP₂ is an important regulator of hDAT function via its interactions with hDAT IL4, and that the PIP₂-hDAT IL4 interactions facilitate AMPH-induced behaviors in *Drosophila*, indicating hDAT IL4 as a significant functional domain participating in AMPH actions.

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Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

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Topic: G.09. Drugs of Abuse and Addiction

Support: R01MH101214

Title: Acute effects of methamphetamine on neuronal activity in the central amygdala

Authors: *T. YANG, A. BOUHUIS, B. LI;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The central amygdala (CeA) is known to have an important role in drug addiction. However, how CeA neurons respond to addictive drugs remains elusive. Here we show that two major CeA populations - the somatostatin-expressing (Sst⁺) neurons and the protein kinase C- δ -expressing (PKC- δ ⁺) neurons - have distinct activities in response to acute methamphetamine administration. *In vivo* single neuron imaging and tracking show that methamphetamine induces an increase in activity in the majority of Sst⁺ CeA neurons, but a decrease in activity in the majority of PKC- δ ⁺ neurons. Strikingly, we find that, at 24 hrs after drug injection, female mice show more sustained excitatory activity in Sst⁺ CeA neurons than male mice. In contrast, male mice show more sustained inhibitory activity in PKC- δ ⁺ CeA neurons than female mice. Overall, our results reveal novel neuronal activity patterns in the CeA in response to methamphetamine, which may help us to better understand the role of the CeA in drug addiction.

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Poster

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Title: Food deprivation modulates heart rate, motor neuron, and locomotion responses to acute administration of d-amphetamine in zebrafish larvae

Authors: *P. BANSAL¹, M. F. ROITMAN², E. E. JUNG¹;

¹Dept. of Mechanical Engin., ²Dept. of Psychology, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Clinical and preclinical studies suggest that physiological states, such as hunger and satiety, affect behavioral responses to abused drugs. Here we investigate the relationship between hunger and drug responses (neuronal and behavioral) in zebrafish larvae. To analyze neural activity and behavioral responses to the psychostimulant drugs, we have used 6 days post fertilization (dpf) Zebrafish (*Danio rerio*) larvae as an animal model and administered them D-amphetamine hemisulfate, a scheduled-II psychostimulant drug, to record direct physiological responses such as locomotion, heart rate and motor neuronal expression in the spinal cord of the larvae *in vivo* linked to locomotor behavior. We performed these experiments in fed and food-restricted larvae, and responses were recorded pre and post-amphetamine administration in drug-treated groups for 10 minutes in the same subjects. To assess the overall change, we also recorded the activities in control groups (without drug administration) for 20 mins, and percentage change in the first and next 10 mins was calculated for both groups. When percentage

changes for both groups were compared, we observed a significant increase in percentage difference by 77% in locomotion activity of food-restricted larvae (n=14 (control groups), n=13 (amphetamine treated groups)) in drug treated groups. Whereas the difference in fed group larvae (n=15 (control groups), n=14 (amphetamine treated groups)) increased only by 10% after amphetamine treatment. These differences imply the relative increment in locomotor responses post-amphetamine in the food-restricted state than in the fed state. A similar trend was observed in heart rate larvae (n=19 (control groups), n=12 (amphetamine treated groups)) and motor neuron activity larvae (n=7 (food-restricted groups), n=6 (fed groups)), where these activities were observed to be higher in the food-restricted state in drug treated groups. These findings can call for further research to investigate the relationship between drug addiction-related neural circuits and hunger mediating neurons.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA 4683256-65

Title: Expression of the glutamate transporter GLT-1 in dopaminergic axons in the medial shell and age-dependent consequences of its deletion on behavioral sensitization to amphetamine

Authors: *K. D. FISCHER¹, A. SHERPA², S. GRISWOLD³, C. AOKI², P. A. ROSENBERG³; ¹Pharmacol. & Toxicology, Indiana Univ. Sch. of Med., Indianapolis, IN; ²Neural Sci., New York Univ., New York, NY; ³Neurol., Boston Children's Hosp., Boston, MA

Abstract: A subpopulation of dopamine (DA) neurons use glutamate as a neurotransmitter, and a primary target of these neurons is the medial shell of the accumbens. We investigated whether dopaminergic axons in this region express the glutamate transporter GLT-1, and the effect of conditionally knocking out GLT-1 in DA neurons in response to amphetamine (AMP) at different ages up to 22 months. Double label EM immunocytochemistry (EM-ICC) for tyrosine hydroxylase (TH) and GLT-1 were used to detect expression of GLT-1 in dopaminergic axons in the medial shell. The GLT-1 gene was inactivated in dopaminergic neurons using a conditional GLT-1 knockout (GLT-1^{flox/flox}) and DAT-IRES-Cre. Locomotor sensitization to AMP (3 mg/kg) was assessed in 8-22 month old males using a five day induction protocol and a challenge 10-14 days after induction. EM immunocytochemistry revealed double labeling of axons for TH and GLT-1. Knockout of GLT-1 restricted to DA neurons using DAT-IRES-Cre (datGLT-1 KO) eliminated the anti-GLT-1 labeling of TH positive axons in the medial shell (p<0.0001). DatGLT-1 KO mice showed a significantly blunted response to AMP on day 1 induction testing compared to WT mice at 8-9 months. At 11+ months, WT mice show a significantly blunted

locomotor response to AMP on day 1 induction testing compared to datGLT-1 KO mice. This reversal of effect appeared to be due to WT mice showing a reduction in their response to AMP as they aged, whereas responses to AMP displayed by datGLT-1 KO mice remained constant. These data demonstrate that GLT-1 is expressed in DA neurons, and suggest that GLT-1 deletion restricted to DA neurons alters the effect of aging on sensitivity to AMP.

Disclosures: **K.D. Fischer:** None. **A. Sherpa:** None. **S. Griswold:** None. **C. Aoki:** None. **P.A. Rosenberg:** None.

Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 485.25

Topic: G.09. Drugs of Abuse and Addiction

Title: Prophylactic Administration of Sigma-1 Receptor Ligands Mitigates Both Physiological and Neuronal Responses to Methamphetamine-Induced Sensitization

Authors: *A. R. ALLEYNE¹, S. W. HARDEN¹, S. O. EANS¹, L. L. WILSON¹, C. R. MCCURDY², C. J. FRAZIER¹, J. P. MCLAUGHLIN¹;
¹Pharmacodynamics, ²Medicinal Chem., Univ. of Florida, Gainesville, FL

Abstract: The U.S. has experienced an eight-fold increase in reported deaths related to psychostimulant use from 2015 - 2021. This surge emphasizes the urgent medical need for therapeutics to address psychostimulant abuse, as no FDA-approved treatments exist. Several studies have implicated a role for the sigma-1 receptor (S1R) in exacerbating the negative effects of psychostimulants, but the mechanism remains unclear. S1Rs are chaperone proteins known to modulate both calcium release within cells as well as sodium and potassium channel activity in the cell membrane. We hypothesize that S1Rs increase neuronal excitability within reward pathways of the brain following exposure to psychostimulants such as methamphetamine (METH) which can be attenuated with the use of S1R ligands. Using *in vivo* and *ex vivo* techniques, we investigated the role of S1Rs in the development of METH-induced sensitization. C57BL/6J male mice were treated twice daily for 7 days with either saline, METH (2mg/kg), or METH + the S1R antagonist, CM304 (30 mg/kg). After one week where no treatments were given, ambulatory activity was recorded following additional exposure to METH (1 mg/kg). While mice that had previously been administered METH alone demonstrated significant hyperlocomotor behavior, mice treated with METH + CM304 had similar ambulatory activity to saline-treated control mice, suggesting therapeutic value of CM304 for the reduction of METH-induced sensitization. In an effort to understand the cellular mechanisms underlying this behavior, we used acute brain slices derived from mice that underwent the METH sensitization protocol for electrophysiology studies. Exposure to METH for 7 days, a 1-week washout, and then re-exposure to METH induced significant increases in action potential (AP) gain of medium spiny neurons (MSNs) in the nucleus accumbens (NAc), a key brain region for reward

processing, suggesting METH-induced sensitization may be driven by increased neuronal excitability within reward circuitry. Following the washout period, MSNs examined in mice that had been treated with METH and the S1R antagonists CM304 (30 mg/kg) or S1RA (30 mg/kg) demonstrated reduced excitability compared to METH sensitization MSNs, implicating S1Rs as a likely driver of this hyperexcitability. Additionally, we found that acute bath application of the S1R agonist SA4503 (10 μ M) causes excitation in NAc MSNs. Taken together, these data suggest that S1Rs may exacerbate psychostimulant use by overstimulating reward centers of the brain, supporting the notion that their therapeutic targeting could prove a beneficial treatment for psychostimulant abuse.

Disclosures: A.R. Alleyne: None. S.W. Harden: None. S.O. Eans: None. L.L. Wilson: None. C.R. McCurdy: None. C.J. Frazier: None. J.P. McLaughlin: None.

Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 485.26

Topic: G.09. Drugs of Abuse and Addiction

Support: T32 DA031115
R01 DA039146

Title: An Alternative Within-Session Approach for Quantifying Economic Demand for Drug Reinforcers in Rats

Authors: *H. I. RISCA¹, A. SULIMA², K. C. RICE³, G. I. COLLINS¹;
¹Pharmacol., Univ. of Texas Hlth. Sci. at San Antonio, San Antonio, TX; ²NIDA and NIAAA, San Antonio, MD; ³NIDA and NIAAA, Bethesda, MD

Abstract: An Alternative Within-Session Approach for Quantifying Economic Demand for Drug Reinforcers in Rats

Risca, Harmony I¹; Sulima, Agnieszka²; Rice, Kenner C² and Collins, Gregory T¹ Department of Pharmacology, The University of Texas Health Science Center at San Antonio, USA; ²Drug Design and Synthesis Section, Molecular Targets and Medications Discovery Branch, NIDA and NIAAA, Bethesda, MD, USA. The relative value of drugs of abuse is known to be influenced by a variety of factors, including genetics, behavioral and pharmacologic histories, the availability of alternative reinforcers, and the sex of the subject. Within-session demand curve analyses provide an efficient and highly translational approach for determining how environmental, behavioral, or pharmacologic manipulations impact the relative value (i.e., economic demand) of drug reinforcers. The current study used a multiple component, fixed ratio (FR) schedule of drug self-administration to rapidly assess economic demand for cocaine (0.032-1.0 mg/kg/inf), 3,4-methylenedioxypyrovalerone (MDPV) (0.0032-0.1 mg/kg/inf), 3,4-methylenedioxy- α -pyrrolidinopropiophenone (MDPPP) (0.032-1.0 mg/kg/inf), α -pyrrolidinovalerophenone (α -PVP)

(0.01- 0.32 mg/kg/inf), and α -pyrrolidinopropiophenone (α -PPP) (0.032-1.0 mg/kg/inf) in male and female Sprague-Dawley rats. Available unit-doses of drug increased across each of 4, 20-min components, with the response requirement (FR1, 5, 10, 18, 32, 56, 100) increased across sessions. Demand for cocaine, MDPV, MDPPP, α -PVP, and α -PPP from the current study resulted in elasticity coefficients, curves, and rank orders which were correlated ($r = .72$) with elasticity coefficients obtained using previously validated drug demand procedures. This alternative demand procedure allows for more rapid testing of a range of doses across different prices while still preserving the integrity of behavioral economic demand theory and results from demand-curve analyses. Validation of this multiple component schedule of self-administration allows for efficient within-subject testing to characterize the reinforcing effectiveness of drugs, investigate pharmacotherapeutic agents and neuronal manipulations more readily.

Disclosures: H.I. Risca: None. A. Sulima: None. K.C. Rice: None. G.I. Collins: None.

Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 485.27

Topic: G.09. Drugs of Abuse and Addiction

Title: Fixed-interval schedule controlled behavior affected by d-amphetamine induced behavioral sensitization in male rats

Authors: *W. HSU¹, S. WU¹, S. CHEN², C. WANG², R. LIAO¹;

¹Dept. of Psychology, ²Inst. of Neurosci., Natl. Chengchi Univ., Taipei, Taiwan

Abstract: Repeated exposure to psychostimulants (e.g., amphetamine) produces behavioral sensitization (BS), a model being used in the study of drug reward and addiction. Although cognitive disruption is frequently observed in the subjects with repeated stimulant drug experience, whether the timing or time perception could be affected by BS of amphetamine remains unclear. It is also unknown about the long-lasting effects of amphetamine-induced BS on timing behavior. This study was then designed to examine the long-lasting effects of d-amphetamine (d-AMP) sensitization on operant behavior acquired and maintained by a fixed-interval 30 sec (FI30) schedule in male rats. The protocol of d-AMP induced BS was first carried out through four stages, 1) pre-test (PreT), 2) development phase, 3) 3-day short-term-withdrawal post-test (STPosT), and 4) 3-week and 6-week long-term-withdrawal post-test (LTPosT). In the PreT, STPosT, and LTPosT, rats received a low-dose (LD; 0.5 mg/kg) of d-AMP or saline, whereas a high-dose (HD; 1 mg/kg) of d-AMP or saline was administered in the development phase given by a seven-time intermittent injection protocol. The BS of locomotor activity was significantly induced by d-AMP as revealed the locomotion increment profoundly manifested in the STPosT and LTPosT compared to PreT. Subsequently, during the 14-day acquisition of a FI30 task, there was no difference detected between d-AMP-sensitized and saline-control groups. At last, to evaluate the dose effects of d-AMP on FI30 behavior, we found

that LD d-AMP injection significantly increased the total responses and decreased post-reinforcement pause in the sensitized group as compared to saline control; in contrast, HD d-AMP injection altered these two measures in the opposite direction. Together, BS of locomotor activity developed to d-AMP were demonstrated following both short- and long-term withdrawal. Despite the absence of disrupted acquisition of FI30 behavior in drug-sensitized rats, d-AMP dose-dependently affected the performance of this behavior after a long-term withdrawal. These results indicate that time-based operant behavior could be associated with neural substrates underlying the stimulant sensitization.

Disclosures: W. Hsu: None. S. Wu: None. S. Chen: None. C. Wang: None. R. Liao: None.

Poster

485. Mechanisms of Amphetamine Reinforcement and Reinstatement

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 485.28

Topic: G.09. Drugs of Abuse and Addiction

Support: DA042110

Title: Methamphetamine Vapor Administration in Female Rats

Authors: *B. J. SAWYER, B. E. SCHMEICHEL;
Biomed. Sci., East Tennessee State Univ., Johnson City, TN

Abstract: In 2020, 2.5 million people aged 12 and older abused methamphetamine in the US (NSDUH). Methamphetamine (MA) is a harmful psychostimulant that can be abused through various routes of administration, including intravenous injection and smoke or vapor inhalation. Historically, intravenous drug self-administration is one of the most commonly used procedures in preclinical models of addiction. The current preclinical studies are aimed at validating a model of vaporized drug self-administration in adult female Wistar rats using electronic delivery of vaporized MA. First, locomotor testing following passive MA vapor exposure compared to systemic MA injection (intraperitoneal; i.p.) was used to determine proper doses for MA vapor self-administration studies. Rats showed comparable baseline locomotor activity following vehicle (60/40 VG/PG or saline) and an increase in locomotor activity following 100 and 200 mg/ml MA vapor exposure to that of 0.5 and 1.0 mg/kg MA (i.p.), respectively. A separate cohort of rats were allowed to self-administer MA vapor (100 mg/mL) for 1-hour sessions daily for three weeks. Rats exhibited discrimination of active lever press (4 secs MA vapor delivery/press; 30 sec time-out) from inactive lever press (no programmed response) by 3 weeks of training, and subsequently showed a concentration-response function as the number of drug vapor deliveries (average of five, consecutive 1hr sessions) was significantly less at 200 mg/ml compared to 100 mg/ml MA. These findings demonstrate the validity of this operant MA vapor self-administration. Ongoing studies will extend this research to male rats to assess possible sex

differences. This rodent model of MA vapor inhalation provides the basis for future research into a resurging area of concern.

Disclosures: B.J. Sawyer: None. B.E. Schmeichel: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA Grant 1F31DA053774-01A1

Title: A Novel Gene Therapy Approach for Lasting Protection from Opioid Use Disorder

Authors: *K. CLEMENZA¹, S. MCELROY¹, E. F. OLIVEIRA², A. FRYC², L. L. SJULSON¹;
¹Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY; ²Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., New York, NY

Abstract: Opioid use disorders pose a substantial public health burden, with consistently high rates of morbidity and mortality. Though several effective interventions exist, nonadherence and treatment failure are sufficiently common to warrant development of new treatment modalities. The reinforcing properties of opioids emerge partly from an ability to drive increases in nucleus accumbens (NAc) dopamine levels, through activation of Gi-coupled mu opioid receptors (MuORs) on ventral tegmental area (VTA) GABAergic neurons, and subsequent disinhibition of VTA dopaminergic neurons. Artificially decreasing opioid-evoked VTA dopamine release is known to reduce drug-seeking behavior, but this information has not been used therapeutically. Inspired by recent work using inhibitory DREADDs to attenuate heroin self-administration, this project seeks to utilize a version of the MuOR itself to suppress opioid reward-related VTA dopamine release in a way that has clear translational value. We propose that a low affinity MuOR mutant, which we refer to as LAMuOR (Low Affinity MuOR), may be exploited to create a genetically encoded tool that, when expressed in VTA dopaminergic neurons, inhibits their activity in the presence of exogenously administered opioids, but not in response to endogenous peptides. We base our LAMuOR on the MuOR variant D114(2.50)N, which is a well-characterized mutation that reduces binding affinity for opioid peptides while leaving binding for exogenous opioids relatively intact. The goal of our project is to test this hypothesis that LAMuOR suppresses opioid taking by responding preferentially to opioids of abuse - such as during free opioid consumption - while remaining relatively unresponsive to physiological rewards. We have found that mice expressing LAMuOR in VTA exhibit reductions in fentanyl-evoked VTA dopamine release as measured by NAc-targeted dLight1.2 photometry, with little effect on cocaine-induced dopamine release. LAMuOR mice also demonstrate reduced fentanyl-induced open field locomotion, an effect that gets stronger as LAMuOR and fentanyl doses increase. LAMuOR mice choose to consume less oxycodone compared to controls in the two

bottle choice assay, with the lowest ingestion levels found in the highest LAMuOR dosed mice. Sucrose preference, a test of physiological reward response, appears to be unaffected by LAMuOR, as do measures of drug-free and cocaine-induced open field locomotion. These preliminary results offer a first look into LAMuOR's effectiveness as a potential therapeutic capable of suppressing opioid-driven VTA dopamine release and the associated opioid consumption that drives opioid use disorder.

Disclosures: **K. Clemenza:** None. **S. McElroy:** None. **E.F. Oliveira:** None. **A. Fryc:** None. **L.L. Sjulson:** None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.02

Topic: G.09. Drugs of Abuse and Addiction

Title: Estradiol Increases Anxiety During Early Morphine Withdrawal

Authors: M. N. ELLIS, *T. S. DENNIS;
Neurosci., St. Mary's Col. of Maryland, St Mary's City, MD

Abstract: While men and male animals have traditionally been the target of addiction research, a growing literature over the past few decades has revealed key differences in the way that women and men (as well as female and male rats) respond to drugs of abuse. We see one such difference in opiate withdrawal, where male rats show more severe symptoms of somatic withdrawal during earlier timepoints and female rats display a more protracted withdrawal syndrome. In addition to sex differences, there is also evidence that ovarian hormones can influence the way that a female population experiences drugs of abuse. For example, high levels of estradiol are typically associated with increases in drug-seeking behavior, whereas progesterone is typically associated with decreases. Much less research, however, has focused on the influence of these hormones on opiate withdrawal. To explore the role of ovarian hormones on somatic withdrawal behaviors and anxiety experienced during opiate withdrawal, our rats underwent a chronic escalating morphine paradigm. Rats were given experimenter-administered morphine (or saline as a control) twice daily for 10 days. After 10 days, rats were allowed to undergo spontaneous withdrawal, during which we measured somatic withdrawal behaviors every 12 hrs (up to 108 hrs into withdrawal). We also measured anxiety using elevated plus maze and light-dark box testing at the 12 hr and 108 hr withdrawal timepoints. Importantly, all female rats received ovariectomy surgeries prior to the initiation of the study and were provided with a four-day repeating cycle of hormone replacement (First Day - 0.1ml 5µg estradiol; Second Day - 0.1ml 5µg estradiol; Third Day - 0.1ml 250µg progesterone; Fourth Day - peanut oil). All male rats received sham surgeries and were administered only peanut oil as a control for the daily hormone injections. Our data revealed that during early morphine withdrawal, female rats given estradiol on first day of the repeating hormone replacement cycle showed greater anxiety when compared

with morphine withdrawn female vehicle controls and morphine withdrawn male rats (in light-dark box testing). Current analysis is underway to understand the extent which hormone treatment influences the severity of somatic withdrawal.

Disclosures: M.N. Ellis: None. T.S. Dennis: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-MBRS Score-1SC2DA047809-02
NIH-RISE-R25GM061838

Title: Deep Brain Stimulation Increases BDNF After Extinction of Morphine CPP

Authors: *M. E. LLORET-TORRES¹, J. L. BARRETO-ESTRADA²;

¹Univ. of Puerto Rico Med. Sci. Campus, Univ. of Puerto Rico Med. Sci. Campus, San Juan, Puerto Rico; ²Anat. & Neurobio., Univ. of Puerto Rico RCM, Rio Piedras San Juan, Puerto Rico

Abstract: Deep brain stimulation (DBS) is a neurosurgical procedure in which stimulation electrodes are implanted in specific brain regions to modulate brain activity by current administration. DBS has been used primarily in movement disorder such as Parkinson's disease, but its uses have recently been expanded to other diseases such as OCD, depression and, addiction. Clinical studies on DBS for addiction have shown positive results although refractory patient hesitancy to participate is caused by many factors including a lack of understanding of DBS's biological mechanism of action. Therefore, more pre-clinical research is needed to improve outcomes and, ease hesitancy. One potential approach is to study DBS' elicited changes in protein expression. Increased expression of brain derived neurotrophic factor (BDNF) has not only been associated with extinction of addictive behaviors, but also it has been shown to increase by DBS. Here we test whether DBS can increase BDNF expression in brain regions of the corticomesolimbic reward system during extinction of morphine place preference. Previously, we have shown that LF-DBS (20-Hz) aimed to the ventral striatum/nucleus accumbens (VS/NAc) during sessions of morphine (5mg/kg) extinction significantly reduced the number of days required to extinguish CPP ($P < 0.001$). After behavioral assessments, Western blots showed that LF-DBS significantly increased BDNF expression (130%) in the hippocampus ($P < 0.05$), while in the VS/NAc, amygdala and medial prefrontal cortex no significant changes were observed. Our data suggest a potential role for hippocampal/accumbal connections in DBS-mediated extinction facilitation, which may involve axonal BDNF transport. This work was supported by NIH-MBRS Score-1SC2DA047809-02 and NIH-RISE-R25GM061838.

Disclosures: M.E. Lloret-Torres: None. J.L. Barreto-Estrada: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.04

Topic: G.09. Drugs of Abuse and Addiction

Support: VA Grant RX002931
Advancing a Healthier Wisconsin

Title: Chronicity of repeated blast traumatic brain injury associated increase in oxycodone seeking in rats

Authors: *C. M. OLSEN¹, B. L. GLAESER¹, R. CHIARIELLO², C. MCCARTHY¹, M. D. BUDDE², B. D. STEMPER³;
²Neurosurg., ¹Med. Col. of Wisconsin, Milwaukee, WI; ³Biomed. Engin., Marquette Univ. & Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Numerous clinical studies demonstrate correlations between traumatic brain injury (TBI) and substance abuse, and it has been estimated that 10-20% of patients develop a new diagnosis of substance use disorder after TBI. We have previously demonstrated that repeated blast TBI (rbTBI) increases oxycodone seeking after self-administration. Here, we measured the effects of time at two experimental periods: time between injury and self-administration and time of oxycodone abstinence after self-administration on the ability of rbTBI to alter oxycodone seeking. We tested durations of 4 days, 4 weeks, and 4 months at each experimental period. Adult male rats were trained to self-administer oxycodone (0.1 mg/kg) after repeated blast or repeated sham injuries, then tested for drug seeking in extinction conditions. Time between injury and oxycodone self-administration did not have a major impact on drug self-administration, but drug seeking was elevated at 4 days and 4 weeks, but not 4 months post-injury. The duration of abstinence showed a similar result, with the 4-week timepoint showing the largest effect of injury. In conclusion, rbTBI prior to oxycodone self-administration had minimal effect on the oxycodone intake, but injury was associated with increased oxycodone seeking at acute and subacute timepoints.

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Poster

486. Opioids: Reward and Reinforcement I

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Program #/Poster #: 486.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NSERC Discovery Grant
Concordia University Bridge Fund

Title: The effects of chemogenetic inhibition of the anterior paraventricular nucleus of the thalamus on heroin seeking in male abstinent rats

Authors: *E. G. AH-YEN, S. PATTERSON, C. BORGES, J. L. CHARLES, U. SHALEV;
Concordia Univ., Montreal, QC, Canada

Abstract: Drug addiction is a chronic disorder characterized by a cycle of excessive drug use, abstinence, and relapse. In human and animal models, chronic food restriction has been shown to increase relapse rates. However, the underlying neuronal mechanisms are not clear. The paraventricular nucleus of the thalamus (PVT) has been shown to have an important role in feeding control and drug seeking. Our laboratory has examined the role of the PVT in heroin seeking and relapse following a forced abstinence period with chronic food restriction. In contrast to previous reports, we found that inhibiting the PVT did not decrease heroin seeking under those conditions. One possible explanation for the discrepancy is the difference between anterior (a) PVT and posterior (p) PVT subregions. In our previous studies of the role of the PVT on heroin seeking, we manipulated the pPVT. There is ample evidence that the aPVT and pPVT differ in their afferent and efferent pathways, as well as in their function. Here, we aimed to identify the role of the aPVT on drug seeking and relapse under a forced abstinence period with chronic food restriction. Male Long Evans rats ($N = 42$) were injected with a viral vector carrying an inhibitory Designer Receptor Exclusively Activated by Designer Drug (DREADD) into the aPVT. Rats were then trained to self-administer heroin (0.1mg/kg/infusion) for 10 days, followed by a forced abstinence period of 16 days. During forced abstinence, rats were either sated or food restricted (FDR) to reach ~ 85% baseline body weight. On day 15 of food restriction, rats were either injected with Clozapine-N-Oxide (CNO) to chemogenetically inhibit the aPVT or with vehicle (VEH) and underwent a 3-hour heroin-seeking test under extinction conditions. Chemogenetic inhibition of the aPVT had no statistically significant effect on heroin seeking in the FDR or sated groups. The lack of significant findings suggests that either the aPVT does not affect heroin-seeking behaviour under these conditions or that the aPVT serves as an inhibitory function. We plan to validate our findings with pharmacological inhibition of the aPVT, and further explore the role of the aPVT using chemogenetic excitation.

Disclosures: E.G. Ah-Yen: None. S. Patterson: None. C. Borges: None. J.L. Charles: None. U. Shalev: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.06

Topic: G.09. Drugs of Abuse and Addiction

Support: Rowan University School of Osteopathic Medicine Seed Funding

Title: Effects of sex and estrous cycle on oxycodone craving and oxycodone-induced alterations in glutamatergic signaling pathways in the nucleus accumbens

Authors: **B. P. PATEL**, J. A. LOWETH;
Cell Biol. and Neurosci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ

Abstract: Opioid use disorder is a chronic, relapsing condition. Significant sex differences in opioid addiction liability have been reported in both preclinical and clinical studies. However, less is known regarding the cellular and molecular mechanisms driving cue-induced oxycodone craving and relapse vulnerability, including the impact of sex and estrous cycle on these measures. Here we assessed the effects of sex and estrous cycle on the withdrawal-dependent increase in or incubation of cue-induced oxycodone craving that occurs during the first few weeks of withdrawal in rats and is thought to reflect increased relapse vulnerability. Adult male and female rats self-administered oxycodone (0.1 mg/kg/infusion) or saline for 10 days, followed by forced abstinence or withdrawal in their home cage. At different withdrawal time-points, animals underwent cue-induced seeking tests in a drug-free state to assess changes in oxycodone craving. Consistent with previous reports, both males and females showed robust escalation of oxycodone intake but no sex differences in oxycodone intake were observed. Also, consistent with previous reports, both males and females showed incubation of cue-induced oxycodone craving. Interestingly, we found a significant difference in seeking behavior between males and females on withdrawal day (WD) 1, with males showing an increase in seeking behavior compared to females. As a result, the magnitude of incubated craving (average percent change in oxycodone seeking from WD44 compared to WD1) was significantly higher in females compared to males, which could indicate increased relapse vulnerability in females. However, no effects of estrous cycle on oxycodone seeking behavior were observed. Given the fact that drug-induced alterations in glutamatergic signaling pathways in the nucleus accumbens (NAc) play a critical role in drug seeking behavior, we will also assess changes in both cell surface and total protein levels of glutamate receptors (AMPA, NMDA, Group I mGluRs) and glutamate transporter expression (GLT-1) in the NAc core and shell in these animals and correlate protein levels with oxycodone seeking behavior and oxycodone intake. Together these findings will begin to identify neuroadaptations underlying withdrawal-dependent changes in oxycodone craving in both males and females and across the estrous cycle.

Disclosures: **B.P. Patel:** None. **J.A. Loweth:** None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.07

Topic: G.09. Drugs of Abuse and Addiction

Support: Startup funds from department of psychology, University of Maryland college park

Title: Role of estrous cycle and orbitofrontal cortex in oxycodone seeking after prolonged abstinence in female rats

Authors: *A. OLANIRAN, H. LIN, M. A. M. BURKE, I. LINSHITZ, X. LI;
Dept. of Psychology, Univ. of Maryland, College Park, MD

Abstract: Relapse to drug use is a major challenge in curbing the ongoing opioid epidemic in the United States. In rats, oxycodone seeking progressively increases after abstinence, a phenomenon termed incubation of oxycodone craving. We have previously shown that the orbitofrontal cortex (OFC) plays a critical role in incubation of oxycodone craving in male rats. Here, we focused on female rats and aimed to investigate: 1) the effect of estrous cycle on oxycodone seeking; 2) whether the critical role of OFC in oxycodone seeking generalizes to female rats. In Exp.1, we trained female Sprague Dawley rats to self-administer oxycodone (0.1mg/kg/infusion, 6 h/d) for 10 days and tested them for oxycodone seeking on abstinence day 14, 15 or 16. We monitored the estrous cycle throughout the experiment. In Exp.2, we used chemogenetics to examine the causal role of the OFC in oxycodone seeking in female rats. We first injected adeno-associated virus expressing hM4Di (AAV8-hSyn-mCherry-hM4Di) bilaterally into OFC. Next, we trained all female rats to self-administer oxycodone as described above. On abstinence day 15, we tested all rats for oxycodone seeking after pretreating them with either saline (vehicle) or J60 (0.3mg/kg). We perfused all rats immediately after the seeking test and quantified mCherry expression in the OFC. We found no differences in oxycodone intake across estrus, proestrus and metestrus phases during training. Oxycodone seeking was also similar between estrus and non-estrus rats after abstinence. Moreover, we observed a trend toward significant decrease of oxycodone seeking in female rats with pretreatment of J60 at 0.3 mg/kg compared with saline. Results suggest that the estrous cycle has no effect on oxycodone intake and seeking under our experimental conditions. In addition, the critical role of OFC in oxycodone seeking may be generalized to female rats, and studies are underway to validate this conclusion by examining the effect of chemogenetic inactivation of the OFC by J60 at 1.0 mg/kg on oxycodone seeking after abstinence in female rats.

Disclosures: A. Olaniran: None. H. Lin: None. M.A.M. Burke: None. I. Linshitz: None. X. Li: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.08

Topic: G.09. Drugs of Abuse and Addiction

Support: DA047858

Title: Negative allosteric modulation of CB1 receptor signaling decreases opioid self-administration and relapse

Authors: *I. OLIVA¹, F. KAZI¹, L. N. CANTWELL², G. A. THAKUR², J. D. CRYSTAL¹, A. G. HOHMANN¹;

¹Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN; ²Dept. of Pharmaceut. Sci., Northeastern Univ., Boston, MA

Abstract: The endocannabinoid system interacts with the reward system to modulate natural reinforcers, as well as drugs of abuse. Previous preclinical studies showed that direct blockade of CB1 receptors could be employed to treat substance use disorder (SUD) but this strategy failed during clinical trials due to severe psychiatric side effects, including anxiety, depression, and even suicidal ideation. Alternative strategies have emerged to circumvent serious side effects of direct CB1 binding by developing allosteric modulators. We hypothesized that pharmacological inhibition of CB1 signaling through negative allosteric modulation would reduce morphine addiction. By employing i.v. self-administration in mice, we studied the effects of the CB1 negative allosteric modulator GAT358 in morphine intake and relapse-like behavior. GAT358 reduced morphine infusion intake during the maintenance phase of morphine self-administration. GAT358 also decreased the relapse of morphine seeking behavior after forced abstinence. Our results suggest that CB1 negative allosteric modulators could represent a viable therapeutic route to decrease opioid addicted behaviors.

Disclosures: I. Oliva: None. F. Kazi: None. L.N. Cantwell: None. G.A. Thakur: None. J.D. Crystal: None. A.G. Hohmann: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.09

Topic: G.09. Drugs of Abuse and Addiction

Support: 5R01DA046818-02
U01DA051947

Title: Dysregulated axonal guidance pathways underlying heroin exposure

Authors: *S. MITRA¹, M. IIDA², C. AN², D. M. DIETZ³;

¹Marshall Univ., Huntington, WV; ²Univ. at Buffalo, Buffalo, NY; ³SUNY At Buffalo, Clarence Center, NY

Abstract: Opioid use disorder is a chronic and debilitating disease that is marked by compulsive opioid seeking. A multitude of drug-elicited neuroadaptations in brain regions governing reward

such as the Nucleus Accumbens (NAc) mediate maladaptive neuroplasticity. Here we show that following heroin self-administration, repulsive axon guidance cue, Slit2 is upregulated in the NAc that is concomitant with increased expression of its receptor Robo2. Further, we show that this increase in Slit2 is specific to astrocytes implicating glial regulation of axonal guidance disruption underlying heroin exposure. We also demonstrate an activated JAK STAT signaling cascade that is known to influence cellular levels of Robo2 through transcriptional regulation. Finally, we provide evidence that the activation of slit2 robo2 pathway leads to the upregulation of epigenetic driver Mecp2 and the activated form of cofilin, a synaptic destabilizer. Together, these data highlight that heroin exposure leads to dysregulated axonal guidance in the NAc that might be mediated through neuro glia crosstalk involving a concerted action of altered epigenetic and synaptic plasticity.

Disclosures: S. Mitra: None. M. Iida: None. C. An: None. D.M. Dietz: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NSERC Discovery grant
Concordia University Bridge Fund

Title: The effect of chemogenetic and pharmacological inhibition of the pPVT on food deprivation-induced relapse to heroin seeking after punishment-imposed abstinence, in male and female rats.

Authors: *C. BORGES, A. DARECKA, E. AH YEN, U. SHALEV;
Concordia Univ., Montreal, QC, Canada

Abstract: Drug addicts often abstain from drug use due to the negative consequences associated with drug use. However, relapse occurs in a large proportion of abstinent users, and the underlying brain mechanisms are not clear. The posterior paraventricular nucleus of the thalamus (pPVT) plays a role in motivational processes and addiction-like behaviours. We assessed the effect of pPVT inhibition on food deprivation stress-induced relapse to heroin seeking in male and female rats using inhibitory DREADDS (N=24) or baclofen + muscimol (B+M) intracranial injections (N=20). All treatment groups were trained to self-administer heroin (0.1 mg/kg/infusion) for 2 weeks under a seeking-taking chain schedule. Self-administration was followed by 7 days of punishment training, during which a mild footshock (0.2 to 0.6 mA) was delivered on 30% of the completed seek lever links instead of access to the take lever. Relapse to heroin seeking was tested after 24 h of food deprivation (FD) and under sated condition, in a within-subjects counterbalanced design. Animals in the DREADD group were injected with a DREADD ligand or Vehicle (i.p) 30 mins prior the heroin seeking tests, and the B + M group

was injected with baclofen + muscimol or vehicle (i.c), 10 mins prior the heroin seeking tests. Under the FD condition, chemogenetic inhibition of the pPVT resulted in a statistically significant increase in heroin seeking compared to the control group, only in male rats. There was no statistically significant effect for pPVT inhibition on heroin seeking under the sated condition. Data from the pharmacological inhibition is still being collected and analyzed. Our results so far support the hypothesis that the pPVT is involved in heroin seeking induced by caloric deficit after voluntary abstinence, in male rats.

Disclosures: C. Borges: None. A. Darecka: None. E. Ah Yen: None. U. Shalev: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.11

Topic: G.09. Drugs of Abuse and Addiction

Support: ZIA-DA000602-06

Title: Mifepristone reduces sufentanil intake in a rat model of opioid use disorder

Authors: A. MCGINN, G. F. KOOB, L. F. VENDRUSCOLO;
Natl. Inst. on Drug Abuse, NIH, Baltimore, MD

Abstract: The increase in opioid overdose deaths over the last several years has led to the declaration of an opioid crisis and public health emergency in the US. Over 50% of opioid overdose deaths have been attributed to the synthetic opioid fentanyl and its analogues (e.g., sufentanil) that are common heroin adulterants. Opioid use disorder is a major contributor to these fatalities and opioid consumption via smoking or vaporizing is very common. We hypothesized that opioid dependence involves an overactivation of stress circuitry in the brain, including extrahypothalamic glucocorticoid signaling. We tested this hypothesis by examining whether the glucocorticoid receptor (GR) antagonist mifepristone would reduce sufentanil dependence-associated behaviors in male rats. For these experiments, we utilized a validated rodent model of vaporized opioid self-administration, where rats were trained to lever press for sufentanil vapor in 1 h (short-access) or 12 h (long-access) sessions. The rats in the short-access group displayed a stable level of self-administration throughout the experiment and showed few signs of opioid withdrawal (nondependent), whereas rats in the long-access group significantly escalated their intake over time and exhibited robust signs of opioid withdrawal, indicative of opioid dependence. Next, we treated these opioid-dependent and nondependent rats with mifepristone daily for 9 days while continuing sufentanil self-administration. Dependent, but not nondependent, rats treated with mifepristone significantly reduced sufentanil intake as well as naloxone-induced increases in sufentanil intake. These results indicate that glucocorticoid receptors are functionally involved in sufentanil dependence-associated behaviors. This work extends our previous findings that mifepristone decreases both heroin and alcohol dependence-

associated behaviors in male and female rats. Ongoing work includes female rats as well as a proteomic analysis of the central nucleus of the amygdala in mifepristone-treated and vehicle-treated dependent and nondependent rats. This information will provide a better understanding of opioid dependence and contribute to the discovery of new pharmacological targets for the treatment of OUD.

Disclosures: A. McGinn: None. G.F. Koob: None. L.F. Vendruscolo: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

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Program #/Poster #: 486.12

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant UL1TR002544
NIH Grant UG3DA050942

Title: The impact of intranasal administration of GDNF plasmid DNA nanoparticles on cue-induced opioid reinstatement and central dopamine pathways in male and female rats

Authors: S. B. ISGATE¹, K. E. BUDGE¹, Y. CHEN¹, F. M. VASSOLER¹, Y. ABUHASAN², S. AGRAWAL³, J. YANG⁴, O. SESENOGLU-LAIRD⁵, R. C. MOEN⁵, M. J. COOPER⁵, E. N. POTHOS⁶, B. L. WASZCZAK⁷, *E. BYRNES¹;

¹Dept. of Comparative Pathobiology, Tufts Univ. Cummings Sch. of Vet. Med., North Grafton, MA; ²Dept. of Immunol., ³Grad. Sch. of Biomed. Sci., ⁴Sch. of Med., Tufts Univ., Boston, MA; ⁵Copernicus Therapeutics, Inc, Cleveland, OH; ⁶Integrat Physiol & Pathobiol, Prog. in Pharmacol & Exp Therapeut. & Neurosci., Tufts Univ. Sch. Med., Boston, MA; ⁷Dept Pharmaceut Sci., Northeastern Univ., Boston, MA

Abstract: Opioid relapse remains a significant public health issue and while medication assisted treatment is the current standard of care, non-opioid based treatments remain elusive. Intravenous self-administration, with reinstatement of drug-seeking behavior following forced abstinence, provides a valid model of relapse. Previous studies reported significantly decreased ethanol reinstatement when glial derived neurotrophic factor (GDNF) was directly infused into the ventral tegmental area (Carnicella et al, 2008; PNAS). In an effort to advance a non-invasive route for delivery of GDNF to the brain, we utilized intranasal administration of GDNF plasmid DNA nanoparticles (NPs; Copernicus Therapeutics, Inc.) and examined effects on relapse to the widely abused opioid, oxycodone. In our preliminary studies, we observed significantly decreased cued reinstatement in abstinent oxycodone self-administering males when examined 30 days after intranasal delivery of pGDNF DNA nanoparticles (NPs). The current study addresses the following questions: 1) Are similar effects of intranasal GDNF NPs observed in females; and 2) Are the effects of intranasal GDNF NPs associated with changes in central dopamine. Adult male and female Sprague Dawley rats were implanted with jugular catheters

and trained to selectively press an active lever for OXY (0.1 mg/kg/infusion; 6 h/day; 12 training days) using a fixed ratio 1 (FR1) schedule for 9 days followed by FR5 for 3 days to increase responding. On Day 13 all animals were tested for their level of motivated responding for OXY using a progressive ratio (PR) schedule. The next day rats were administered either intranasal saline vehicle or pGDNF NPs (90 µg DNA), with groups counterbalanced based on both their total drug intake (comparable OXY exposure) and their responding during PR (comparable motivated responding). All animals then underwent forced abstinence for 30 days and were then tested for cue-induced reinstatement (90 min). One set of rats were immediately euthanized at the end of reinstatement to quantify Fos positive tyrosine hydroxylase neurons in the ventral tegmental area using immunohistochemistry with fluorescence as well as gene expression of dopamine receptors (D1 and D2) and the dopamine transporter in regions of the cortex and nucleus accumbens using qPCR. Finally, a separate group of animals were processed to examine dopamine release dynamics in the nucleus accumbens, dorsal striatum and medial prefrontal cortex using *ex vivo* carbon fiber amperometry. These studies are ongoing but will be discussed in the context of effects of intranasal pGDNF NPs on the attenuation of reinstatement in males and females.

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Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.13

Topic: G.09. Drugs of Abuse and Addiction

Title: Adolescent Social Isolation Increases Resilience to Voluntary Opioid Consumption in Adulthood in Rats

Authors: P. LEMEN, *H. CHEN;
Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Social stress during adolescence can cause behavioral changes lasting into adulthood and is a risk-factor for substance use disorder, but the effect varies between individuals. This study characterizes how social isolation in adolescence affects opioid use and anxiety-like behavior in adulthood using WKY/NCrl rats. We compared oxycodone intake and behaviors measured in an elevated plus maze (EPM) between rats either group-housed or isolated for 6 weeks post-weaning. We develop a method (PeerPub) to measure operant oral intake of two rats in the same chamber to better model human social condition. PeerPub uses a RaspberryPi computer and touch sensor to count the number of licks on spouts. It delivers 60 µl of oxycodone under a fixed ratio schedule. A radio-frequency identification chip implanted on top of rat's skull allows identity-tracking during licking. Preliminary data showed female rats isolated during

adolescence (n = ~10/group) developed resilience to oxycodone consumption in adulthood (F(1,15)=3.78, P=0.00036 in females but F(1,11)=6.50, P=0.81 in males). At the dose of 0.1 mg/ml, intake by female group-housed rats was ~1 mg/kg body weight, while intake by isolated rats was ~0.4 mg/kg. Both male group-housed and isolated rats' intake was ~0.3 mg/kg. We also demonstrate differences between groups in anxiety-like behavior using EPM. For example, isolated females spent significantly more time in open arms during baseline measurements when compared to open arm time after 4hrs of oxycodone self-administration (P = 0.03), but group housed females did not see significant changes and, instead, spent less time in open arms throughout the entire experiment. This indicates that female rats in the isolated adolescence group have less anxiety-like behavior before undergoing self-administration. Additionally, male group housed rats demonstrated significantly more open arm time after self-administration (P = 0.023) when compared to open arm time during withdrawal. Male group housed rats overall spent less time in open arms throughout the entire experiment when compared to the isolated group, especially during withdrawal (P = 0.028). This suggests that male rats group-housed in adolescence are more vulnerable to the negative effects of oxycodone consumption. These data demonstrate a need for better understanding the role of social environments in resilience to drug use. In future studies, we plan to examine underlying molecular mechanisms associated with the resilience phenotype.

Disclosures: P. Lemen: None. H. Chen: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.14

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA044353
BBRF Grant NARSAD YIA 26771
NIH Grant DA049531

Title: Micro RNA gene regulation of mRNA expression in the nucleus accumbens in the development of oxycodone addiction-like behaviors in rats

Authors: S. B. ISGATE, S. FLICK, K. E. BUDGE, E. M. BYRNES, F. M. VASSOLER;
Tufts Univ. Cummings Sch. of Vet. Med., North Grafton, MA

Abstract: Opioid addiction is a lifelong disease resulting from long-lasting molecular and behavioral changes. Micro RNAs (miRNAs) are key modulators of gene regulation and are known to be involved in substance use disorder and addiction. It is the goal of the present studies to measure miRNA expression within the nucleus accumbens (NAc) and correlate those expression changes with mRNA changes in the same region in rats that were trained to self-administer oxycodone utilizing either short or long access and compare them to yoked controls.

Male rats were trained to self-administer oxycodone (0.1 mg/kg/infusion, i.v.) for either 2h or 6h daily sessions. Each animal had a yoked saline control animal that received a saline infusion every time the leader received oxycodone. All animals were euthanized 1 hour following the last self-administration session. Total RNA was extracted from the NAc. Both miRNA and mRNA were sequenced from the NAc of the short access animals. Targets were developed based on the sequencing analysis and follow-up PCR was performed in the short and long access animals. Preliminary evidence demonstrates that miRNAs regulate the development of addiction-like behavior within the accumbens of male rats.

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Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.15

Topic: G.09. Drugs of Abuse and Addiction

Support: MBRS-SCORE-1SC2DA047809
NIGMS-RISE-R25GM061838
NeuroID

Title: BDNF expression in the mesolimbic reward system in the extinction of morphine place preference

Authors: *W. V. RICHIEZ MATEO¹, M. E. LLORET², P. BONILLA RULLAN³, R. PAGÁN AYALA⁴, L. HERNÁNDEZ ROMÁN³, J. L. BARRETO ESTRADA⁵;

¹Biol., Univ. of Puerto Rico, Bayamon Campus, Bayamon, PR; ²Univ. of Puerto Rico Med. Sci. Campus, San Juan, PR; ³Biol., Univ. of Puerto Rico, Rio Piedras Campus, San Juan, PR; ⁴Anat. and Neurobio., Univ. of Puerto Rico, Med. Sci. Campus, San Juan, PR; ⁵Anat. & Neurobio., Univ. Puerto Rico, Med. Sci. Campus, San Juan, PR

Abstract: Opioid addiction is the leading cause of overdose in the United States, leading to the deaths of over 100,000 in 2021 (CDC, 2021-22). Substance use disorder (SUD) is a cognitive disorder of chronic relapse in which an organism develops a dependency to a substance. Although it is not fully understood, studies suggest that aberrant learning patterns cause neuroplasticity changes in the circuits of the corticomesolimbic dopaminergic system, resulting in the lack of extinction of persistent drug-seeking. Previously, we showed that male rats that extinguished drug-seeking behavior after being subjected to morphine-induced conditioned placed preference (CPP) exhibited a higher transcript of brain derived neurotrophic factor (Bdnf) in the ventral striatum/nucleus accumbens (VS/NAc). Therefore, this research will 1) evaluate whether the bdnf transcript expression correlates with BDNF protein expression in the VS/NAc, 2) determine BDNF expression in the amygdala (AMY) and the hippocampus (HPC), 3)

preliminarily, compare morphine conditioning and extinction between males and females, and 4) determine frequency of rears and side changes, as a measure of withdrawal symptoms and exploratory-based anxiety, respectively. In males, three distinctive behavioral phenotypes were observed: the sham-extinction group (rats that remain in their home cage; n=5), the extinction group (rats that extinguished CPP; n=12), and the extinction resistant (rats that did not extinguish CPP; n=7). Preliminary results showed similar conditioning patterns between male and female rats, however, thirteen (13) percent (2 out of 16) of female rats were able to extinguish their morphine CPP, as compared to fifty (50) percent (12 out of 24) in males. Rears and side-changes in males significantly decreased in animals that received extinction training, compared to sham-extinction animals. In females, there was a similar pattern in each test, however, preliminary baseline data shows less rears and side changes in all groups. At the molecular level, BDNF expression was not affected in the VS/NAc, although it was significantly increased in the HPC of animals in the extinction group. In contrast, BDNF expression in AMY was increased in both extinction and extinction-resistant groups. In females, preliminary results showed increased BDNF expression in the HPC, similar to males. Overall, our data shows that although increased BDNF expression in the AMY might be responsible for contextual learning during extinction training, the increased BDNF expression in the HPC plays a key role in the successful extinction of opioids seeking behavior.

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Poster

486. Opioids: Reward and Reinforcement I

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Program #/Poster #: 486.16

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R01DA012513-22 (PWK)
NIDA Grant T32DA007288-30 (BNK)

Title: Ventral pallidum exhibits opposing cell- and circuit-specific regulation of heroin seeking and refraining behavior

Authors: *B. N. KUHN¹, J. L. HOPKINS¹, E. DERESCHEWITZ¹, A. M. KALASKY¹, P. W. KALIVAS²;

¹Neurosci., Med. Univ. of South Carolina, Charleston, SC; ²Neurosci. Res., Med. Univ. S Carolina, Charleston, SC

Abstract: Substance use disorder is a chronic neuropsychiatric disorder whereby compulsive drug seeking and relapse occurs regardless of attempts to refrain from drug use. Considerable scientific progress has been made toward disentangling the complex neurobiological mechanisms underlying addiction-related behaviors. However, the neurobiological basis of drug seeking

versus refraining (i.e. withholding from drug seeking) has yet to be thoroughly assessed. The ventral pallidum (VP) exhibits opposing regulation of appetitive and aversive motivated behaviors, with the dorsolateral (dl) sub-compartment explicitly showing cell-specific regulation over cocaine seeking versus refraining behavior. In the current studies, male and female transgenic mice expressing Cre recombinase selectively in either GABA, enkephalin or glutamate cells underwent heroin self-administration training (12 days), followed by a week of forced abstinence and extinction training (12 sessions). Tests for cued reinstatement then followed using a within-subject design. In the first study, a Cre-dependent stimulatory DREADD (designer receptor exclusively activated by designer drugs) was injected in the dlVP. Selective chemogenetic stimulation of dlVP GABA or enkephalin cells enhanced cue-induced reinstatement of heroin-seeking behavior, whereas dlVP glutamate neuron stimulation promoted refraining behavior. These results compliment previous findings identifying dlVP cell-specific regulation of cocaine-seeking behavior. Next, an intersectional chemogenetic approach was used to assess the contribution of cell-specific dlVP efferent pathways in mediating heroin seeking versus refraining behaviors. Current results show that activation of dlVP GABAergic projections to the subthalamic nucleus, a region involved in reward seeking, augments cued heroin seeking behavior. In contrast, chemogenetic activation of dlVP glutamatergic projections to the lateral hypothalamus, a critical component of the motive circuitry, enhanced refraining behavior. Ongoing work is assessing the functional role of dlVP enkephalin projections to the subthalamic nucleus. Additional work using viral tracing is identifying alternative dlVP cell-specific projections that may contribute to these opposing behaviors. Together, these results emphasize the cell and pathway-specific functional regulation of the dlVP in mediating heroin seeking and refraining, contributing to our understanding of the neurobiology of heroin relapse.

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Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.17

Title: WITHDRAWN

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.18

Topic: G.09. Drugs of Abuse and Addiction

Support: 5R01DA046818
U01DA051947

Title: Histone demethylase JMJD3 mediates neuroadaptations underlying heroin relapse in male rats.

Authors: S. MITRA¹, P. H. GOBIRA², J. A. MARTIN³, C. T. WERNER⁴, S. THOMAS⁵, M. IIDA⁶, R. L. NEVE⁷, M. LOBO⁸, *D. DIETZ⁹;

¹Pharmacol. & Toxicology, The State Univ. of New York (SUNY) At Buffalo, Buffalo, NY;

²Univ. of São Paulo, Ribeirao Preto, Brazil; ³Dept. of Physiol. and Biophysics, Univ. of Buffalo, Buffalo, NY; ⁴NIDA, Dayton, MD; ⁵Jacobs Sch. of Med. and Biomed. Sci., Buffalo, NY;

⁶Nagoya Univ. Grad. Sch. of Med., Nagoya, Aichi, Japan; ⁷MIT, Boston, MA; ⁸Anat. and Neurobio., Univ. of Maryland, Baltimore, MD; ⁹SUNY At Buffalo, Buffalo, NY

Abstract: Opioid use disorder is a chronic and debilitating disease that is marked by relapse after periods of abstinence. A multitude of drug-elicited neuroadaptations in brain regions governing reward such as the NAc, mediate maladaptive craving behaviors leading to relapse. One of the persistent changes underlying neuroadaptive mechanisms during abstinence from drugs of abuse is epigenetic modifications of DNA and histones. Here we show that at prolonged abstinence from heroin self-administration, histone demethylase JMJD3 is increased in the NAc. Further, we show that this increase is specific to D2 medium spiny neurons, indicating a cell-type-specific regulation. To demonstrate the functional significance of these changes, we employed both pharmacological and viral-mediated approaches to modulate JMJD3 expression in the NAc, which showed that JMJD3 levels and activity are sufficient to regulate cue-induced heroin seeking. We also show that these epigenetic modifications are themselves regulated through the TGF β super-family, bone morphogenetic pathway, which we have previously demonstrated to be essential for drug-seeking behaviors. Together these data highlight that JMJD3 is essential in mediating persistent cellular and behavioral adaptations following heroin exposure.

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Poster

486. Opioids: Reward and Reinforcement I

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Program #/Poster #: 486.19

Topic: G.09. Drugs of Abuse and Addiction

Support: DA25267
DA048353

Title: Pharmacotherapeutic and Abuse Potential of Mitragynine and its Active Metabolites in Rats Trained to Self-Administer Remifentanyl

Authors: *J. D. ZUARTH GONZALEZ¹, C. C. MAZPULE-CARRIGAN³, R. C. PATEL⁴, M. MOTTINELLI⁵, S. MUKHOPADHYAY⁵, F. LEON⁵, C. R. MCCURDY⁵, J. L. WILKERSON¹, T. HIRANITA¹, L. R. MCMAHON²;

¹Pharmaceut. Sci., Texas Tech. Univ. Hlth. Sci. Ctr., Amarillo, TX; ²Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; ³Pharmacodynamics, ⁵Medicinal Chem., ⁴Univ. of Florida, Gainesville, FL

Abstract: Kratom (*Mitragyna speciosa*), a natural product from Southeast Asia, has gained popularity in recent years and is widely available in the United States. Kratom users claim great success self-treating opioid dependence, yet scientific evidence is lagging. Amidst the current opioid epidemic finding pharmacotherapies for opioid use disorders (OUD) is a public health priority. The 40+ alkaloids within kratom serve as natural leads in the development of OUD treatments. For the current study, we examined the most abundant alkaloid in the plant: mitragynine (MG) as well as its metabolites 7-hydroxy-mitragynine (7-OH-MG) and mitragynine pseudoinoxyl (MG-P). Intravenous (i.v.) self-administration for the opioid agonist, remifentanyl, was established in both male and female (4 males and 4 females) Sprague Dawley rats during the light cycle. The experiment was conducted within subjects and each session was made up of five (30-minute) components starting under extinction condition and increasing remifentanyl doses per component (0.1, 0.32, 1, 3.2 µg/kg/infusion). In these rats trained to self-administer remifentanyl, the selectivity of intraperitoneally (i.p.) administered test compounds (MG, 7-OH-MG, and MG-P) to antagonize responding for remifentanyl vs. a non-opioid, cocaine, was assessed. The abuse potential of MG, 7-OH-MG, and MG-P was also studied within the same cohort. Remifentanyl maintained self-administration above extinction levels at most doses tested (0.32, 1, and 3.2 µg/kg/infusion). Each of the kratom alkaloids, MG, 7-OH-MG, and MG-P, were 3- to 4-fold more potent to decrease remifentanyl response rates (alkaloid ED₅₀ values: 47.8, 1.99, and 3.36 µg/kg, respectively) than they were to decrease the maximum cocaine cross-administration response rates (alkaloid ED₅₀ values: 135, 7.67, and 10.6 µg/kg, respectively). That is, the antagonistic effects of MG, 7-OH-MG, and MGP were relatively specific for the reinforcing effects of remifentanyl over those of cocaine. When the test compounds were substituted for i.v. remifentanyl, self-administration responding above extinction was maintained by 7-OH-MG and MGP, but not MG. Despite the abuse potential of active metabolites of MG, these results in rats suggest low MG abuse potential. The lack of obvious MG reinforcing effects may suggest little, if any contribution of MG to the *in vivo* opioid-like activity of kratom. The metabolites 7-OH-MG and MGP might play a more pivotal role to opioid-like activity of kratom *in vivo*.

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Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.20

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute of General Medical Science of the National Institutes of Health
1SC3GM130430-04 to R.R.

Title: Environmental enrichment reverses addiction-related neuronal adaptations

Authors: *A. VASHISHT¹, E. D. BARRERA¹, A. C. MANGAL², H. GOLDSTEIN², E. J. GALAJ³, R. RANALDI²;

¹Biol., Grad. Center, City Univ. of New York, New York, NY; ²Psychology, Queens College, City Univ. of New York, Queens, NY; ³Psychology, Colgate Univ., Hamilton, NY

Abstract: Environmental enrichment (EE) has been shown to be an effective strategy in reducing heroin relapse. Cue-induced reinstatement of drug seeking in rats, following heroin intravenous self-administration (IVSA) training, is reduced using EE. However, the mechanisms driving the effectiveness of EE are still unknown. Dopamine receptors D1 and D3 as well as mu-opioid receptors (D1R, D3R and MOR, respectively) are implicated in heroin self-administration and may undergo changes as a result of chronic drug-taking, which are potentially reversed by EE. To test our hypothesis, we designed a study intended to identify the underlying neuronal mechanisms involved in the capacity of EE to reduce heroin relapse. We used 16 male Long-Evans rats in an experiment consisting of 2 components. The first component had 2 groups: saline (control) or IVSA training for 15 days, to confirm neural adaptations caused by heroin intake. The second component had another 2 groups: all trained on heroin IVSA for 15 days and followed by assignment to standard (non-EE) or enriched (EE) housing for 15 days, to test if EE could reverse those neural adaptations. All brains were collected, sectioned, and processed using RNAscope *in situ* hybridization to visualize D1R, D3R and MORs in the nucleus accumbens (NAc) and insula, two regions strongly implicated in drug addiction. D1R, D3R and MOR mRNA was counted per cell to obtain the average puncta density per cell. When comparing saline to heroin IVSA, we found D1R expression significantly increased specifically on D3R cells in the NAc in the heroin IVSA groups. When comparing IVSA/non-EE to IVSA/EE we found a significant reversal of these changes in rats that were exposed to EE. Conversely, D1R expression was downregulated in the insula in heroin IVSA compared to saline IVSA rats and the effect was significantly reversed in rats that were exposed to EE. These interesting findings indicate that heroin self-administration increases D1Rs in the NAc but decreases them in the insula. Importantly, these results strongly indicate that EE is able to reverse the heroin-caused upregulation of D1 receptors in NAc and downregulation of D1 receptors in insula. Therefore, these data suggest that a reversal of these neuronal adaptations by EE is a mechanism by which EE reduces the propensity to relapse in heroin seeking.

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Poster

486. Opioids: Reward and Reinforcement I

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.21

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant P01DA047233

Title: Transcriptome profiling of the brain's reward circuitry in heroin self-administration identifies a ventral hippocampus gene network related to relapse susceptibility

Authors: C. J. BROWNE¹, R. FUTAMURA¹, A. RAMAKRISHNAN¹, X. ZHOU¹, A. MINIER-TORIBIO¹, F. MARTINEZ-RIVERA¹, M. ESTILL¹, A. GODINO¹, E. M. PARISE¹, M. SALERY¹, A. TORRES-BERRIO¹, A. M. CUNNINGHAM¹, J. GARON¹, P. J. HAMILTON², D. M. WALKER³, B. ZHANG¹, Y. L. HURD¹, L. SHEN¹, E. J. NESTLER¹; ¹Icahn Sch. of Med. at Mount Sinai, New York, NY; ²Virginia Commonwealth Univ., Richmond, VA; ³Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Opioid addiction exacts a devastating toll on individuals, their families, and the healthcare system. Treatment is made exceptionally difficult by prolonged susceptibility for relapse into compulsive drug-seeking and taking, often triggered by re-exposure to drug-associated cues or the drug itself. Lasting relapse susceptibility is thought to be mediated in part by persistent changes in gene expression within interconnected reward-processing regions of the brain. However, few studies have performed transcriptome-wide analyses throughout brain reward regions following volitional opioid intake. Here, we combine heroin self-administration in mice, RNA sequencing (RNA-seq), and advanced bioinformatics approaches to identify novel genes and gene networks in the reward circuitry that are regulated by opioid intake and drug-seeking. First, mice underwent 15 daily 4 hr self-administration sessions wherein lever pressing lead to intravenous delivery of saline or heroin (FR1, 0.05 mg/kg/inf). Mice were then euthanized either from their homecage 24 hr after the last session or after a 30-day homecage forced abstinence period. In the 30-day group, mice also received a challenge injection of saline or heroin (1 mg/kg) and were placed back into self-administration chambers for a 2 hr drug-seeking test, after which mice were immediately euthanized. This design enabled comparisons of multiple addiction-relevant outcomes, including first-ever heroin exposure, early withdrawal from chronic use, context-induced drug-seeking, and combined drug/context-induced drug-seeking. RNA-seq was conducted on six brain regions involved in reward-processing: medial prefrontal cortex, nucleus accumbens, dorsal striatum, basolateral amygdala, ventral hippocampus, and ventral tegmental area. Bioinformatic analysis of this rich dataset has uncovered numerous patterns of differential gene expression in a region- and condition-dependent manner. Further, exploratory factor analysis was used to link gene expression patterns across brain regions with behavioral profiles relevant to an addiction-like state. Employing multiscale embedded gene co-expression network analysis (MEGENA), we also identified gene networks that associate with susceptibility to relapse, changes especially prominent in the ventral hippocampus. Current work focuses on modulating expression of hub genes within this network and studying the impact on heroin self-administration and drug-seeking behavior.

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Poster

486. Opioids: Reward and Reinforcement I

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.22

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant P50DA037844

Title: Intermittent-access to heroin leads to higher drug motivation independent of overall intake similarly for males and females compared to continuous-access paradigm

Authors: ***E. RAKOWSKI**¹, C. P. KING², B. M. THOMPSON³, K. KATZ², P. J. MEYER²; ²Psychology, ¹State Univ. of New York, Univ. at Buffalo, Buffalo, NY; ³Psychology, Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: Previous research with cocaine has shown that the temporal pattern of self-administration, not just overall intake, promotes drug-directed motivation and better represents human patterns of drug intake. The intermittent-access (IntA) paradigm creates alternating high and low drug concentrations in the brain that are not seen in continuous-access (ContA) models. The current study helped determine if similar results could be replicated with heroin as the reinforcer. Adult female (n=29) and male (n=35) heterogeneous stock rats were trained to self-administer heroin (20 µg/kg/infusion), then assigned to either ContA or IntA self-administration patterns. Motivation was measured with progressive-ratio (PR) and behavioral economics threshold tests. Conditioned reinforcement tests were conducted 7 and 14 days after the last exposure to heroin. Male rats in the ContA group earned more infusions than females during self-administration, while IntA rats earned a similar number of infusions regardless of sex. IntA rats exhibited a “spiking” pattern in drug-taking, with nearly all infusions earned during the first two minutes of each drug-available period, while ContA rats showed steadier rates of intake across a session. IntA rats had larger increases in PR breakpoints, particularly when a lower dose was given. Threshold tests revealed more responding in the IntA group for lower doses compared to the ContA group. All rats responded more during the conditioned reinforcement test on withdrawal day 7 compared to withdrawal day 14, regardless of group or sex. These data show that IntA rats showed more motivation to expend effort to receive heroin reinforcement despite having lower heroin intake overall. These results suggest that the higher “spikes” of heroin in the brain related to IntA induce more robust motivation to work for subsequent heroin infusions and create an experience unique from lower, constant levels seen in ContA. Further research into the distinct neural mechanisms involved in IntA can contribute to improved therapeutic interventions for heroin abuse.

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Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

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Program #/Poster #: 486.23

Topic: G.09. Drugs of Abuse and Addiction

Support: ERC grant 802885

Title: Do opioid analgesics boost subjective well-being? A prospective observational study of acute opioid effects before surgery

Authors: *S. LEKNES¹, M. EIKEMO², I. MEIER⁴, G. E. LØSETH³, N. ØRSTAVIK², E. JENSEN⁵, M. TRØSTHEIM⁴, G. ERNST⁵;

¹Univ. Oslo, Univ. Oslo, Oslo, Norway; ²Postboks 1039 Blindern 0315, ³Dept. of Psychology, Univ. of Oslo, Oslo, Norway; ⁴Oslo Univ. Hosp., Oslo, Norway; ⁵Anaesthesia, Kongsberg Hosp., Kongsberg, Norway

Abstract: Millions of people receive opioid analgesics every year as part of their surgical experience. Acute opioid treatment is associated with improved mood, e.g. euphoria and anxiety relief. However, laboratory drug studies in healthy non-opioid users tell a different story, with little consistent mood improvement. Many people dislike opioid drug effects. Here, we determined how two commonly used opioid analgesics affected patients' well-being in standard clinical practice. Day surgery patients rated their mood in the minutes before and after open-label infusion of remifentanyl (N=159) or oxycodone (N=110) while on the operating table before anaesthesia. One minute after infusion, both opioids induced substantial intoxication (>6/10 points). As hypothesised, anxiety was reduced after opioids; this anxiolytic effect was however modest (remifentanyl Cohen's $d=0.21$; oxycodone $d=0.31$) and most patients (65%) reported the same or worse anxiety levels. Unexpectedly, we found moderate to strong evidence against a concurrent boost in positive affect (Bayes Factors >6). After remifentanyl, ratings of feeling good were even significantly reduced from pre-infusion ratings ($d=0.28$). After oxycodone, only 1 in 3 participants felt better. Ordered logistic regression showed that the likelihood of a positive mood boost after opioids was linked to prior drug exposure: opioid-naïve patients were unlikely to feel better (21%); for those with a history of longterm opioid use the odds were >4 times higher (adjusted OR=4.3). In sum, positive opioid effects on mood may be substantially less common than previously thought. These results are particularly striking given the ecological setting with no use of blinding or placebo control, since patients and physicians typically expect opioids to enhance well-being. We speculate that opioids primarily improve well-being via pain relief rather than by directly enhancing mood.

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Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

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Program #/Poster #: 486.24

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of intra-Acb administration of μ -opioid receptor agonist DAMGO and palatable diet on morphine conditioned place preference

Authors: *Y. CAM, C. G. KOCUM, E. R. KONRAD, T. K. HOUSKA, T. A. SCHWEIZER, E. M. STEVENS, M. J. WILL;
Univ. of Missouri, Columbia, MO

Abstract: While many environmental factors are known to contribute to the onset or relapse to drug use and addiction, the role of diet has received comparatively little attention. Individuals with opioid use disorder display increased craving for sweets and palatable food, a type of feeding known as hedonically-driven feeding. Animal models have characterized this feeding pattern to be driven by activation of the nucleus accumbens (Acb). Here, we investigated the effects of hedonic feeding circuit activation (i.e., intra D-Ala²,N,Me-Phe⁴,Gly-ol⁵-enkephalin (DAMGO) treatment + access to palatable food) on the expression of morphine conditioned place preference (CPP) and reinstatement in male and female rats. Following a 30-min preexposure session to entire CPP apparatus, rats received counterbalanced conditioning assignment to drug (4mg/kg morphine) and saline paired sides. After four 45min drug and saline pairings, the expression of morphine CPP was assessed first following no-treatment (NT), and then in a counter-balanced order following intra-Acb DAMGO (0.25 μ g/0.5 μ l/side) injection with or without access to 2gr of high-fat diet for 20 min, before being placed in CPP apparatus for the test of preference. During the initial NT test of preference, males and females showed similar morphine CPP. In female rats, results showed that DAMGO injections were able to eliminate morphine CPP. Although males showed significant morphine CPP after all treatments, the activation of hedonic feeding mechanisms by intra-Acb DAMGO + HF reduced morphine CPP expression, compared to the only DAMGO + No Fat treatment. Next, animals went through an extinction phase, during which they were placed in each side of the box after sham i.p. injections for 45 min. After 12 extinction sessions, females showed extinction, but males did not. Following extinction, animals were tested again for CPP reinstatement in response to the same 3 treatments as before. Only DAMGO + HF treatment was able to reinstate morphine CPP in females. Although we did not find a potentiating effect of hedonic mechanism activation on the expression of morphine CPP, it reinstated morphine CPP expression in females. The results also revealed sex differences in locomotor sensitization and persistency of morphine CPP.

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Poster

486. Opioids: Reward and Reinforcement I

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Program #/Poster #: 486.25

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R36DA051703
NIH Grant T32GM132494

Title: The G protein-biased kappa opioid receptor agonist nalfurafine reduces the reinforcing properties of hydrocodone while potentiating hydrocodone-induced antinociception in C57BL/6J mice

Authors: *A. N. WHITE, J. G. LAMP, V. SETOLA;
Neurosci., West Virginia Univ. Sch. of Med., Morgantown, WV

Abstract: The kappa opioid receptor (KOR) agonist nalfurafine has been shown to reduce the reinforcing properties and to potentiate the anti-nociceptive effects of morphine and of oxycodone in animal models of reward and of pain. Others, including us, have proposed that the desirable effects of nalfurafine on reward and pain are due the drug's bias towards G protein-dependent KOR signaling. Here, we tested whether nalfurafine has a similar salutary effect on hydrocodone, another widely prescribed opioid painkiller, in C57BL/6J mice. Hydrocodone (5 mg/kg, ip) alone induced marked conditioned place preference; however, nalfurafine (0.015 mg/kg, sc) plus hydrocodone (5 mg/kg, ip) resulted in significantly less conditioned place preference, suggesting that nalfurafine antagonizes the reinforcing properties of hydrocodone. Notably, we determined that hydrocodone itself, at a reinforcing dose (5 mg/kg, ip), was not significantly anti-nociceptive in the hot-plate test of supraspinal nociception; however, nalfurafine (0.015 mg/kg, sc) plus hydrocodone (5 mg/kg, ip) exhibited marked anti-nociceptive behavior. We are currently testing hydrocodone alone or in combination with nalfurafine in the intravenous self-administration model of reward and in the warm-water tail withdrawal test of spinal nociception. We are also exploring the extent to which nalfurafine's G protein bias at the KOR contributes to its effects on hydrocodone-associated reinforcement and on hydrocodone-induced antinociception. Altogether, the effects of nalfurafine on opioid painkiller-induced reinforcement and anti-nociception suggest that, in human subjects, a formulation of hydrocodone, oxycodone, or morphine containing nalfurafine may result in a combination pain therapy with reduced abuse potential and heightened pain relief. In this regard, nalfurafine has been used as an antipruritic in Japan since 2009 with an exceptional record of safety and efficacy in humans. As such, we submit that clinical trials of nalfurafine as an anti-addictive, dose-sparing adjuvant to opioid painkillers could and should be fast tracked.

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Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

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Program #/Poster #: 486.26

Topic: G.09. Drugs of Abuse and Addiction

Title: Impact of the ghrelin system on oxycodone-motivated behaviors studied in Wistar rats: Sex differences and the effects of ghrelin receptor deletion and antagonism

Authors: *Z.-B. YOU¹, S. PARI¹, M. CRISSMAN¹, G.-H. BI¹, L. LEGGIO², E. L. GARDNER¹;

¹Mol. Targets and Medications Discovery Br., NIDA-IRP, NIH, Baltimore, MD; ²Section on Clin. Psychoneuroendocrinology and Neuropsychopharmacology, TAMB, NIDA-IRP and NIAAA, NIH, Baltimore, MD

Abstract: The orexigenic hormone ghrelin has recently emerged as a critical biological substrate implicated in drug reward. However, the reciprocal interactions between the endogenous ghrelin system and drug-motivated behaviors remain largely to be studied. Using a variety of experimental approaches, we have recently found that oxycodone self-administration significantly elevates plasma ghrelin levels and acquisition of oxycodone self-administration, and significantly upregulates ghrelin receptor mRNA levels in dopamine neurons in the ventral tegmental area (VTA), a brain region critical to drug reward. Pretreatment with JMV2959, a selective ghrelin receptor (GHS-R) antagonist, dose-dependently reduces oxycodone self-administration and decreases the breakpoint for oxycodone self-administration under progressive ratio reinforcement in male Long-Evans rats. The inhibitory effect of JMV2959 on oxycodone self-administration is selectively mediated by ghrelin receptors – as JMV2959 shows a similar effect in Wistar wildtype but not in ghrelin receptor knockout male rats. In this follow-up study, we expanded our work to study the effects of the ghrelin system on oxycodone-motivated behaviors in female Wistar rats. We found that female rats acquired oxycodone self-administration significantly faster than their male littermates – tested at 0.1 mg/kg/infusion unit dose. Genetic deletion of GHS-R (GHS-R knockout) tended to slow the acquisition of oxycodone self-administration in both male and female rats. In male rats, acquisition of oxycodone self-administration was associated with significantly higher locomotor activity induced by systemic injection of oxycodone (1 mg/kg, i.p.) in GHS-R knockout rats compared to wildtype littermates while no significant differences were found in female rats. Pretreatments with JMV 2959 significantly decreased oxycodone self-administration in both male and female rats, and significantly inhibited reinstatement of oxycodone seeking triggered by oxycodone (1 mg/kg, i.p.) in female wildtype rats after extinction of drug-taking behaviors. JMV2929 had no effect on oxycodone-motivated behaviors in either male or female GHS-R knockout rats. These findings reveal a critical role for GHS-R in controlling oxycodone-motivated behaviors in Wistar

rats regardless of sex and suggest that targeting the GHS-R may be a viable novel treatment approach for opioid use disorder. Supported by NIDA-IRP

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Poster

486. Opioids: Reward and Reinforcement I

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Program #/Poster #: 486.27

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant

Title: A novel μ -opioid receptor transgenic Cre rat: cellular and behavioral characterization

Authors: *J. M. BOSSERT¹, C. A. MEJIAS-APONTE², T. SAUNDERS³, L. K.-P. ALTIDOR¹, M. A. EMERY³, I. FREDRIKSSON¹, A. BATISTA¹, S. CLAYPOOL¹, K. E. CALDWELL¹, V. KUMAR³, A. SEASHOLTZ³, E. HUGHS³, W. FILIPIAK³, B. K. HARVEY¹, C. T. RICHIE¹, F. VAUTIER¹, M. MICHAELIDES¹, J. L. GOMEZ¹, B. L. KIEFFER⁴, S. J. WATSON³, H. AKIL³, Y. SHAHAM¹;
¹NIH, NIDA, IRP, Baltimore, MD; ²Lab. of Sensorimotor Res., Natl. Eye Inst., Bethesda, MD; ³Univ. of Michigan, Ann Arbor, MI; ⁴Univ. of Strasburg and ISERM, Strasburg, France

Abstract: Background: To specifically manipulate MOR-expressing neurons, we developed a transgenic rat to co-express Cre-recombinase and MOR under the endogenous *Oprm1* gene promoter. We performed validation experiments to show expression patterns of both *Oprm1* and Cre-recombinase and assess impact of targeting Cre to the *Oprm1* gene on opioid-mediated pain responses and heroin self-administration (SA). **Methods:** We used RNAscope, fluorescence in situ hybridization chain reaction (HCR RNA-FISH), and autoradiography to verify that the knock-in manipulation had no effect on *Oprm1* mRNA expression, and that iCre co-expresses with *Oprm1*. We test basal response to pain, morphine analgesia and tolerance. We trained male and female heterozygote (HET) rats and wildtype (WT) littermates to self-administer heroin and tested them in three relapse measures. We also tested the effect of nucleus accumbens (NAc) AAV1-EF1a-Flex-taCasp3-TEVP (Caspase3) injections on initiation and maintenance of heroin SA. **Results:** There were no differences between HET and WT rats in NAc MOR expression and function. Preliminary results showed co-localization of *Oprm1* with iCre in HET rats. There were no differences in pain sensitivity or response to morphine, and no genotype-related differences for heroin SA, extinction responding, context-induced reinstatement, and heroin reacquisition. NAc Caspase3 lesions decreased MOR expression and function in HET but not WT. Additionally, the lesions had sex-specific effects on initiation and maintenance of heroin SA maintained by different drug doses and different fixed-ratio reinforcement schedules. **Conclusions:** The novel *Oprm1*-Cre transgenic rat can be used to study the role of brain *Oprm1*-

expressing cells in opioid addiction- and pain-related behaviors, as well as other opioid-mediated learned and innate behaviors.

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Poster

486. Opioids: Reward and Reinforcement I

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Program #/Poster #: 486.28

Topic: G.09. Drugs of Abuse and Addiction

Support: The research was supported by NIDA-IRP.

Title: Role of ventral subiculum neuronal ensembles in incubation of oxycodone craving after electric barrier induced voluntary abstinence

Authors: *I. FREDRIKSSON¹, A. BATISTA¹, A. SHEKARA¹, S. V. APPLEBEY¹, D. J. REINER¹, A. MINIER-TORIBIO¹, L. ALTIDOR¹, C. CIFANI², X. LI³, F. J. RUBIO¹, B. T. HOPE¹, J. M. BOSSERT¹, Y. SHAHAM¹;

¹IRP/NIDA/NIH, Baltimore, MD; ²Univ. of Camerino, Sch. of Pharm., Univ. of Camerino, Sch. of Pharm., Camerino, Italy; ³Univ. of Maryland Col. Park, Dept. of Psychology, College Park, MD

Abstract: Background: We recently developed a rat model of incubation of oxycodone craving after voluntary suppression of drug self-administration by adverse consequences of drug seeking. Here, we studied the role of ventral subiculum (vSub) neuronal ensembles in this incubation, using the activity marker Fos, muscimol-baclofen (GABAergic agonists) inactivation, and Daun02 chemogenetic inactivation. **Methods:** We trained Sprague-Dawley or *Fos-lacZ* transgenic male and female rats to self-administer oxycodone (0.1 mg/kg/infusion, 6-h/d) for 14 days. The rats were then exposed for 14 days to an electric barrier of increasing intensity (0.1 to 0.4 mA) near the drug-paired lever that caused voluntary abstinence or were exposed to 14 days of forced abstinence. We tested Sprague-Dawley rats for relapse to oxycodone seeking without shock and drug on abstinence day 15 and extracted their brains for Fos-immunohistochemistry, or tested them after vSub vehicle or muscimol-baclofen injections on abstinence days 1 and 15. We performed Daun02 inactivation of relapse-activated vSub Fos neurons in *Fos-lacZ* transgenic rats on abstinence day 15 and then tested them for relapse on abstinence day 18. **Results:** Relapse after electric barrier-induced abstinence increased Fos expression in vSub. Muscimol-baclofen inactivation or Daun02 selective inactivation of vSub Fos-expressing neuronal

ensembles decreased “incubated” oxycodone seeking after voluntary abstinence. Muscimol-baclofen vSub inactivation had no effect on non-incubated opioid seeking on abstinence day 1 or incubation after forced abstinence. **Conclusions:** Results demonstrate a selective role of vSub neuronal ensembles in incubation of opioid craving after cessation of drug self-administration by adverse consequences of drug seeking.

Disclosures: **I. Fredriksson:** None. **A. Batista:** None. **A. Shekara:** None. **S.V. Applebey:** None. **D.J. Reiner:** None. **A. Minier-Toribio:** None. **L. Altidor:** None. **C. Cifani:** None. **X. Li:** None. **F.J. Rubio:** None. **B.T. Hope:** None. **J.M. Bossert:** None. **Y. Shaham:** None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.29

Topic: G.09. Drugs of Abuse and Addiction

Support: This research was supported by funds to the NIDA Intramural Research Program - Yavin Shaham

Title: Choice between social interaction and remifentanyl - an investigation in dopamine signaling in the nucleus accumbens core

Authors: ***J. M. CHABOT**, J. J. CHOW, K. M. COSTA, G. SCHOENBAUM, Y. SHAHAM; NIDA IRP, NIDA IRP, Baltimore, MD

Abstract: Background: We previously characterized how operant social interaction functions as a reinforcer in rats. We also introduced a choice procedure that engenders dose-dependent choice between social interaction and the opioid agonist remifentanyl. Here, we investigate dopamine signaling during choice between social interaction and remifentanyl using fiber photometry. Methods: We first trained male and female rats to self-administer social interaction (15 s) for 10 days (15-trials/day or 1 h) and remifentanyl (10 µg/kg/infusion, i.v.) for 10 days (15-trials/day or 1 h). We then trained the rats on controlled reinforcer frequency choice procedure for remifentanyl (0, 1, and 10 µg /kg/infusion) vs. social interaction (15 s); during which we conducted probes using a discrete-trials choice procedure. We then recorded dopamine using fiber photometry via GRAB-DA in the nucleus accumbens core during choice. Results: Rats trained for social interaction vs. remifentanyl choice showed preference for social interaction at 0 µg/kg/infusion, indifference at 1 µg/kg/infusion, and preference for remifentanyl at 10 µg/kg/infusion under both choice procedures. Preliminary fiber photometry data indicate dopaminergic peaks during choice presentation, choice action, and reward delivery. Conclusions: We can observe dopamine signaling using fiber photometry via GRAB-DA during choice between social interaction and remifentanyl. In an ongoing experiment, we will determine if there are any unique dopaminergic signaling across the different choice conditions that correlate and predict choice behavior. We will present these results at the meeting.

Disclosures: J.M. Chabot: None. J.J. Chow: None. K.M. Costa: None. G. Schoenbaum: None. Y. Shaham: None.

Poster

486. Opioids: Reward and Reinforcement I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 486.30

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program - Yavin Shaham

Title: Escaping from shock and precipitated withdrawal - procedures for negative reinforcement in male and female rats

Authors: *J. J. CHOW¹, J. M. CHABOT¹, R. ITO², Y. SHAHAM¹;

¹NIDA IRP, NIDA IRP, Baltimore, MD; ²Univ. of Toronto, Univ. of Toronto, Toronto, ON, Canada

Abstract: Background: Currently, there is limited research directly investigating avoidance of opioid withdrawal in preclinical models. Here, we attempt to adapt a primate-model of opioid negative reinforcement for rats. **Methods:** We first screened male and female rats to lever-press to escape a mild footshock (0.1 mA to 0.27 mA; 1-s on/2-s off for 10-45 s) for 21 sessions (30 trials/sessions). Next, we implanted the rats with osmotic minipumps containing 10 mg/kg/day methadone. We then paired non-contingent naloxone (20 µg/kg/infusion) with a compound light+tone cue 4 times (single session). Next, we trained the rats to avoid naloxone (1 µg/kg/infusion) injections for 8 sessions (30 trials/session). We also conducted a naloxone dose-response and tested the rats for conditioned avoidance responding to the withdrawal cue under extinction conditions 1 and 15 days after minipump removal (incubation tests). **Results:** Almost all rats (53/60 rats, 88%) learned to lever-press to escape a mild footshock while the success to escape naloxone infusions was lower (30/44 rats, 68%). Rats were also sensitive to the different doses of naloxone, showing fewer escapes at the higher doses. Finally, the rats showed greater lever pressing to avoid the naloxone-paired cue on day 15 than on day 1 during incubation tests. No sex differences were observed for either shock or naloxone escape. **Conclusions:** We introduced a rapid method to train rats to lever-press to escape shock. We adapted a negative reinforcement primate model to rats. Currently, we are validating the opioid negative reinforcement and will present these results at the meeting.

Disclosures: J.J. Chow: None. J.M. Chabot: None. R. Ito: None. Y. Shaham: None.

Poster

487. Neural Mechanisms of Unimodal Perception

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 487.01

Topic: H.02. Perception and Imagery

Support: McNair Foundation

Title: Differential modulation of the default mode network underlie distinct cognitive processes

Authors: ***E. DEVARA**, B. A. METZGER, R. K. MATHURA, A. ANAND, H. DANG, S. REDDY, B. R. PASCUZZI, J. ADKINSON, M. ROBINSON, S. A. SHETH, K. R. BIJANKI, B. SHOFTY;

Baylor Col. of Med., Houston, TX

Abstract: The default mode network (DMN) is a widely distributed, intrinsic brain network thought to play a crucial role in internally-directed cognition. It is involved in self-referential thinking, recollection of the past, mind wandering, and creativity. It is unknown, however, how the DMN is electrophysiologically modulated during the variety of distinct processes it is implicated in. We implanted stereoelectroencephalography (SEEG) depth electrodes in 12 human patients undergoing monitoring for epilepsy, providing recordings with high spatiotemporal local-field potential (LFP) resolution. We recorded LFP activity from 425 total electrode contacts within the canonical DMN while patients performed tasks designed to engage the network - an alternate-uses-task (AUT) and a mind-wandering task (MW). In addition, a visual attention task (ATT) was performed as control. Spectral power was calculated across the range of frequency bands from 0-150 Hz and fit to linear-mixed effects models (LME). The LME included fixed effects of task, window, and their interaction as well as random effects of subject, and channel nested within subject. The LME revealed a significant fixed effect of task condition, such that the AUT and MW tasks evoked decreased theta (4-8 Hz) and alpha (8-14 Hz), as well as increased gamma (30-70 Hz) and high gamma (70-150 Hz) power relative to the ATT task. We also observed a significant interaction of task and window, namely that gamma and high gamma band activity was higher during the stimulus window for the AUT task and higher during the response window for the MW task. These results suggest that DMN activity is flexibly modulated as a function of specific cognitive processes. Frequency band power is differentially modified to subserve different task demands, with distinct differences in activity promoting mind wandering and creativity. Our findings add insight into a more complete characterization of how the DMN underlies creative cognition.

Disclosures: **E. Devara:** None. **B.A. Metzger:** None. **R.K. Mathura:** None. **A. Anand:** None. **H. Dang:** None. **S. Reddy:** None. **B.R. Pascuzzi:** None. **J. Adkinson:** None. **M. Robinson:** None. **S.A. Sheth:** F. Consulting Fees (e.g., advisory boards); Boston Scientific, Zimmer Biomet, Neuropace. **K.R. Bijanki:** None. **B. Shofty:** None.

Poster

487. Neural Mechanisms of Unimodal Perception

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 487.02

Topic: H.02. Perception and Imagery

Support: NSERC

Title: Saccadic time compression is associated with network entropy and specific cortical hubs

Authors: *A. GHADERI¹, M. NIEMEIER², J. D. CRAWFORD¹;

¹York Univ., Toronto, ON, Canada; ²Univ. of Toronto Scarborough, Toronto, ON, Canada

Abstract: Perceived time is dramatically affected just before and during saccades, and this perceptual distortion can highly affect our sense of time in various visual or even nonvisual experiences. However, despite multiple studies that investigated behavioural saccadic time distortion effects, the underlying neural mechanism is still unclear. To address this, we recorded EEG via 64 channels in 21 participants during the following paradigms. In the fixation condition, a sequence of 1-3 reference stimuli (three horizontal lines) were presented below central fixation, followed by a 300ms, and then a 70 ms test stimulus (three vertical lines; same size/location). In the saccade condition, participants shifted gaze between left and right positions across centre, 100ms before the test stimulus. Finally, participants judged the duration of test stimulus in comparison to reference. 250ms EEG segments (corresponding to the presaccadic interval) were selected for source localization and graph theoretical analysis. In previous studies, we showed that this paradigm results in an interaction in visual and motor signals that can be observed at both the behavioral (Ghaderi et al. *Heliyon* 2022) and neural levels (Ghaderi et al. *Cerebral Cortex* 2022). Here, we analyzed network dynamics and topology based on the perceptual decision (comparing underestimated duration versus trials with correct judgment). We computed functional connectivity (lagged coherence) between current densities in 84 Brodmann areas, then calculated network measures associated with functional brain network integration, segregation, synchronization, complexity, and network hubs. Our results showed significant differences in functional connectivity ('hubness') of right Brodmann area 46 (including dorsolateral prefrontal cortex) and left Brodmann area 24 (corresponding with anterior cingulate cortex). These areas are associated with time perception, attention allocation and decision-making processes, but this is the first study that implicates them in saccadic time compression. Further, we observed a significant difference in network complexity (measured by Shannon entropy of network). The latter observation is consistent with the notion that entropy equates with physical time, and thus the vector model of time perception that best explained our behavioral results (Ghaderi et al. *Heliyon* 2021). Funded by NSERC

Disclosures: A. Ghaderi: None. M. Niemeier: None. J.D. Crawford: None.

Poster

487. Neural Mechanisms of Unimodal Perception

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 487.03

Topic: H.02. Perception and Imagery

Support: This work was supported by the NIMH Intramural Program.

Title: A functionally time-resolved reconstruction technique for high-resolution fMRI.

Authors: ***T. MORGAN**¹, Y. CHAI², R. MANSOOR¹, J. TEVES¹, P. A. BANDETTINI¹;
¹Natl. Inst. of Mental Hlth., Bethesda, MD; ²Univ. of Illinois, Urbana-Champaign, IL

Abstract: Functional MRI (fMRI) with sub-millimeter spatial resolution is a promising technique for probing the human brain's mesoscopic scale [1]. However, typical spatial resolutions remain too coarse to sample individual human columnar and laminar structures. Moreover, high-resolution fMRI measurements using echo-planar trajectories (EPI) and blood oxygen-dependent (BOLD) contrast suffer from spatial distortions and T2* blurring due to long readout trains. Recently, time-resolved reconstruction methods have alleviated some of these issues by keeping track of the timing of data acquisition in reference to signal properties [2] or physiological cycles [3]. We utilize this conceptual framework to time resolve data with respect to events in a neuroscience experiment. The current work has high spatial resolution (0.5 mm) and is not affected by phase-encoding distortions yet reconstructs brain responses with reasonable temporal resolution (500 ms).

We acquired data from 3 participants (2 male; ages 23, 23 & 25) on a Siemens MAGNETOM 7T+ with a Nova 32-channel head coil. We collected a multi-echo 2D-GRE sequence (TR=31 ms, TE_s=[4.22, 8.38, 12.54, 16.7, 20.86, 25.02] ms, slice thickness=0.8 mm, matrix=360x270, no acceleration or Partial Fourier). We tracked the time of each acquisition line by sending an external trigger to a stimulation computer. The experimental paradigm consisted of a 10 Hz flashing radial checkerboard presented for 2 s (15 s ISI). We reconstructed data via low-rank tensor completion [4] with modes for k-space, receivers, echoes and response time. The resulting reconstruction depicts brain responses from -2 to 32 seconds after stimulus presentation. Primary visual cortex displayed a prominent dip in T2* decay times in middle layers, allowing us to identify infra- and supra-granular layers. Functional responses peaked between 2.5 and 3 s after the short stimulus presentation and superficial layers showed larger peak response and post-stimulus undershoot amplitudes, similar to reports in rodents [5].

We have presented an fMRI reconstruction method which incorporates experimental designs into the image reconstruction process to capture high spatial and temporal resolution brain responses. These features expand the arsenal of tools available to non-invasively examine mesoscopic responses in the human brain.

[1] M. Moerel, et al., J. Neurosci. 2018

[2] F. Wang et al., Magn. Reson. Med. 2019

[3] A. G. Christodoulou et al., Nat. Biomed. Eng. 2018

[4] M. A. O. Vasilescu, University of Toronto Thesis. 2009

[5] P. Tian et al., Proc. Natl. Acad. Sci. 2010

Disclosures: **T. Morgan:** None. **Y. Chai:** None. **R. Mansoor:** None. **J. Teves:** None. **P.A. Bandettini:** None.

Poster

487. Neural Mechanisms of Unimodal Perception

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 487.04

Topic: H.02. Perception and Imagery

Support: UCLA and CAMH unrestricted grant

Title: Somatomap 3D: a digital avatar to identify brain networks associated with own body size and shape estimation accuracy

Authors: J. GUO¹, T. D. MOODY², V. CAZZATO³, S. S. KHALSA⁴, C. RALPH-NEARMAN⁵, L. BREITHAUPT⁶, A. NARAINDAS⁷, *J. FEUSNER⁸;

¹Ctr. for Multimodal Imaging and Genet., Univ. of California, San Diego, CA; ²Semel Inst. for Neurosci. and Human Behavior, Univ. of California, Los Angeles, CA; ³Liverpool John Moores Univ., Liverpool, United Kingdom; ⁴Laureate Inst. for Brain Res., Tulsa, OK; ⁵Univ. of Louisville, Louisville, KY; ⁶Harvard Med. Sch., Boston, MA; ⁷Univ. Col. Dublin, Dublin, Ireland; ⁸UCLA Semel Inst. For Neurosci. and Human Behavior, Toronto, ON, Canada

Abstract: Body size and shape misperception is a marker of several psychiatric disorders including eating disorders and body dysmorphic disorder and may involve distorted body representation. Yet, there is a dearth of evidence surrounding the brain networks representing mental processes of Body Size Estimation (BSE). We previously developed Somatomap 3D, a digital tool to visually assess one's mental representation of body-part sizes and shapes (Ralph-Nearman et al., 2019; Ralph-Nearman et al., 2021). In this study, we test whether brain connectivity within the salience, central-executive, and attention networks (Blanke et al., 2010, Skottnik and Linden, 2019) is associated with BSE accuracy. Thirty-six healthy female (n = 25) and male (n = 11) adults, estimated their own-body size and shape using Somatomap 3D. These scores were subtracted from their corresponding actual body part sizes to produce a "discrepancy score." Principal component analysis was applied to reduce the dimension of standardized residuals on weight, height, and BMI of discrepancy scores from 12 body parts to one principal component (PC). Resting state fMRI data was collected and fMRIprep preprocessed and registered data were entered into FSL MELODIC ICA to compute 30 group Independent Components (ICs). We spatially correlated these ICs to reference canonical networks (Power et al., 2012) to identify the three networks of interest. Dual regression was utilized to examine brain regions associated with BSE by applying PC loadings of standardized residuals of body discrepancy scores to the imaging data. Significance was determined using permutation tests, corrected across voxels and across ICs representing the networks of interest. The majority of the 12 discrepancy standardized residual scores showed positive correlations with each other. The PC1 explained 47.0% of the variability. Nine ICs were significantly associated with canonical salience, central-executive, and attention networks. PC1 scores were significantly associated with connectivity in clusters in the left premotor cortex, left superior parietal lobule, and the right temporoparietal junction. These results demonstrate that size and shape estimation accuracy of body representation is associated with brain regions consistently reported in previous studies of body perception. In sum, Somatomap 3D may provide a useful tool to quantify body image

distortions in body image disorders and potentially identify disturbances in associated brain connectivity.

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Poster

487. Neural Mechanisms of Unimodal Perception

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 487.05

Topic: H.02. Perception and Imagery

Title: Temporal coupling reduces entropy in neuronal spiking patterns: a basis of perception and voluntary movements

Authors: *D. S. GUPTA;

Col. of Sci. and Humanities and Col. of Hlth. and Pharm., Husson Univ., Bangor, ME

Abstract: Gupta and Bahmer (2019) had previously proposed that sensory input leads to an increase in entropy in spiking activities of the cortex, which is reduced by an increase in mutual information due to an increase in the probability of joint activation of pairs of neurons. The increase in mutual information, a measure of correlated activities, reduces the entropy in the cortical spiking pattern given the sensory stimuli. This reduction in entropy, which represents surprise or uncertainty, is the gain in the knowledge by the brain about external stimuli, which is perception. We propose that temporal coupling increases the probability of joint activation of pairs of cortical neurons. We further propose that successful interaction with the physical world, a critical aspect of survival, drives temporal coupling. Temporal coupling results when many neuronal events must occur on the time axis, represented in the brain, to match the sequence of external events on the physical (actual) time axis. In an example of successful interaction with the physical world, catching a fruit falling from a tree, there is a sequence of events in the physical world, that is, the vertical position of the fruit, as it falls from different heights of tree branches of trees, which is directly inputted into the brain by visual stimulation. To catch a fruit falling from a tree branch, muscle contractions must occur at a specific speed and at particular coordinates on the time axis to reach the tree at a given distance and fruit falling from a tree branch at a given height. Accordingly, neuronal events controlling sequential contractions of muscles for successful catch must occur at specific coordinates on the neural representation of the time axis, which must match the physical time axis directly inputted by visual stimulation by falling fruit at different positions along its trajectory. Thus, for an attempted catch, which can prevent fruit from becoming inedible, the neuronal events must be tightly located at specific coordinates along the time-axis represented in the brain. This will lead to the temporal coupling of pairs of neurons in visual and motor areas. Accordingly, cortical patterns underlying the movements to execute the catch will reduce entropy in the cortical spiking patterns, resulting from the visual stimulation of falling fruit, which will lead to the perception of the falling fruit.

Likewise, cortical activity patterns, underlying visual stimulation, will also reduce entropy in the cortical spiking patterns resulting from attempting the catch - an increase in the knowledge about the motor activity - voluntary movement.

Disclosures: D.S. Gupta: None.

Poster

487. Neural Mechanisms of Unimodal Perception

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 487.06

Topic: H.02. Perception and Imagery

Support: This is a research project that was supported by a grant from the research center for College of Education, Deanship of Scientific Research at King Saud University.

Title: Performance in Visual-Orientation Discrimination Task Can Be Predicted by Peak Gamma Frequency and Visual Evoked Potential N1 Peak Amplitude

Authors: *A. BIN DAWOOD;

Dept. of Psychology, King Saud Univ., Riyadh, Saudi Arabia

Abstract: Excitation-inhibition (E-I) balance has been indirectly inferred from psychophysical measures (i.e., performance in orientation discrimination task [ODT]) and neurophysiological measures (i.e., gamma frequency oscillations, and amplitudes of visual evoked potential (VEP)) due to their associations with gamma-aminobutyric acid (GABA), the major inhibitory transmitter. Although previous studies have found an association between enhanced ODT performance and higher peak gamma frequency, the relationship between ODT performance and VEP activity has not yet been explored. Therefore, the current study investigates to what extent performance in ODT could be predicted by gamma frequency activities (peak gamma frequency and gamma frequency power) and amplitudes of VEP components (N1 and P2). Forty-nine healthy adult participants completed an ODT comprising vertical and oblique conditions and an EEG visual task that has been shown to elicit strong peak gamma frequency and VEP activity. The results of multiple linear regression analyses showed that only performance in the oblique condition of ODT but not in the vertical condition could highly be predicted by neurophysiological measures. Specifically, enhanced performance in the oblique ODT is associated with higher peak gamma frequency and/or lower VEP-N1 peak amplitude. These findings support the suggested association between increased cortical inhibition (indicated by enhanced performance in the ODT), higher peak gamma frequency, and lower VEP-N1 amplitude.

Disclosures: A. Bin Dawood: None.

Poster

487. Neural Mechanisms of Unimodal Perception

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 487.07

Topic: H.02. Perception and Imagery

Support: Einstein Center for Neurosciences, Charite Berlin
Max Planck Society
DFG, GRK 2386/1, Extrospection

Title: Neural correlates of stimulus expectations in somatosensory perception

Authors: *C. FORSTER^{1,2,3}, M. GRUND¹, E. PANAGOULAS^{1,4}, E. AL¹, V. NIKULIN¹, A. VILLRINGER^{1,2,4};

¹Dept. of Neurol., Max Planck Inst. for Human and Cognitive Brain Sci., Leipzig, Germany;

²Einstein Ctr. for Neurosciences, Charite Universitätsmedizin, Berlin, Germany; ³Bernstein Ctr. for Computat. Neurosci., Charite Universitätsmedizin, Berlin, Germany; ⁴Fac. of Philosophy, Berlin Sch. of Mind and Brain, MindBrainBody Inst., Humboldt-Universität zu Berlin, Berlin, Germany

Abstract: According to Bayesian integration, what we perceive is a combination of sensory evidence and prior beliefs (Knill & Richards, 1996). A prior belief can be based on information about the probability of a stimulus occurring in a given situation. Expectations about the probability of a sensory stimulus should therefore alter the perception of a weak stimulus. It is unknown how, where, and when this top-down influence of stimulus expectations is processed in the human brain. Here, we address whether stimulus expectations bias somatosensory perception and confidence, and how this is reflected in brain activity measured with 64-channel EEG. 43 female and male, healthy human adults received near-threshold electrical stimulation on their left index finger in 360 out of 720 trials. Participants reported stimulus presence or absence, and binary decision confidence with a button press. Stimulus expectations were manipulated in a within-subject design. Each mini block contained either three near-threshold trials (25%; low expectation) or nine near-threshold trials (75%; high expectation). Probability cues matched the actual probability of stimulus presence. Analysis based on Signal detection theory (Green & Swets, 1966), indicated that participants used a more conservative threshold to report a stimulus in the low stimulus probability condition, while there was no significant difference in sensitivity. Reaction times were faster, and confidence was higher in hits in blocks with a high stimulus probability. Detected trials showed a stronger early potential (P50) in centro-frontal sensors as well as a stronger and earlier peak in the time window from 300 to 500 milliseconds poststimulus in centro-parietal sensors. Analysis of prestimulus power replicated the well-known excitability effect of lower alpha and beta power for detected stimuli. Contrasting low and high probability conditions in the prestimulus window, we found higher beta power in the low probability condition in right centro-parietal electrodes. We show for the first time in the somatosensory system that informative cues of lower stimulus probability increase the subjective threshold to report a weak stimulus. While we replicated the well-established finding of higher excitability indexed by lower alpha power before detected stimuli, preliminary results suggest that not alpha,

but beta power encodes top-down expectations about stimulus probability.

Green, D. M., & Swets, J. A. (1966). Signal detection theory and psychophysics (Vol. 1, pp. 1969-12). New York: Wiley. Knill, D. C., & Richards, W. (Eds.). (1996). Perception as Bayesian inference. Cambridge University Press.

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Poster

487. Neural Mechanisms of Unimodal Perception

Location: SDCC Halls B-H

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Program #/Poster #: 487.08

Topic: H.02. Perception and Imagery

Support: NSF-BCS-1829470 "Collaborative Research: RUI: Isolating neural mechanisms of perceptual awareness from post-perceptual processes"
TWCF-2022-30267 "Bifurcation Dynamics in a No-Report Paradigm"
Canadian Institute for Advanced Research Fellowship to M.A.C.

Title: EEG bifurcation dynamics in the absence of report in a visual masking paradigm

Authors: *C. DEMBSKI¹, K. ORTEGO², C. STEINHILBER¹, M. COHEN^{3,4}, M. PITTS¹;
¹Dept. of Psychology, Reed Col., Portland, OR; ²Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH; ³Dept. of Psychology and Program in Neurosci., Amherst Col., Amherst, MA; ⁴McGovern Inst. for Brain Research, Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Neural correlates of perceptual awareness are often difficult to distinguish from correlates of (1) unconscious processing of physical stimulus properties, and (2) task-related cognition involved in generating reports about one's perception. To isolate awareness-specific neural activity from unconscious sensory encoding, we designed a new EEG experiment similar to a previous study by Del Cul et al. (2007) in which we used backward masking to manipulate physical stimulus properties in a linear fashion by systematically varying stimulus onset asynchrony (SOA) between targets and subsequent masks such that that each target fell below, at, or above the perceptual threshold. In one condition ('report' condition), participants reported after each trial whether or not they saw the target, as in the experiment by Del Cul et al. (2007). In addition, we added a novel 'no-report' condition to circumvent the task-related confounds inherent in purely report-based paradigms.

Del Cul et al. (2007) found that amplitudes of early sensory responses (P1) scaled linearly with the SOA manipulation regardless of whether the targets were consciously perceived, while the later P3b displayed a bifurcated pattern of amplitude modulation corresponding closely with participants' visibility reports. They concluded that the early responses reflected unconscious accumulation of sensory evidence and that the P3b was likely a correlate of conscious

perception.

Our results in the report condition closely replicated Del Cul et al. (2007), but in the no-report condition, while the linear P1 modulations remained the same, the P3b and its bifurcation dynamics completely disappeared. However, eliminating the P3b revealed a mid-latency, fronto-central ERP that showed signs of bifurcation dynamics corresponding to participants' seen/unseen responses in the report condition, which suggests that this ERP might correlate with perceptual awareness. These results may help improve and refine contemporary models of conscious perception such as the Global Neuronal Workspace.

Del Cul, A., Baillet, S., & Dehaene, S. (2007). Brain dynamics underlying the nonlinear threshold for access to consciousness. *PLoS biology*, 5(10), e260.

Disclosures: C. Dembski: None. K. Ortego: None. C. Steinhilber: None. M. Cohen: None. M. Pitts: None.

Poster

487. Neural Mechanisms of Unimodal Perception

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 487.09

Topic: H.02. Perception and Imagery

Title: Exploring a new subregion of the posteromedial cortex (Gyrus S) and its involvement in representing sense of bodily self with intracranial EEG and direct electrical stimulation in the human brain

Authors: *D. LYU, J. PARVIZI;
Stanford Univ., Palo Alto, CA

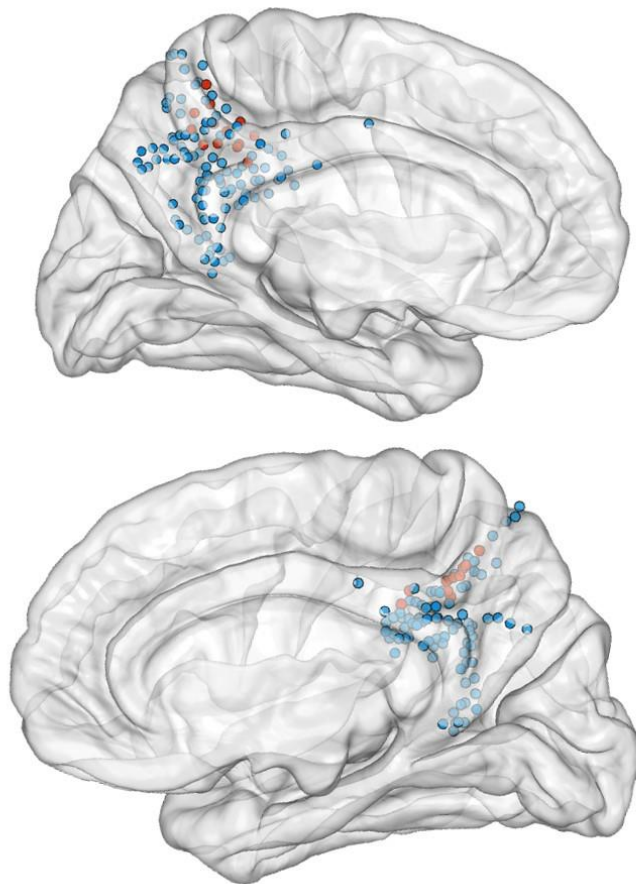
Abstract: Exploring a new subregion of the posteromedial cortex (Gyrus S) and its involvement in representing sense of bodily self with intracranial EEG and direct electrical stimulation in the human brain

Dian Lyu and Josef Parvizi

Self-dissociation is an altered state of consciousness where the integrity of the sense of self is disrupted. We recently reported a case of self-dissociation due to seizures stemming from the posteromedial cortex (PMC) and by the electrical stimulation of the epileptic tissue or its homologues region in the contralateral hemisphere. Along with the previous single case, we explored the phenomenology of self-dissociation in 5 more human subjects who have no epileptic abnormality in their PMC. Using direct bipolar 50Hz electrical stimulation of different sites within the PMC in each individual subject's brain, we confirmed that the altered sense of bodily self is only caused when the stimulation involves the anterior PMC within the mid-region of an S-shaped vertical gyrus (hence, Gyrus-S). We next used resting-state fMRI and seed-based functional connectivity (FC) analysis in the native brain space as well as intracranial 0.5Hz single pulse electrical stimulation (SPES) to compare the connectivity of PMC sites whose stimulation caused self-dissociation versus those that did not. Our findings showed a clear

distinction in the profile of cortical and thalamic connectivity of the Gyrus-S, which marked an important functional watershed from the neighboring PMC areas. In brief, self-dissociation-eliciting sites (i.e., hot zones) are not part of the default mode (DM) network, but causally and bilaterally connected with DM, somatomotor, limbic, ventral attention networks, as well as the anterior nuclei of the thalamus and pulvinar but not the mediodorsal nucleus. Our findings feature a special anatomical and functional landscape of an unexplored region of the PMC (Gyrus S) that is distinct from a hub of DMN but causally related to it.

A. Spatial localization of hot (red) and cold (blue) electrodes.



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Poster

487. Neural Mechanisms of Unimodal Perception

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Program #/Poster #: 487.10

Topic: H.02. Perception and Imagery

Support: UCLA Faculty Research Grant
APA Dissertation award

Title: Specialized neural mechanisms for self-recognition from whole-body movements

Authors: *A. KADAMBI¹, G. ERLIKHMAN¹, M. JOHNSON¹, M. IACOBONI², M. M. MONTI¹, H. LU¹;

¹UCLA, Los Angeles, CA; ²Psychiatry, UCLA Sch. of Med., Los Angeles, CA

Abstract: Humans can identify their actions from point-light displays of the whole body, even when the actions are visually degraded and depicted in unfamiliar viewpoints. To date, we lack a direct test of the underlying neural circuitry for self-action recognition of the whole body. In the present study, across two sessions, we motion-captured a range of actions from 20 participants, who returned after a delay period for functional neuroimaging. Using univariate, multivariate, and connectivity analyses, we found that self-processing of own actions recruited the action observation network (AON) spanning the frontoparietal to the temporooccipital regions. The frontoparietal regions showed increased activity and functional connectivity to the temporooccipital regions during self-processing, while the temporooccipital regions primarily decoded identity regardless of self-specificity. The featural space of the temporooccipital regions appeared to further represent lower-level features related movement dynamics and body structure when encoding the identity of actions. The findings together suggest a specialized neural circuitry for whole-body self-recognition of actions, with prioritized roles of frontoparietal regions of the AON for self-recognition.

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Poster

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Title: Beta and alpha entrainment preferentially enhance seen and unseen information

Authors: *P. CHENG¹, S. SANKARANARAYANAN², S. DAVIES¹, J. FRAGETTA¹, S. GROVER¹, W. WEN¹, D. SOTO³, R. REINHART¹;

¹Psychological and Brain Sci., ²Biomed. Engin., Boston Univ., Boston, MA; ³Brain and Language, Basque Ctr. on Cognition, San Sebastian, Spain

Abstract: Understanding the causal neural architecture of human conscious experience remains one of the most challenging endeavors in all of psychology and neuroscience. Neural rhythms have been a major mechanism under study in consciousness research, and correlated with both seen and unseen information. Here, we examine whether rhythms play a causal role in visual consciousness. Further, previous research emphasized the difference in rhythmic scope depending on access to consciousness: long-range synchronized rhythms were associated with conscious processing, and local rhythms with unconscious processing. We explore an uncharted territory in consciousness research - rhythmic frequency. In particular, we investigated whether specific frequency bands selectively modulate most relevant information depending on conscious access. Guided by electrical field modeling, we developed a novel high-definition transcranial alternating current stimulation (HD-tACS) protocol in a between-participants ($n = 72$; 29 men; mean age: 20.56 years \pm 0.41), sham-controlled experiment to entrain non-harmonic beta (20Hz), alpha (11Hz) or theta (6Hz) activity in primary visual cortex, and implemented a novel modification of a rated backward masking paradigm containing gatings. Surprisingly, we revealed dissociable causal mechanisms for improving seen and unseen information. Specifically, entraining beta rhythms enhanced discriminability for orientation information only when it was seen. By contrast, entraining alpha rhythms enhanced discriminability for orientation information only when it was unseen. Theta and sham conditions did not see the effect. The perceptual enhancements were rapid, long-lasting and frequency specific. Signal detection analyses revealed that the enhancements were restricted to orientation information, which was most relevant to task performance. These results demonstrate, for the first time, casual and dissociable rhythms depending on conscious access. The benefit of beta modulation on information available to consciousness is the first causal evidence, and consistent with theories of consciousness. However, the benefit of alpha modulation on information unavailable to consciousness is not predicted by any theory of consciousness. Therefore, our findings call for critical modifications to consciousness theories.

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Poster

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Title: Mirror exposure intervention improved an activation in the left superior parietal lobule: preliminary results from a randomized controlled trial

Authors: *Y. HAMAMOTO, K. OBA, R. ISHIBASHI, Y. DING, R. NOUCHI, M. SUGIURA; IDAC, Tohoku Univ., Sendai, Japan

Abstract: Body image disturbance, overestimation and negative evaluation of one's body, is one of the biggest risk factors for eating disorders, which is examined as the gap between perceived-self and actual body sizes (overestimation) and the gap between perceived-self and ideal body sizes (negative evaluation). It is implied that exposure to one's body using mirrors (mirror exposure) can reduce body image disturbance by promoting functional body image processing (e.g., how a body part works) instead of emotional processing. However, the neural correlates of the mirror exposure effect remained unclear. Thus, we investigate its neural correlates: we hypothesized that the mirror exposure would reduce brain activation related to negative evaluation of one's body and increase activations related to body location, shape, and movements. We recruited 24 healthy right-handed young females and equally assigned them to two intervention groups by block randomization: the mirror exposure (ME) and the control (CT) groups. Participants in the ME looked at their bodies in the mirror and described their body parts using terms related to color, texture, shape and so on (e.g., My palm is pink) instead of using critical or subjective terms such as beautiful, big or fat. Participants in the CT described their bodies in the same way as the ME by imaging their bodies instead of using the mirror. Both interventions took 40 minutes and participants performed either intervention once. Before and after the intervention, we measured fMRI data while participants evaluated their actual and ideal body size using their silhouette images distorted only in width, which provided behavioral index of body image disturbance. The fMRI data from the evaluation of one's actual and ideal body were pooled to investigate body image processing and contrasted against the baseline. Change in body image disturbance pre- and after post-intervention was the primary outcome and change in fMRI data was the secondary outcome. There was no significant change in body image disturbance by the mirror exposure (overestimation: $p = 0.41$, negative evaluation: $p = 0.09$; Student t-test). The increase of activation in the left superior parietal lobule (SPL) was greater in the ME after the intervention compared with the CT (ME[post-pre] > CT[post-pre], $p < 0.05$ FWE corrected at cluster level); activation of the left SPL in post increased in the ME while decreased in the CT compared with pre. The lack of significant behavioral improvements may be due to the insufficient length of the mirror exposure. The activation in the left SPL by the mirror exposure was consistent with our hypothesis given the role of this region in visuospatial processing.

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Poster

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Title: Comparative analysis of mental simulation and motor execution during a serial reaction time task using a probabilistic structure and EEG

Authors: *P. SILVA DE CAMARGO¹, A. GALVES², A. FRAZÃO HELENE³;
¹Inst. of Psychology, ²Inst. of Mathematics and Statistics, ³Dept. of Physiology, Inst. of Biosci., Univ. of São Paulo, São Paulo, Brazil

Abstract: Mental Simulation (MS) is characterized by the performance of an action only in the pre-execution stages, avoiding sensorial feedback effects, while Motor Execution (ME) occurs with motor activation and sensory feedback. In the present study we propose to evaluate in a Serial Reaction Time Task using a sequence of stimulus generated by a probabilistic structure defined by a context tree, the imagined/motor performance, and the electroencephalography measurements. We aim to evaluate the impact on probabilistic structure performance in absence of sensorial feedback, analyzing MS and ME groups reaction times (RT) and EEG event-related potentials, considering the specific single stimulus probability and the probability considering the last time that it occurred on the last stimulus appearance. The experiment (CAAE 15274718.8.0000.5464) involved healthy volunteers divided into ME (n=10) and MS (n=10) Groups. Edinburgh Handedness Inventory (EHI) and Kinesthetic/Visual Motor Imagery Questionnaire (KVIQP-10, Brazilian version) were applied. The task consisted of 750 auditory stimuli into 5 blocks. Each stimulus was answered with a specific button or by imagined action of pressing the correct button. MS executed a contralateral stereotyped action to trigger imagination. The probability of occurrence of each one of 3 possible auditory stimuli follows a context tree that imposes 25%, 75%, or 100% of individual probability. A GLM univariate ANOVA suitable for a Poisson distribution was applied, followed by a post-hoc Tukey test. Groups showed compatible scores on EHI (ME:82,73±13,75; MS:86,51±15,3) and KVIQP-10 (Kinesthetic - ME:18,4±3,53; MS:14±4,06 / Visual - ME:19,3±2,83; MS:14,4±4,84). The RT analysis concerning the probability of each stimulus showed - ME: effects of Probability (F19,3=33,225,p<0,001), Block (F19,4=3,220,p=0,012), and Block/Probability (F19,12=1,900,p=0,030); MS: effects of Block (F19,4=141,933,p<0,001) and Probability (F19,3=15,331,p<0,001). Performance as a function of the impact of the past probability associated of the last time the same stimuli appeared showed - ME: effects of Probability (F7,3=15,789,p<0,001) and Probability/Last Transition (F7,3=9,405,p<0,001); MS: effects of Probability (F7,3=8,584,p<0,001) and Probability/Last Transition (F7,3=7,490,p<0,001). The performance of the MS compared to the ME presented a learning curve of the sequence much more compatible with the context tree. Such an experimental model seems to offer a consistent preparatory approach to the way the Nervous System deals with the contingencies imposed by the probabilistic framework, without sensorial feedback effects.

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Poster

487. Neural Mechanisms of Unimodal Perception

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Program #/Poster #: 487.14

Topic: H.02. Perception and Imagery

Title: Differences in imagery of dominant versus non-dominant hand movements: An electroencephalographic investigation

Authors: ***K. LAMBERT**¹, **C. DONOFF**¹, **J. ELKE**¹, **C. R. MADAN**², **Y. Y. CHEN**³, **A. SINGHAL**¹;

¹Univ. of Alberta, Edmonton, AB, Canada; ²Psychology, Univ. of Nottingham, Nottingham, United Kingdom; ³Dept. of Neurosurg., Baylor Col. of Med., Philadelphia, PA

Abstract: How we interact with our surroundings is thought to be driven by mental representations of our bodies. A popular way to examine these presentations is through motor imagery, which refers to the imagination of a motor act in the absence of its physical performance. This internal process can occur in two different modalities: visual and kinaesthetic. The visual modality emphasizes ‘seeing’ the imagined movement, and is associated with increased activity in the alpha rhythm (8-14 Hz) measured over the occipital regions. The kinaesthetic modality emphasizes ‘feeling’ the imagined movement, and is associated with decreased activity in the mu rhythm (8-14 Hz) measured over the sensorimotor cortices. These two modalities can be engaged in isolation or in combination with one another. We recorded continuous EEG activity while 37 participants (17 left-hand dominant, 20 right-hand dominant) completed an objective motor imagery task of fine motor hand movements. The task was evenly divided into imagery of left-hand and right-hand movements. Right handers exhibited significant differences in activity between occipital regions and motor regions only during imagery of left-hand (non-dominant hand) movements. This difference was not observed during imagery of right-hand (dominant hand) movements. Left handers showed the opposite pattern, exhibiting significant differences between the two regions only during imagery of right-hand (non-dominant hand) movements. Additionally, a strong positive correlation was found between alpha and mu activity. Given that decreased mu is associated with kinaesthetic motor imagery and increased alpha with visual motor imagery, this correlation suggests a trade-off between the two modalities. These findings indicate that individuals imagine movements with their non-dominant hand differently from movements with their dominant hand.

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Poster

487. Neural Mechanisms of Unimodal Perception

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Program #/Poster #: 487.15

Topic: H.02. Perception and Imagery

Title: A retinotopic reference frame aligns visual and memory systems

Authors: *A. STEEL¹, E. H. SILSON², B. D. GARCIA¹, C. E. ROBERTSON¹;

¹Dartmouth Col., Hanover, NH; ²Psychology, Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: We encode the visual world retinotopically, imposing a spatial reference frame on visual information processing. However, models of brain organization generally assume that retinotopic coding is replaced by abstract, amodal, non-retinotopic codes as information propagates through the visual hierarchy towards memory systems. This view raises a puzzle for constructive accounts of visual memory: how can mnemonic and visual information interact effectively if they are represented in different reference frames? To address this question, participants (N=15) underwent population receptive field (pRF) mapping during fMRI. As expected, we observed retinotopic coding throughout the visual system, including the scene perception areas (occipital place area (OPA) and parahippocampal place area (PPA)). Critically, consistent with prior work, we observed robust and reliable pRFs just beyond the anterior edge of visually-responsive cortex in high-level areas previously considered amodal, including anterior ventral temporal and lateral parietal cortex. A large proportion of these high-level pRFs were located immediately anterior to scene-selective visual areas OPA and PPA, in memory-responsive cortex (Steel et al, 2022). To better characterize these anterior pRFs, we localized each participant's OPA, PPA, and place memory areas (lateral (LPMA) and ventral (VPMA)) (Steel et al, 2022). Unlike visual areas OPA and PPA that contained almost exclusively prototypical positive pRFs, we observed a striking inversion of pRF amplitude in memory areas LPMA and VPMA, such that they exhibited spatially-selective negative BOLD responses. The visual field representation of negative pRFs in mnemonic areas closely matched their perceptual counterparts', suggesting a common reference frame between perceptual and mnemonic regions. Finally, during a visual memory task, trial-wise activity of the positive and negative pRFs within the perceptual and memory areas was negatively correlated, suggesting a competitive push-pull dynamic between these neural systems. These results suggest that retinotopic coding, a fundamental organizing principle of visual cortex, persists in high-level, mnemonic cortex previously considered amodal. This shared code may provide a robust communication system aligning these neural systems.

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Poster

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Topic: H.02. Perception and Imagery

Support: BioTechMed-Graz Young Researcher Group Grant

Title: Parietal area IPS0/V7 represents illusory shapes in a topographic manner

Authors: *A. ARSENOVIC^{1,2}, A. ISCHEBECK^{1,2}, N. ZARETSKAYA^{1,2};

¹Dept. of Cognitive Psychology and Neurosci., Inst. of Psychology, Univ. of Graz, Graz, Austria;

²BioTechMed-Graz, Graz, Austria

Abstract: Visual illusions give rise to percepts that are more complex than the incoming sensory information, thereby exposing the inferential nature of perception. It has been shown that early visual cortex (EVC) represents not only physical input, but also illusory content of perception. Specifically, during perception of an illusory shape, the topographic representation of the shape's surface is enhanced, while the regions surrounding it are either suppressed or unchanged. Recently, we have shown that the topographic maps of the intraparietal sulcus (IPS), respond to illusory Kanizsa figures [1]. However, it remains unclear if these parietal maps contain a fine-scale activity pattern like the one observed in the EVC. To test this, we measured brain activity from 30 healthy human participants using functional magnetic resonance imaging (fMRI). The participants performed a central detection task with two difficulty levels while passively observing a surrounding configuration of four flickering inducers. The inducers formed either a bilateral illusory diamond, a unilateral illusory triangle, or no illusory figure. An additional functional localizer scan allowed us to identify voxels uniquely representing subregions of the visual space (either the area corresponding to the illusory surface or the background) and define them in the EVC (V1, V2, V3) and in a posterior parietal topographic region IPS0/V7 in most participants ($n = 28$). We then tested for a subregion-specific illusory response modulation, as well as its dependence on task difficulty within the EVC and IPS0 in the main experiment. Our results confirmed the previously reported enhanced illusory surface and suppressed background activity within all areas of the EVC. Crucially, we also observed a differential response across subregions within IPS0/V7, which exhibited the illusory surface enhancement, but no background suppression. None of the subregion-specific responses were affected by the task difficulty. Our results thus demonstrate that fine-scale topographically specific illusory shape responses are present far beyond the EVC, within the topographic map IPS0/V7 of the intraparietal sulcus. They also show that there are some differences in how higher-level topographic maps separate illusory content from the surround compared to the EVC. While both show an enhancement in the representation of illusory surface, in contrast to the EVC IPS0/V7 exhibits no background suppression. [1] Arsenovic, Ischebeck & Zaretskaya (2022). *bioRxiv*. <https://doi.org/10.1101/2022.04.12.488016>.

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Poster

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Topic: H.02. Perception and Imagery

Title: An interoceptive perspective of time perception in spinal cord injury.

Authors: *M. L. DE MARTINO^{1,2}, E. LEEMHUIS², A. SCUDERI^{1,2}, M. PAZZAGLIA^{1,2};
¹Sapienza Univ. of Rome, Rome, Italy; ²Fondazione Santa Lucia - IRCCS, Rome, Italy

Abstract: How implicit aspects of time perception can be related to interoception remains still relatively unexplored. Interoceptive states of the body would create our experience of duration. The physiological sense of time would thus be associated with the temporal integration of signals from the interoceptive system. Here, we investigate and relate changes in temporal expectation and interoception in cases of brain-body disconnection. 32 participants with spinal lesions (age range: 19-46 years) in the chronic injury phase were grouped according to level of spinal cord lesion, resulting in two groups: 16 patients with cervical lesion and 16 patients with thoracic lesion. Interoceptive accuracy was measured using a heartbeat tracking task consisting of four trials with varying interval durations of 25, 35, and 45 seconds played in a randomized order. Participants were instructed to internally count the heartbeats they perceived and report the number at the end of each trial. The actual number of heartbeats was measured using electrocardiographic signal. Physiological sense of time was measured using temporal expectation task (TT) and controlled by a color control task (CT). Briefly, a white circle (diameter: 12.8 degrees of visual angle) was shown on a gray background, with a gray annulus superimposed at an eccentricity of 6.4 - 9.6 degrees of visual angle, acting as an occluding band. In each trial, a purple ball moved from the center of the display toward the periphery in a fixed direction and at a constant speed. In the TT, participants were then asked whether the ball re-emerged earlier or later than expected. In the CT, participants assessed whether the ball reappeared more reddish or bluish than before. The Mann-Whitney U test indicated that the cervical lesion group shows lower interoceptive accuracy than the thoracic lesion group ($p < 0.01$). Therefore, higher deafferentation in SCI patients can decrease interoceptive accuracy. Interoceptive accuracy was also significantly correlated with the difficulty in temporal (0.69; $p < 0.002$) but no control color task ($p < 0.17$) as discriminated by temporal offsets. Our findings suggest that a reduced interoceptive accuracy play a role in the subjective perception of time in participants with higher SCI lesions. The predictive nature of our temporal task is closely related to the predictive feelings depending on bodily signals. Thus, a lower ability to detect and update the interoceptive inputs seems to reflect a distortion in perceiving pulses emitted by the “pacemaker” of the human internal clock. Alignment of interoceptive and temporal dimensions is consistently with the theoretical framework of the *embodied* models of timing.

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Title: Body representation changes after repeated lower-limb exoskeleton use in patients with a chronic spinal cord injury.

Authors: *E. LEEMHUIS¹, S. TRANQUILLI¹, G. SCIVOLETTO¹, L. DE GENNARO², A. GIANNINI², M. PAZZAGLIA^{2,1};

¹Fondazione Santa Lucia IRCCS, Rome, Italy; ²La Sapienza, Univ. of Rome, Rome, Italy

Abstract: Exoskeleton use in patients with spinal cord injury may bring health benefits but little information is available about its impact on body perception and cognition. Aim of the study is to explore how repeated use of a powered lower-limb exoskeleton modulates the user’s body representation. **Methods:** Exo group: 13 SCI patients (all male, mean age 43 ± 14.54 y; time since lesion: 11 ± 6.34 years). Lesions ranged from thoracic (T3) to lumbar (L2): 7 participants had complete lesions (ASIA Impairment Scale [AIS] A), and 6 patients featured sensory and motor incomplete (AIS B) lesions. This group followed an exoskeleton assisted training program (8 sessions in 4 weeks). SCI control group: the group was selected to match the experimental group (mean age 44 ± 14 ; years since lesion: 8 ± 8.22 years; all p ’s > 0.4). Lesions range was T3-L1 with 8 AIS A and 5 AIS B. Control activities consist in passive movements, standing, and strengthening the upper part of the body. Healthy controls: similar age to SCI groups, 8 session wheelchair training program. The body image task (BIT) - administered before (T0) and after (T1) the training period- collects quantitative measures of perceived body spatial organization. On a touchscreen, the participants indicate the location of target body part using an anchor image (head or feet) as a reference. The trials include 14 targets (e.g. left elbow or right knee) for a total of 4 blocks of 52 trials each. Body targets are presented on the screen in written form. Then, an anchor image appears randomly in one of four locations. The participants touch the screen to record the perceived position of the body target. **Results:** Measurements are expressed as the difference between the perceived length, width, or height and true body sizes as a proportion of the true size (distortion index). To compare the body distortion patterns of the rehabilitation programs, a mixed-model analysis of variance (ANOVA) with a T0/T1 condition, body part sizes as within-subject factor, and group as between-subject factor was used. The exoskeleton group perceived hips as narrower (difference T0-T1 ratio = 0.39 to 0.33; $p < 0.017$), lower arms longer (difference T0-T1 ratio = -0.21 to -0.10; $p < 0.0001$), and upper legs longer (difference T0-T1 ratio = -0.38 to -0.25; $p < 0.0008$). The changes go in the direction of real body proportions. **Conclusion:** exoskeleton use can modulate perception of specific body parts. Active control of the exoskeleton (agency), jointly with postural and sensorimotor changes, may facilitate wheelchair dis-embodiment. These events may promote the emergence of body representations formed before the injury.

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Poster

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Title: Mental rotation of body parts and embodied assistive tool in patients with spinal cord injury

Authors: *F. FAVIERI^{1,3}, G. FORTE^{2,3}, A. GIANNINI¹, M. PAZZAGLIA^{1,3};

¹Dept. of Psychology, ²Dept. of Dynamic and Clin. Psychology and Hlth. Studies, Sapienza Univ. Di Roma, Rome, Italy; ³Body and Action Lab., IRCCS SANTA LUCIA FOUNDATION, Rome, Italy

Abstract: The adoption of innovative assistive wearable robotics (WR), including exoskeletons (EXO) as gait rehabilitation and assistance after SCI has an impact on brain reorganization influencing body representation, body ownership and sense of agency. Traditionally, researchers have studied embodiment of assistive tool by quantifying how users interact with the environment with a focus on wheelchair and the improvement on motor imagery after training. We investigated individuals with spinal cord injuries (SCI) included that participated to an EXO training to find out: 1. how the loss of sensory sensitivity and motor function in the body part below the level of injury influences body imagery; 2. if a user reacts to rotation of an assistive tool in a similar way they might react to rotation of their own body. To this aim a group of 20 male patients with SCI (lesion from T4 to L2, time since lesion: 11 ± 6.34 years) was compared to 20 age matched healthy control considering differences and impact of training in body representation and embodiment of biological (i.e., lower and upper limbs) and non-biological (i.e., assistive tools: wheelchair, EXO) objects. A mental rotation task adopting the two categories of objects (biological; non biological) were administered (16 practice trials; 48 experimental trials). Participants verbally indicated whether the stimulus, with four different orientations (0° ; 90° ; 180° ; 270°) was presented in left or right side despite spatial orientation. Reaction times (RTs) were collected. Two measurements were collected for both the groups (T_0 and T_1). For SCI patients T_1 took place after assisted EXO training program (8 sessions in 4 weeks) Mixed ANOVAs on RTs considering Between Groups differences and Within factors (type of stimulus; measurement times) were carried out. No significant between-group differences in general RTs emerged, but faster RTs in biological limb rotation in both the groups were highlighted. Interestingly, healthy participants performed slower RTs than SCI in wheelchair and lower limbs rotation. Moreover, within-group differences in SCI highlighted a significant effect of training in RTs of EXO rotation (RTs of $T_0 >$ RTs of T_1). The study highlighted two main results. Firstly, the pattern of response times to stimuli at various orientations suggests that SCI patients didn't show a linear increase in reaction time as a function of rotation angle of the foot pictures; and secondly, the improvement of EXO rotation indicated a positive effect of training in WR embodiment, which can promote a positive effect of both motor and sensory rehabilitation.

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Poster

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Title: The challenge of neuropathic pain in Spinal Cord Injuries: the advances of exoskeleton use.

Authors: *G. FORTE^{1,3}, F. FAVIERI^{2,3}, S. TRANQUILLI^{2,3}, A. GIANNINI², M. PAZZAGLIA^{2,3};

¹Dept. of Dynamic and Clin. Psychology and Hlth. Studies, ²Dept. of Psychology, Sapienza Univ. Di Roma, Roma, Italy; ³Body and Action Lab., IRCCS SANTA LUCIA FOUNDATION, Rome, Italy

Abstract: Spinal Cord Injuries (SCIs) include alteration of the somatosensory system and development of chronic and neuropathic pain. Although various treatments are available, pain is refractory in many people with SCIs since it appears to be related to somatosensory cortices changes. The need for better access to effective non-invasive treatment options to alleviate pain makes the effect of powered exoskeletons on pain of particular interest. Since training with an exoskeleton has been found to affect cortices organization, possibly reverting maladaptive plasticity, we hypothesized that intensive long-term powered exoskeleton training could positively affect pain perception and the intensity of neuropathic pain deafferentated body parts. Twelve patients with complete (n=5) and incomplete (n=8) traumatic SCI in a chronic pathology phase participated in a weekly two-hour training program with the exoskeleton. We analyzed the variation of Tactile Threshold (TT) and pain intensity, collecting data both before the beginning of the training protocol and after nine training sessions to assess long-term changes. TT was detected through a computerized pressure algometer, while pain intensity was measured by a Numerical Rating Scale (NRS). We observed a significant reduction in the TT and pain intensity after the training. Specifically, we found tactile perception to have a strong improvement in the upper part of the leg but not in the lower. These results sustain the idea of neuropathic pain as a consequence of maladaptive plasticity. Indeed, the prolonged use of the exoskeleton would allow the stimulation of spared sensitive fibres and the upload of the altered body image. This would re-establish a functional cortical organization, particularly for the upper part of the legs, which encounter major changes from the wheelchair to the exoskeleton. Modern assistive Wearable Robots are more flexible and powerful than traditional rehabilitation devices; this could be helpful in the clinical challenge of user-centred neuroprosthetic technologies, which is a key element for natural learning. These potential aspects should also be used to improve the chronic complications of SCI, such as pain.

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Poster

487. Neural Mechanisms of Unimodal Perception

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 487.21

Topic: H.02. Perception and Imagery

Support: cod. RF 18.82 "Ministero della Salute"

Title: Rebuilding the body from the inside: interoception and the bodily self following spinal cord injuries.

Authors: *A. SCUDERI^{1,2}, M. L. DE MARTINO¹, E. LEEMHUIS², M. PAZZAGLIA^{1,2};
¹Sapienza, Univ. of Rome, Rome, Italy; ²Fondazione Santa Lucia IRCCS, Rome, Italy

Abstract: Spinal cord injury (SCI) significantly impacts body awareness by disrupting brain-body communication. Attempts have been made to enhance the sense of self-identification with the body post-SCI by intervening at a multisensory level. However, the contribution of the interoceptive domain remains largely unaddressed. We explored the role of interoception in modulating the sense of body ownership following high or low thoracic SCI. This distinction allowed us to discriminate in terms of visceral and cardiac interoceptive afferents spared. We selected male participants in the chronic phase of the disease divided into two groups: 15 patients with T1-T4 lesions, mean age 33 ± 7 years, mean time since lesion 869 ± 512 days; 15 patients with T8-T12 lesions, mean age 36 ± 8 years, mean time since injury 906 ± 479 days. The level of the neurological lesion was determined by using AIS grade. Exclusion criteria were the presence of brain injury, head trauma, neurological, cardiovascular, or psychiatric diseases. Our procedure included physiological, behavioural and metacognitive measurements. Body ownership changes were assessed using the Rubber Hand Illusion (RHI) paradigm (comprising RHI questionnaire and proprioceptive drift assessment) with simultaneous recording of cardiac activity. The interoceptive accuracy index was obtained via the Heartbeat Tracking Task. Then, the degree of interoceptive awareness was rated using a 10-point VAS (0=no heartbeat awareness, 10=full awareness). The Cambridge Depersonalisation Scale (CDS) was used to investigate the presence of any symptoms of depersonalisation or derealisation. The Group T1-T4 exhibited: stronger proprioceptive drift ($F(1,28) = 6.2, p = 0.02, \eta^2 > 0.18$); reduced interoceptive accuracy ($z(15) = 3.73, p = 0.0001$); higher CDS scores ($z = -2.47, p = 0.014$). Interoceptive accuracy correlated negatively with CDS score. Our data revealed a link between autonomic completeness and the level and severity of SCI. We exclude that our findings depend on impaired proprioception or posture. No differences were found between the two groups in global body ownership, but patients in the T1-T4 group were more sensitive to multisensory stimulation of the RHI than those with low lesions. As the bodily experience relies on various signals, several factors may be at play, ranging from vision to interoception. Enhancing interoceptive information thus will be the next step in enhancing and normalising the bodily experience post-SCI.

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Poster

487. Neural Mechanisms of Unimodal Perception

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Topic: H.02. Perception and Imagery

Title: Temporal attention-mediated enhancement of N60 toward facial affect: From the perspectives of awareness and trait anxiety

Authors: *M. SUZUKI¹, T. URAKAWA², T. IMANI¹, O. ARAKI¹;

¹Dept. of Applied Physics, Tokyo Univ. of Sci., Tokyo, Japan; ²Integrated Control Syst. Develop. Div., Mazda Motor Corp., Hiroshima, Japan

Abstract: Our previous study showed that temporal attention-mediated augmentation of N60 (a negative VEP at a latency of about 60 ms) is relevant to the facilitation of subliminal affective priming (Imani et al., 2020). This result raises an important question of whether the N60's augmentation was related to visual awareness of the subliminal primer. This is because the latency of N60 is much earlier than the previously reported spatial attention-mediated enhancements of N1/N2 at the latency of 130-300 ms (Koivisto et al., 2009). The purpose of the present study is to clarify whether the temporal attention-mediated augmentation of N60 reflects the visual awareness for the target. Furthermore, considering the influence of affective faces, we attempted to clarify if the personal trait anxiety influences N60 and the awareness. In our experiments, a backward masking paradigm was used; the target (happy or fearful face) was presented for 20 ms and followed by a mask image (neutral face). To control temporal attention, a visual cue indicating the timing of the target's presentation was presented at the beginning of each trial. Participants (24 males, age 22.2 ± 1.26 (mean \pm SD)) were asked to report the perceived affect of the target and rate the levels of confidence for their reports. Results showed that the confidence level of visual awareness was significantly higher with temporal attention (Two-way repeated-measured ANOVA; $P < 0.05$), but the mean N60 amplitude was not changed by the temporal attention. Inter-individual analyses for the fearful target to which the temporal attention was paid showed a significant positive correlation between enhanced visual awareness and N60's enhancement ($\rho(17) = 0.569$, $P < 0.05$). This finding means that the visual consciousness enhanced by temporal attention is reflected in the early VEP at about 60 ms after the target's onset. On the other hand, in terms of trait anxiety, inter-individual analyses for the temporally attended fearful target showed a significant negative correlation between the STAI-T score (a measure of trait anxiety) and N60's enhancement ($\rho(20) = -0.501$, $P < 0.05$), and they also showed a significant negative correlation between the STAI-T score and enhanced visual awareness ($\rho(20) = -0.563$, $P < 0.05$). These results suggest that N60's enhancement and enhanced visual awareness by temporal attention are influenced by trait anxiety. Since none of the correlations were significant for happy faces, it is suggested that amygdala-mediated processing evoked by a fearful face modulates the relation between the attention-mediated N60 and visual awareness.

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Poster

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Program #/Poster #: 487.23

Topic: H.02. Perception and Imagery

Title: Effect of change-related response on access processing to visual awareness

Authors: *Y. KURITA¹, T. URAKAWA², K. UENO¹, M. MITANI¹, O. ARAKI¹;

¹Dept. of Applied Physics, Fac. of Sci., Tokyo Univ. of Sci., Tokyo, Japan; ²Integrated Control Syst. Develop. Div., Mazda Motor Corp., Hiroshima, Japan

Abstract: In binocular rivalry, for a change of visual stimulus, change-related response (e.g. N1) was more enhanced when the perception is altered than when the perception is not altered (Veser et al., 2008). On the other hand, a previous study showed a significant positive correlation across participants between enhancement of visual mismatch negativity (vMMN) and facilitation of perceptual alternation on binocular rivalry when the deviant stimulus was presented unconsciously (Kurita et al., 2021). While the unconscious deviant stimulus significantly promoted perceptual alternation, the conscious one suppressed it. These results showed that vMMN, which reflects automatic visual change detection of temporal regularity, is relevant to the access processing in which the unconscious stimulus is consciously perceived. The purpose of this study is to clarify whether the amplitude of change-related N1 also correlates enhancement of perceptual alternation in binocular rivalry. In the present study, we focused on inter-individual variability between N1 and the proportion of perceptual alternation from before to after the presentation of visual stimulus changes. In stimulation, we continuously presented a sinusoidal grating and abruptly changed its orientation by 90 degrees (a change without temporal regularity) during binocular suppression using the modified experimental paradigm of our previous study. Under this scheme, we recorded N1 amplitude/latency and calculated the proportion of perceptual alternation in binocular rivalry. Nineteen healthy volunteers (14 males and 5 females, age 22.0 ± 1.49 (mean \pm SD)) participated in this study. All participants' data for N1 amplitude/latency and the proportion of perceptual alternation were included within the mean \pm 3SD. Behavioral data showed that the change of the unconscious stimulus more increased the proportion of perceptual alternation than that without a change ($t(18) = 9.47$, $p < 0.01$, Cohen's $d = 2.58$, a post-hoc t -test with a Bonferroni correction). In correlation analyses, the Spearman's rank order correlation coefficient was calculated. As a result, we found that an enhancement of N1 did not significantly correlated with an increase of the proportion of perceptual alternation when the change of the visual stimulus was presented unconsciously (Amplitude: $\rho(19) = 0.265$, $p = 0.272$; Latency: $\rho(19) = 0.236$, $p = 0.330$), which is different from vMMN. These results indicated that change-related N1 is relevant to the access processing. However, the correlational difference in vMMN and change-related responses implies that the underlying neural mechanisms play different roles in the access processing.

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Poster

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Program #/Poster #: 487.24

Topic: H.02. Perception and Imagery

Title: Context-based association in a spreading-activation network composed of Izhikevich neurons

Authors: *H. MATSUMURA¹, O. ARAKI²;

²Dept. of Applied Physics, ¹Tokyo Univ. of Sci., Tokyo, Japan

Abstract: The spreading-activation theory provides a prominent network model in which activation of a concept spreads and activates related concepts through links between them (Collins & Loftus, 1975; Anderson, 1983). Although this model explains many psychological phenomena such as semantic processing and priming, how to implement the spreading activation is an essential issue. Since most of the previous spreading-activation networks are not represented by a neural network model, the calculation method has been an algorithmic procedure (Siew, 2019), which qualitatively differs from neurons in the brain. In biological neural networks, spreading activation in the network depends on the dynamics of neurons and networks. The purpose of this study is to attempt to construct a neural network model with spreading activation, which is composed of Izhikevich model neurons (Izhikevich, 2003) and explain a context-based association as a target function. In the context-based association, the concept “Hard” in the context of “Bread” will remind you of solid bread such as “Baguette”, for example. On the other hand, in the context of “Computer”, “Hard” will recall hardware. In the proposed model, each concept is represented by a cluster which contains 100 Izhikevich neurons (80% excitatory & 20% inhibitory). Some excitatory neurons (60%) in a cluster have synaptic projections on randomly selected 20% neurons in the cluster of a related concept. In addition, every neuron in a cluster has recurrent connections to 5% of neurons in itself. The mean value of connection delays with a stochastic distribution is set to 50 ms. We designed some clusters as follows: an attribute concept “Hard” which was used as a cue to evoke context-based associations; “Bread”, “Computer” and “Work” for contextual concepts; and other concepts “Baguette”, “Muffin”, “HDD”, etc. In computer simulations, inputs to a specific cluster for ignition of activation were realized by less frequent Poisson trains (avg. 10 Hz) with relatively larger amplitude, and background inputs to all neurons were frequent Poisson trains (avg. 50 Hz) with smaller amplitude. After the inputs to one of the context clusters for a second, the cue “Hard” was activated by the Poisson trains for 300 ms. As a result, the populational activity in the cluster relevant to the activated context was larger than the others. This is because the activation in the context spread to neighboring conceptual clusters and they were maintained by reverberation circuits within the cluster’s recurrent connections. It is thought that the nonlinearity

of neurons and the heterogeneous network have an important role in the stable self-organization of reverberation.

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Poster

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Topic: H.02. Perception and Imagery

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Title: Cortical synchronization at the theta-band is relevant to exogenously-driven perceptual alternation of the binocular rivalry: A transcranial alternating current stimulation study

Authors: *H. GOTO¹, T. URAKAWA², Y. KURITA¹, K. UENO¹, O. ARAKI¹;

¹Dept. of Applied physics, Tokyo university of science, Tokyo, Japan; ²Integrated Control Syst. Develop. Div., Mazda Motor Corp., Hiroshima, Japan

Abstract: Visual mismatch negativity (vMMN), which is evoked by violating an established regularity in a sequence of visual stimuli, is postulated to induce exogenous orienting of attention (e.g., Urakawa et al., 2010). An enhancement of vMMN which was evoked by an unconsciously presented deviant was relevant to the exogenously-driven perceptual alternation of the binocular rivalry (Kurita et al., 2021). The present study further focused on the θ -band (4-8 Hz) cortical phase synchronization centered on the occipital area which reflects vMMN (Wu et al, 2020). Specifically, by using transcranial alternating current stimulation (tACS) at 7 Hz (a frequency within the θ -band) over the occipital area, we evaluated whether the θ -band network phase synchronization invoked by an unconsciously presented deviant would be relevant to the exogenously-driven perceptual alternation. In the present experiment, the intermittent binocular rivalry paradigm was employed with an unconsciously presented deviant which was made by changing the grating's orientation in one of the two eyes. Twenty-five healthy adults [19 males, 6 females, age 22.11 ± 1.29 (mean \pm SD)] gave consent to participate in this study. For the functional connectivity analysis, we adopted the phase lag index (PLI). We computed the phase-locked pair of electrodes in which the PLI under the deviant condition was significantly higher than the standard condition [$p < 0.05$, the permutation test (10000 surrogates) with an FDR correction]. The behavioral results showed that the deviant significantly increased perceptual alternation of the binocular rivalry over that by the standard from before to after the unconsciously-presentation of the deviant [$p < 0.01$, A repeated-measures two-way ANOVA with a Bonferroni correction]. Furthermore, the number of a pair of θ -band phase-locked fronto-occipital connection gradually increased after the deviant stimulus was presented. To evaluate the relation between phase-locked pairs and exogenously-driven perceptual alternation, we divided the subjects into two groups according to whether proportion of perceptual alternation

was increased or decreased after the tACS. In the promoted group by tACS modulation (8 subjects), there were significant increase in the number of θ -band phase-locked fronto-occipital connections. Conversely, in the suppressed group (17 subjects), the number of θ -band phase-locked pairs significantly decreased. These consistent results suggest that the θ -band phase-locked fronto-occipital connection invoked by an unconsciously presented deviant plays a key role in the exogenously-driven perceptual alternation of the binocular rivalry.

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Poster

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Topic: H.02. Perception and Imagery

Support: Blattmachr Family
Loughridge Williams Foundation

Title: Modality-independent role of subcortical arousal systems during transient changes in attention

Authors: *A. KHALAF¹, H. BLUMENFELD²;

¹Yale Univ. Sch. of Med., Yale Univ., New Haven, CT; ²Yale Univ. Sch. of Med., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Subcortical arousal systems have been known to play a role in controlling sustained changes in arousal and attention. However, the possible role of these subcortical systems in dynamic changes in attention has not been fully investigated. In fact, most of the studies investigating transient changes in attention focus on cortical networks involved in stimulus salience and top-down control. In this study, we investigate the role of subcortical arousal systems in transient changes in attention across different sensory modalities using block and event-related fMRI paradigms. To identify subcortical networks that are common for all sensory modalities, we analyzed fMRI data collected while the subjects (N=1556) are performing visual, auditory, tactile, and taste perception tasks. Ten tasks (5 visual, 2 auditory, 2 taste, 1 tactile) selected from five public datasets were employed for the analysis. The public datasets included Human Connectome Project dataset (visual and auditory tasks), UCLA Consortium for Neuropsychiatric Phenomics LA5c dataset as well as three datasets collected at Yale University (taste perception task), Glasgow University (auditory task), and the Hebrew University (tactile task). We performed a model-free fMRI analysis by calculating percentage change in BOLD fMRI signal for the whole brain. To identify the statistically significant changes in percentage change BOLD signals with respect to the baseline, we employed a spatiotemporal cluster-based permutation test. A conjunction analysis was performed on the statistically thresholded brain

maps to identify the subcortical regions sharing common activity across different tasks and sensory modalities at both block-onset and individual task events. The conjunction analysis revealed a common network of subcortical arousal systems that show transient fMRI increases in midbrain tegmentum, thalamus, nucleus accumbens, nucleus basalis and striatum. Identifying such subcortical networks is of great importance to understanding fundamental mechanisms of normal attention, and may help facilitate optimal subcortical targeting of therapeutic neuromodulation to restore consciousness in patients with neurological disorders.

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Poster

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Topic: H.02. Perception and Imagery

Support: Blattmachr Family
Loughridge Williams Foundation

Title: Covert detection and investigation of brain networks in auditory perception

Authors: *S. L. AERTS¹, A. MANGLA², S. I. KRONEMER⁴, Q. XIN⁵, T. YADAV³, S. MAJUMDER³, L. KIM², K. L. CHRISTISON-LAGAY⁶, H. BLUMENFELD⁶;

¹Yale Univ. Interdepartmental Neurosci. Program, ³Yale Univ., ²Yale Univ., New Haven, CT; ⁴NIH, NIH, Chevy Chase, MD; ⁵Yale Neurol., Yale Univ. Interdepartmental Neurosci. Program, New Haven, CT; ⁶Yale Univ. Sch. of Med., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Decoupling conscious perception from the post-perceptual processes required to report perception is integral to determining the neural correlates of consciousness itself. Using a visual paradigm, we recently succeeded in disentangling brain activations related to stimulus detection from those associated with overt report using a novel computational approach based on transient changes in pupillometry and eye tracking. Our goal is to explore whether a similar approach can be used to separate brain signals for perception versus reported perception of auditory stimuli. Subjects were presented at-threshold auditory stimuli in one ear and were instructed to report their perception in this target ear only (Report Condition); during the same trial, an asynchronous sound was presented to the nontarget ear (No-Report Condition). Eye metrics (pupillometry, microsaccades and blinks) were recorded throughout. In the Report condition, we found that subjects (n=9) performed well: perception rate was 63% when a target was present; false positive rate was 12% for blank trials; accuracy for perceived target sounds was 82%, and for not-perceived targets was 35% (chance: 33%). Further, we find that eye metrics differed between perceived and not perceived auditory stimuli. Permutation testing between perceived and not perceived pupil response shows significant differences 200-2500ms post stimulus, with larger pupil dilations occurring in perceived stimuli. Future work will use

eye-metric data from the Report condition to train a model to classify trials as perceived or not; this model will then be applied to the data from No-Report stimuli to classify these trials as perceived or not perceived (i.e., under No-Report conditions, overt report is replaced by machine learning-based predictions of stimuli perception). Based on findings in the visual domain, pupil dilation, a pause in microsaccades, and increased eye blink rate are hypothesized to be predictive of perceived auditory stimuli in the absence of report. The classifier will then be used to investigate brain signals (EEG, fMRI) for reported perceived vs. classified perceived vs. not perceived auditory stimuli. If eye metrics can be used to classify auditory perception, it has the potential to be used widely to study conscious perception purified from the confound of overt report.

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Poster

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Topic: H.02. Perception and Imagery

Support: Blattmachr Family
Loughridge Williams Foundation

Title: Subcortical mechanisms of visual perception: preliminary results from intracranial EEG and thalamic stimulation

Authors: *Q. XIN¹, S. I. KRONEMER², S. MAJUMDER³, S. AERTS⁴, D. S. JIN³, A. KHALAF⁵, T. YADAV³, C. J. CHU⁸, I. H. QURAIISHI⁶, M. J. CROWLEY⁷, H. BLUMENFELD⁹;

¹Yale Neurol., Yale Univ. Interdepartmental Neurosci. Program, New Haven, CT; ²NIH, Chevy Chase, MD; ³Yale Univ., ⁴Yale Univ. Interdepartmental Neurosci. Program, ⁵Yale Univ. Sch. of Med., ⁶Yale Sch. of Med., ⁷Yale Univ., New Haven, CT; ⁸Massachusetts Gen. Hosp. / Harvard Med. S, Massachusetts Gen. Hosp. / Harvard Med. S, Cambridge, MA; ⁹Yale Univ. Sch. of Med., Yale Univ. Sch. of Med., New Haven, CT

Abstract: The neural mechanisms of consciousness have broad scientific and clinical implications. Most previous studies have focused on cortical signals that underlie conscious awareness while subcortical signals have been largely neglected despite their known role in arousal. In a previous study, a novel thalamic awareness potential (TAP) that is contingent upon conscious perception was revealed by intracranial recordings (Kronemer et al., 2021). However, it remains unclear whether this signal has a modulatory role in conscious perception. Therefore, to test whether stimulation in the thalamus can augment conscious processing, one patient with

epilepsy who was chronically implanted with depth electrodes targeting the centromedian nucleus of the intralaminar thalamus (Responsive Neurostimulator System, NeuroPace, Inc.) was recruited to complete a report-based visual perceptual-threshold task. The task includes three conditions: (1) no stimulation; (2) thalamic stimulation (300ms, 3.0mA, and 100Hz) delivered during the onset of visual stimulus; (3) delayed stimulation of the same parameters but delivered two seconds after stimulus onset. In the no-stimulation condition, a perception-specific TAP (peak latency=388±28ms after stimulus onset) can be observed, which is similar to the results of the previous study (peak latency = 441±16ms; Kronemer et al., 2021). Notably, there was an increase in the percentage of perceived trials (58.7±7% v. 46.3±7%) when thalamic stimulation is delivered during visual stimulus onset compared to no stimulation. In comparison, thalamic stimulation delivered two seconds after stimulus onset does not have this augmentation effect as compared to the no-stimulation condition (41.7±5% v. 46.3±7%). These preliminary results suggest that thalamic stimulation could augment perceptual awareness when delivered concurrently with visual stimuli. Together, the intracranial electrophysiological and stimulation results support a modulatory role of the thalamus in conscious perception. Future work may better shed light on this modulatory role by recruiting more patients as well as testing the effect of thalamic stimulation on cortical responses measured by EEG. **Reference:** Kronemer SI, Aksen M, Ding J, et al. Brain networks in human conscious visual perception. *bioRxiv*. January 2021:2021.10.04.462661. doi:[10.1101/2021.10.04.462661](https://doi.org/10.1101/2021.10.04.462661)

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Poster

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Topic: H.02. Perception and Imagery

Support: Blattmachr Family
Loughridge Williams Foundation

Title: Investigating the brain networks of tactile conscious perception with intracranial EEG

Authors: *T. YADAV¹, S. MAJUMDER¹, T. BUI², K. L. CHRISTISON-LAGAY¹, D. JIN¹, J. Z. DING², N. FREEDMAN², M. GUSSO¹, S. I. KRONEMER¹, S. AERTS¹, I. FREEDMAN², K. WU³, I. H. QURAIISHI¹, A. SIVARAJU¹, E. DAMISAH³, H. BLUMENFELD³;
¹Neurol., ³Neurosurg., ²Yale Univ., New Haven, CT

Abstract: Understanding the mechanisms of conscious perception is a major goal of current neuroscience research, with significant headway being made in recent years. Our lab has previously investigated perception of threshold visual and auditory stimuli using intracranial

EEG and revealed that while activity in Not Perceived trials is limited to early sensory areas, Perceived trials are characterized by increases in sensory areas and widespread cortical association networks. To further investigate whether different sensory modalities share similar patterns of activity, a tactile perceptual threshold task was used with intracranial EEG (icEEG). The task involved delivery of a vibration (40ms) at the participant's perceptual threshold to their non-thumb fingers via vibrating tactor in a randomized fashion with no vibration delivered in 14% of the total trials. The perceptual threshold of each participant was estimated from trial to trial using a minimized expected entropy staircase method such that a vibration could be detected in only 50% of the trials. After each trial, participants reported whether a vibration was felt or not, and on which finger was it felt. Behavioral findings indicate that participants (n=7) perceived a vibration in 53.2% (SEM 5.2%) of vibration-present trials; the false positive rate was 9.5% (SEM 5.2%) for blank trials. When participants reported perception of the stimulus, they correctly indicated its location in 81.6% (SEM 3.4%) of trials; when they reported no perception, finger localization accuracy was 26.5% (SEM 1.6%; chance level 25%). icEEG recordings were analyzed for power in the high gamma frequency range (40-115 Hz), because of its known relationship to local neural activity. In Not Perceived trials, post-stimulus activity was dominated by a spatiotemporally limited increase in gamma power in the somatosensory regions mainly contralateral to the stimulus presentation. However, in Perceived trials, the observed increases in gamma power in somatosensory regions expanded outward to association regions including widespread areas of the frontal and parietal association cortex bilaterally. These findings are consistent with our previous results in visual and auditory paradigms, indicating that there are shared mechanisms of conscious perception involving a broad network of cortical regions across sensory modalities.

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Poster

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Loughridge Williams Foundation

Title: Neural mechanisms of awareness of action

Authors: *D. S. JIN¹, O. AGDALI⁵, S. MAJUMDER², M. C. MCCUSKER¹, M. KHURANA², I. FU², S. I. KRONEMER⁶, A. KHALAF³, K. L. CHRISTISON-LAGAY³, S. L. AERTS¹, E. J. SIFF¹, T. XIN¹, J.-J. LI², M. J. CROWLEY⁴, H. BLUMENFELD³;

¹Yale Univ. Interdepartmental Neurosci. Program, ³Yale Univ. Sch. of Med., ⁴Yale Child Study Ctr., ²Yale Univ., New Haven, CT; ⁵Hunter Col., New York, NY; ⁶NIH, NIH, Chevy Chase, MD

Abstract: Loss of awareness of action (AoA), the ability to name complex motor actions just performed, is a common phenomenon in everyday experience. We hypothesize that actions performed with and without AoA can be characterized by differences in static (long-term, state-based) and dynamic (transiently changing, event-based) neural activity as compared to those without. Here, we probe differences in static mechanisms through long-duration blink metrics, and differences in dynamic mechanisms through event-related potentials in EEG. We employ an experimental paradigm based on the classic move-based board game Rush Hour. As subjects (N=19) play the full game, periodically, the puzzle disappears, and subjects may be shown a quiz (50% likelihood) asking about the move just performed in a multiple choice question. After answering, subjects indicate their confidence in their choice on a sliding scale. We considered correct/high-confidence answers to be aware, and incorrect/low-confidence to be unaware. High (above 75th percentile) and low (below 25th percentile) confidence were defined relatively within the subject's slider selections. Long blinks (400-1000 ms), have been noted to indicate drowsiness and overlap microsleep, and we investigated them as a state-based metric. We investigated these in the seconds prior to the action. The incidence of long blinks decreased significantly in aware moves preceding an action, from 6.03% in the 4-5s preceding a move to 0.29% in the 1-2s preceding a move in 19 subjects. A much smaller decrease was seen in unaware moves, from 4.70% to 2.35% in the same timespan. In the EEG domain, the execution of a move was preceded by a robust readiness potential, and the subsequent disappearance of the board elicited a series of ERPs. Pre-action readiness potential onset was noted to begin earlier in unaware actions as opposed to aware actions. In addition, post-board disappearance N200 and P300 were noted to be decreased in amplitude in the unaware actions. Clear state-based and event-based differences characterize actions performed with and without awareness as operationalized with the Rush Hour task. These can be seen in increased long blink onsets and changes to readiness potentials and ERPs in unaware moves. Additional studies of AoA through other methods such as functional neuroimaging will help further elucidate the neuroanatomical origins of these signals.

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Poster

488. Cortical Control of Decision Making in Primates

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 488.01

Topic: H.03. Decision Making

Support: NIH: R01-MH121448

Title: Flexible state-dependent valuation in non-human primates

Authors: *T. W. ELSTON, J. D. WALLIS;

Helen Wills Neurosci. Inst., Univ. of California at Berkeley, Berkeley, CA

Abstract: The value of a choice option can vary across contexts. Thus, optimal choice depends on flexibly assigning value to choice options in a contextually appropriate manner. Despite the ubiquity of such state-dependent valuation in choice outside of the laboratory, how the brain flexibly switches between value-representations in different states remains unknown. To address this question, we trained a rhesus monkey in a novel state-dependent valuation task in which the monkey flexibly updates the values of 8 choice options on a trial-by-trial basis according to a cued task-state. Critically, the state-cue precedes and is not present during choice, requiring the animal to simultaneously update the 8 option values prior to seeing them. This design allows us to probe model-based value-updating because the animal has no chance at trial-and-error (i.e. model-free) reinforcement learning.

The animal performed the task at a high level, choosing the contextually most-valuable option on over 90% of trials. The animal could generalize the structure of the task and could perform at a high level with novel stimuli after, on average, 2 sessions (as compared to several months of initial learning). Taken together, our paradigm provides a substrate for future investigations of state-dependent valuation and its neural implementation.

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Poster

488. Cortical Control of Decision Making in Primates

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Topic: H.03. Decision Making

Title: Cognitive maps underlying model-based behavior in nonhuman primates

Authors: *E. HU, J. D. WALLIS;

Univ. of California, Berkeley, Berkeley, CA

Abstract: Animals use their knowledge of the environment to make inferences that guide decision-making in novel or dynamic situations. This knowledge may be organized in the brain as a ‘cognitive map’: a network of associations that specifies the relationships that underpin a task or environment. Much effort has been dedicated to characterizing the precise nature of cognitive maps in the brain, but little is known about how such maps are used to compute decision-relevant variables that inform choices. A framework that can characterize this process is model-based RL (MB RL). MB RL algorithms learn a model that describes the structure of an environment, and then use this model to infer reward predictions and evaluate the best course of action. Here, we trained macaques to perform a novel sequential decision-making task that probes behavior during MB RL. On every trial, the subject is shown 9 squares spatially arranged

in a 3x3 grid, with each square representing a node of an underlying graph. At the start of each trial, a random node is highlighted to represent the subject's start location. The subject is allowed to move to adjacent nodes, performing this movement by fixating on their intended next location. The subject continues to move through the graph via fixation this until they arrive at a hidden reward node. Once the subject has repeatedly found the optimal path to the reward location (90% accuracy over 35 trials), a new reward location is cued to the subject, requiring the subject to change its navigation policy over the state space. If subjects are engaged in MB RL, they should be able to find the new reward location immediately following the reward-shift. Preliminary results from show that subjects can successfully solve this task using MB RL. We have fully trained one subject up to expert performance on the task. Across all reward locations, he finds the optimal path to the reward 95.2% of the time. Furthermore, on the trial immediately following a change to the reward location, the subject infers the optimal path to the new reward 91±8% and takes the optimal path on the second trial following a reward-shift 96±6% of the time. In future work we will use this paradigm to study how interactions between the hippocampus and prefrontal cortex support MB RL.

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Poster

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Title: WITHDRAWN

Poster

488. Cortical Control of Decision Making in Primates

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Title: Decoding dynamics of self-guided attention in lateral PFC

Authors: *N. MUNET, J. WALLIS;
Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA

Abstract: During decision-making, attention is a powerful mechanism to selectively sample information most relevant to the decision while filtering out irrelevant alternatives. To date, most systems neuroscience studies in attention have involved an explicit attentional cue to manipulate attention. However, in many real-world decision-making contexts, attention is guided not only by explicitly cued rules but also by intrinsic processes that direct attention according to one's subjective goals and biases. Here we leverage recent advances in neural population decoding to decode a context-general visuospatial attention signal from single-unit populations in lateral prefrontal cortex (LPFC). First, we trained macaques on a two-alternative, cued change-detection task, which provides us with ground-truth conditions in which the monkey's attention is explicitly biased to one of six discrete locations on-screen. After training an LDA decoder on the cued trials to predict the attended (cued) location, we successfully confirmed high-accuracy decoding in each time bin from stimulus onset to the time of choice. Importantly, we also analyzed decoder performance on a separate set of uncued probe trials, involving pairs of unbiased targets with equal change probabilities. In these probe trials, monkeys had to direct their attention toward one target or the other in a self-guided manner to optimally detect the change. By testing the decoder on probe trials, we were able to analyze the dynamics of self-guided attentional control at single-trial resolution in the absence of extrinsic cues. We found that prior to change onset, increased attention toward the chosen target predicted faster reaction times. Moreover, on each trial, LPFC tended to stably represent only one of the two target locations, rather than alternating between locations, suggesting sustained selective attention in our task. These results validate our paradigm as a novel approach for decoding attention dynamics in decision-making contexts outside of traditional cued attention paradigms. Emerging work from our lab will use this decoding paradigm to assess the relationship between neural correlates of attention and value during a deliberative, value-based decision-making task.

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Poster

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Title: The neuronal congruency effect in monkey frontal eye field

Authors: *T. YAO¹, W. VANDUFFEL^{1,2,3,4};

¹KU Leuven, Leuven, Belgium; ²Leuven Brain Inst., Leuven, Belgium; ³MGH Martinos Ctr., Charlestown, MA; ⁴Harvard Med. Sch., Boston, MA

Abstract: The interplay between task-relevant and task-irrelevant information induces conflicts that impair behavioral performance, a.k.a. a behavioral congruency effect. The neuronal mechanisms underlying behavioral congruency effects, however, are poorly understood. We recorded single unit activity in monkey prefrontal cortex using a task-switching paradigm and discovered a neuronal congruency effect (NCE) that is carried by target and distractor neurons which process target and distractor-related information, respectively. The former neurons provide more signal, the latter less noise in congruent compared to incongruent conditions, resulting in a better target representation. Such NCE is dominated by the level of congruency, and is not determined by the task rules, reaction times (RT), the length of the delay period, nor the response levels of the neurons. We propose that this NCE can explain behavioral congruency effects in general, as well as previous fMRI and EEG results in various conflict paradigms. Moreover, by analyzing the time-course of the NCE in both target- and distractor-processing neurons, we found that the temporal dynamics of the NCE are independent of the specific task rules (i.e., a spatial or color rule), yet they are substantially different in target- and distractor-processing neurons. Furthermore, the conflict is much faster detected by FEF neurons (already ~20-30ms after the visual response) than predicted based on human EEG results, which suggests that conflicts are fast and automatically detected in the FEF. Resolving the conflict at neuronal level, however, is a more complex process being influenced by interindividual strategy differences, task rules, working memory and maybe other cognitive processes.

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Poster

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Title: Intracranial electrophysiological evidence for a novel neurocomputational mechanism of exploration-exploitation decision-making in humans

Authors: *X. YAN¹, S. KOENIG¹, B. A. EBITZ², D. DARROW¹, A. HERMAN¹;
¹Univ. of Minnesota, Twin Cities, Minneapolis, MN; ²Neurosciences, Univ. de Montréal, Montréal, QC, Canada

Abstract: The exploration-exploitation trade-off is ubiquitous in daily life. Should we continue to exploit the known option with known reward or should we explore other options to gather more accurate information about the uncertain world? Many computational models have been developed to understand the value computation and updating process when humans resolve this dilemma. Evidence from neuroimaging and neurophysiological studies suggests that regions in human prefrontal cortex are important for value encoding and support the flexible transition between exploration and exploitation. However, how different PFC regions dynamically track multiple changing rewards and perform decision-value updating remains poorly understood. In the current study, we recorded local field potentials (LFPs) directly from PFC regions, including the orbitofrontal cortex (OFC), medial prefrontal cortex (mPFC), and anterior cingulate cortex (ACC) in human epilepsy patients (363 channels in 14 patients with 7353 total trials) performing a restless 3-armed bandit task. We conducted complex Morlet wavelet convolution analysis to obtain the induced power of each trial. Utilizing single-trial model-free analysis, we found that theta band (4-8 Hz) power in prefrontal cortex is more dominant in switch trials than stay trials prior to decision-making. Further, we fit choice behavior with a novel adaptive reinforcement learning model and conducted single-trial model based brain-behavior analysis. Interestingly, the gamma band (above 35 Hz) dominantly tracked the trial-based exploit value in prefrontal regions prior to choice, and theta band (4-8 Hz) after trial-feedback tracked the potential to obtain a reward from exploration (all $P < 0.005$, Bonferroni-Holmes Family-Wise-Error corrections). Our findings reveal the dynamic patterns of the value system in prefrontal cortices when people perform exploration-exploitation decision-making. The current study may shed new light on potential invasive neuromodulation targets for improving cognitive flexibility and improving psychiatric symptoms related to cognitive rigidity.

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Poster

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Title: Geometry of spatial and non-spatial signals in the prefrontal cortex during reinforcement learning

Authors: *H. LIANG¹, C. DONAHUE³, D. LEE²;

¹Johns Hopkins Univ., ²Neurosci., Johns Hopkins Univ., Baltimore, MD; ³Wispr AI, South San Francisco, CA

Abstract: Neurons in the prefrontal cortex modulate their activity according to multiple types of past and present events during decision-making in reinforcement learning tasks. However, little is known about how differentially task-relevant and task-irrelevant mnemonic signals are retained across multiple trials such that the value functions could be updated adaptively only based on task-relevant signals. Here, we examined the population activity in the dorsolateral prefrontal cortex (DLPFC) of monkeys performing a probabilistic reversal learning task. During this task, target colors indicated the reward probability of each target ($p=0.2$ or 0.8) in a block of trials and were therefore task-relevant. On the other hand, target locations with different reward probabilities were randomized across trials and therefore were irrelevant for subsequent choices. In addition, the magnitude of reward from each target was indicated by the number of small yellow disks presented around each target. To investigate how the neural signals related to the color and location of the targets chosen in the previous trial might be combined with those in the current trial, we applied a regression model that includes the interaction between the previous and current choices. We found that the signals related to the previous and current color choices in the DLPFC often reflected whether the animal's color choice was repeated or not, rather than the specific target colors. By contrast, the signals related to the previous and current location choices tend to reflect the actual target locations instead of their interaction. We also quantified the transfer performance of a cross-condition neural decoder and applied non-linear dimensionality reduction methods to the choice signals recorded from the DLPFC. Results from these methods identified the trajectory of population activity in the DLPFC consistent with the results from the regression analysis. These results suggest that the signals related to previous and current choices might be combined differently for task-relevant and task-irrelevant dimensions during reinforcement learning.

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Poster

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Support: NIMH IRP ZIA MH002886
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Title: The role of medial prefrontal cortex in using counterfactual outcomes

Authors: *M. WANG, D. QUETEL, E. A. MURRAY;
Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Counterfactual outcomes refer to what would have happened had a subject chosen differently. They allow subjects to evaluate and switch to alternative courses of actions flexibly. Rhesus macaques can understand, represent, and use counterfactual outcomes. Previous work has shown that the medial prefrontal cortex (mPFC) represents counterfactual outcomes and predicts general behavioral changes (Fouragnan et al., 2019; Heilbronner & Hayden, 2016). To test whether mPFC is essential for using counterfactual outcomes, we tested rhesus macaques with neurotoxic lesions of mPFC (n = 2) and unoperated controls (n = 2) on a task designed to probe this ability. Monkeys chose among three new stimuli, each associated with a high, medium, or low reward probability. The high and low probabilities reversed in the middle of each session. On each trial, two out of the three options were randomly presented for choice. This required the monkeys to maintain and use the value of the unavailable third option (i.e., the counterfactual outcome) to guide the decision of whether to choose it, when it next became available. We found that both control (t-stat=2.32, p=0.02; logistic regression) and lesion (t-stat=2.01, p=0.04) groups used the counterfactual outcome to guide whether to choose the unavailable option when it came online, before probability reversal. After the reversal, however, the groups differed. Whereas controls (t-stat=2.41, p=0.02) continued to use counterfactual outcomes to guide choice, monkeys with mPFC lesions did not (t-stat=1.181, p=0.24). In addition, monkeys with mPFC lesions showed significantly lower choice accuracy (percent of choosing the option with higher reward probability) than controls (X²=4.38, p=0.04; chi-square test), especially after probability reversal (X²=16.77, p<0.01). In a control task, we presented only two options, each associated with a high and low reward probability, which made choices less dependent on the use of a counterfactual outcome to guide behavioral changes. Both control and lesion groups performed similarly in the control task, suggesting intact switching behavior when maintaining the representation and use of counterfactual outcomes is not heavily demanded to solve the task. These preliminary findings suggest that mPFC is necessary for learning and using counterfactual outcomes to guide flexible behavioral adaptation, especially after environmental changes (here, probability reversal).

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Poster

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Title: WITHDRAWN

Poster

488. Cortical Control of Decision Making in Primates

Location: SDCC Halls B-H

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Program #/Poster #: 488.10

Topic: H.03. Decision Making

Title: Learning attentional templates in frontal and parietal cortex

Authors: *C. I. JAHN¹, N. T. MARKOV¹, B. M. MOREA¹, T. J. BUSCHMAN^{1,2};
¹Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; ²Dept. of Psychology, Princeton university, Princeton, NJ

Abstract: Attention filters the flood of sensory inputs, allowing us to focus on goal-relevant information. Importantly, attention is not static – as the environment, or our goals, change, attention adapts to focus on what is currently relevant. To study how the brain adapts its attention to the current situation, we trained monkeys to perform a novel attention-learning task. On each trial, the monkeys searched a visual array for a color that best matched a ‘template’ color; the closer the selected color was to the template, the more reward the animal received. However, the monkeys were never instructed as to the template color. Instead, monkeys had to learn the template through trial and error by choosing a color, getting feedback (amount of reward), and updating their internal attentional template. After the monkeys learned a template, the template would unexpectedly change, requiring the animal to repeatedly relearn a new attentional template. Behavioral modeling showed the monkeys responses were well explained by a reward-learning model (Q-learning with function approximation) that included a reset for large reward prediction errors.

Previous work has shown neurons in frontal and parietal cortex represents attentional templates. To test how these template representations emerged during learning, we recorded from large populations of neurons in frontal and parietal cortex. Using multi-class classifiers, we found template representations in neuron populations recorded from lateral intraparietal (LIP) cortex, frontal eye field (FEF) and lateral prefrontal cortex (LPFC). This is consistent with fronto-parietal networks representing an attentional template. Additional classifiers found choices were represented in the same way for all template colors while value representations ‘rotated’ to match the current template color. Together, these results suggest learning of new attentional templates re-maps value representations to the currently attended stimulus but does not change decision representations. Together, our results provide new insight into the behavioral basis of attentional template learning and its neural substrates in the frontal and parietal cortex.

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Poster

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Title: Intrinsic neural timescales and their pharmacological manipulation in the rhesus macaque brain

Authors: *A. M. MANEA¹, A. ZILVERSTAND², A. CUSHNIE³, S. R. HEILBRONNER³, J. ZIMMERMANN³;

¹Dept. of Neurosci., Univ. of Minnesota, Twin Cities, Minneapolis, MN; ²Dept. of Psychiatry & Behavioral Sci., Univ. of Minnesota, MINNEAPOLIS, MN; ³Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Hierarchical temporal dynamics are a fundamental computational property of the brain; however, there are no whole brain, noninvasive investigations into timescales of neural processing in animal models. While traditionally investigated in electrophysiology, recent advancements in fMRI make it possible to study hemodynamic timescales in nonhuman primates (NHPs). We used ultra-high field fMRI to probe timescales across the whole macaque brain, moving investigations from single cells to macroscale dynamics. Timescales of baseline neuronal activity vary across the brain, from fast timescales in sensory areas, to slower timescales in association areas. These intrinsic fluctuations in the neural signal, also described as intrinsic neural timescales (INTs), are thought to provide a time window during which brain regions integrate inputs. We uncovered within-species consistency between INTs estimated from fMRI and electrophysiology (n = 9). Crucially, we not only replicate electrophysiological hierarchies, but we extend these to whole-brain topographies. Since we provide a direct comparison within the same species, our results show for the first time that INTs estimated from fMRI and electrophysiology reflect similar phenomena. Despite efforts to understand the relationship between connectivity and INTs, the relationship to functional connectivity (FC) has not been elucidated yet since high-resolution fMRI data in NHPs is scarce. We show that the macroscale functional organization of the brain, as reflected by FC and INTs, is organized along gradual topographies. Moreover, the FC and INT topographies are closely related. Finally, we show that investigating the macaque brain with fMRI can bring an invaluable translational contribution. For example, it has been previously shown that schizophrenic patients undergoing antipsychotic treatment show abnormal hierarchical INT patterns. However, there is no data on how antipsychotic medication affects INTs or FC. Here, we administered antipsychotic medication to NHPs (n = 2). We show that antipsychotic treatment and schizophrenia have opposite effects on INTs, with schizophrenia lowering and antipsychotics increasing them. Moreover, antipsychotics bring subjects to a common functional state, as reflected by an increase in their whole-brain INT and FC similarity.

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Poster

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Title: Distinct beta frequencies reflect categorical decisions

Authors: *E. EL RASSI¹, Y. ZHANG², G. MENDOZA⁴, H. MERCHANT⁵, S. HAEGENS³;
¹Donders Inst. for Brain, Cognition and Behaviour, Donders Inst. for Brain, Cognition and Behaviour, Nijmegen, Netherlands; ²Columbia Univ., New York City, NY; ³Dept. of Psychiatry, Div. of Systems Neurosci., Columbia Univ., New York, NY; ⁴Inst. de Neurobiologia UNAM, Campus Juriquilla, Inst. de Neurobiologia UNAM, Campus Juriquilla, Querétaro, Mexico; ⁵Inst. de Neurobiologia UNAM, Inst. de Neurobiologia UNAM, Queretaro, Mexico

Abstract: Beta oscillations are involved in a variety of cognitive functions beyond their traditional sensorimotor role. Based on prior findings of content-specific beta synchronization during working memory and decision making, we hypothesized that beta activity supports the activation and reactivation of cortical representations by mediating neural ensemble formation within and between brain regions. We here found that beta activity in monkey dorsolateral prefrontal cortex (dlPFC) and in pre-supplementary motor area (preSMA) reflected the content of a stimulus in relation to the task context, regardless of its objective properties. In multiple versions of a categorization task, we changed a categorical boundary between sessions, such that a stimulus which belonged to one of two categories during one session could belong to the other category during the next session. During a delay in which monkeys had to hold their categorical decision in mind, we found that two distinct beta-band frequencies were consistently associated with the same two relative categories, and that activity in these respective bands predicted the animals' responses. We characterized beta at these frequencies as transient bursts with distinct temporal profiles. We further showed that dlPFC and preSMA were connected via these distinct frequency channels, with dlPFC driving the frequency separation, a result supported by Granger causality and spike-field coherence analyses. In sum, these results provide support for the role of beta in forming neural ensembles, and further show that such ensembles synchronize at different beta frequencies.

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Poster

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Title: A cognitive map for value-guided choice in ventromedial prefrontal cortex

Authors: *S. VESELIC¹, T. MULLER¹, T. E. BEHRENS^{2,1}, L. T. HUNT³, J. BUTLER¹, S. W. KENNERLEY¹;

¹Univ. Col. London, London, United Kingdom; ²FMRIB Centre, Univ. Oxford, Oxford, United Kingdom; ³Univ. of Oxford, Oxford, United Kingdom

Abstract: The prefrontal cortex (PFC) is crucial for economic decision making. Neural representations for both the value of options subjects choose between, as well as their difference, have been repeatedly found across different subregions of the PFC (Kennerley et al. 2011; Hunt et al. 2018). Here we reframe economic decision-making in PFC guided by findings of how structure is represented within the medial temporal lobe (MTL): we framed choice between different options as a navigation process in value space (Behrens et al. 2018; Bongioanni et al. 2021). We looked for evidence of a value space in PFC in a task where rhesus macaques choose between two options parametrized by magnitude and probability of reward. We found that the angle between options subjects had to decide between was represented with a grid-like code in LFP theta frequency within ventromedial PFC (vmPFC) right before subjects made their choice. Crucially, this grid code was also present in single neurons recorded from nearby locations, confirming for the first time that population level measures of grid coding can be found in firing rates of neurons. In addition to a map-like representation of value at choice, we also found sharp-wave ripples, another computation important for flexible behaviour and planning. The observed ripple events were dynamically modulated by both reward and success of each trial. In sum, we have shown vmPFC represents the structure of a value space using a grid-like which is utilized right before subjects make choices, together with exhibiting sharp wave ripples throughout the choice process. This means PFC employs two fundamental map-like computations during value guided choice, similarly to MTL during spatial navigation.

Behrens, T. E. J. et al. (2018). What Is a Cognitive Map? Organizing Knowledge for Flexible

Behavior. Bongioanni, A. et al. (2021). Activation and disruption of a neural mechanism for novel choice in monkeys. Hunt, L. T. et al. (2018). Triple dissociation of Attention and decision computations across prefrontal cortex. Kennerley, S. W., Behrens, T. E. J. & Wallis, J. D. Double dissociation of value computations in orbitofrontal and anterior cingulate neurons.

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Poster

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Title: Inhibiting prepotent responses increases low frequency power in human dorsolateral prefrontal cortex and basal ganglia

Authors: *A. KHAN¹, Z. IRWIN¹, A. ROLLER¹, R. T. KNIGHT³, K. M. VISSCHER⁴, B. GUTHRIE¹, H. C. WALKER⁵, K. A. ZAGHLOUL⁶, N. BENTLEY²; ²Neurosurg., ¹Univ. of Alabama at Birmingham, Birmingham, AL; ³California Clin. Trials, Berkeley, CA; ⁴Neurobio., Univ. of Alabama, Birmingham, Birmingham, AL; ⁵Neurol., UAB, Birmingham, AL; ⁶NINDS, Bethesda, MD

Abstract: The ability to monitor conflicting stimuli and withhold responses until the correct decision can be made is essential in daily human life. This ability, termed inhibitory control, has been studied across species and is thought to be mediated by the frontal cortex and subthalamic nucleus (STN). Prior studies have shown conflict-related increases in functional connectivity between midline frontal cortex and the STN. Further, the dorsolateral prefrontal cortex (DLPFC), in particular, has been shown to be active during response inhibition in fMRI experiments. Previous work has also demonstrated spike-phase coherence both within DLPFC and between DLPFC and anterior cingulate during conflict. However, the spectral power changes in the DLPFC during response inhibition has not been shown in human intracranial recordings. Moreover, while the STN has been extensively implicated in cognitive control, little is known about the role of globus pallidus. Here, we investigated prefrontal and basal ganglia networks involved in response inhibition by simultaneously recording from DLPFC and basal ganglia in Parkinson's disease (PD) patients undergoing deep brain stimulation (DBS) for motor dysfunction. We measured performance on a Go/No-go task and recorded electrocorticographic local field potentials (LFP) using a subdural strip and subcortical LFP from the DBS electrode. During No-go trials, we found increases in high delta and low theta power (3-6 Hz) compared to Go trials in the DLPFC, globus pallidus interna (GPi), and STN. We also found significant increases in beta power in the No-go trials compared to Go following the response time in the

STN. This effect was also observed in GPi, but was not statistically significant. We also observed increased coherence between DLPFC and the two basal nuclei in the No-go trials compared to Go. Overall, our data suggest a coupled role for DLPFC, STN, and GPi delta and theta oscillations in inhibiting prepotent responses.

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Poster

488. Cortical Control of Decision Making in Primates

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 488.15

Topic: H.03. Decision Making

Support: ANR-18-CE37-0012-01

Title: Neuroimaging evidence of the functional interplay within the fronto-amygdala network during behavioural adaptation in human

Authors: ***C. GIACOMETTI**¹, **D. AUTRAN-CLAVAGNIER**², **L. VIÑALES**¹, **F. LAMBERTON**³, **E. PROCYK**¹, **F. HADJ-BOUZIANE**⁴, **C. AMIEZ**¹;

¹INSERM U1208 - Stem Cells and Brain Res. Inst., Bron, France; ²Inovarian - Life Sci. and Res. Ctr., Paris, France; ³La Structure Fédérative de Recherche Santé Lyon-Est, CNRS UAR 3453, INSERM US7, Lyon 1 Univ., Lyon, France; ⁴INSERM, U1028, CNRS UMR5292, Lyon Neurosci. Res. Center, ImpAct Team, Lyon, France

Abstract: A hallmark of our survival in the real world is our ability to show behavioural adaptation (BA). BA is a challenging process that requires to know whether to link our failures to our own actions (i.e. our actions' feedback -FB-) or to unexpected changes in our environment (i.e. when facing Action-Independent Events -AIDEs-, e.g. rules changed). The aim of this study is to identify how the functional interplay in the network formed by the midcingulate cortex (MCC) and the amygdala (AMG) is modulated by BA. To understand AMG and MCC functional interplay, we first assessed their functional connectivity by means of resting-state fMRI (rs-fMRI). Following up, we scanned 22 human subjects with fMRI while they were performing a new behavioral task in which they had to learn by trial and error how to adapt when facing both FB and AiDEs, AiDEs influencing or not the meaning of the FB. Rs-fMRI results showed that MCC and AMG present a negative correlation, suggesting an antiphase functional connectivity pattern. Accordingly, fMRI task results show that the functional interplay between the AMG and MCC changes during the learning process of AIDEs significance: 1) the AMG is activated and the MCC deactivated at the first occurrence of AiDEs (i.e., when subjects do not know yet the meaning of the AiDEs), 2) then the MCC becomes activated and the AMG deactivated when subjects understood that an AiDEs signal a need to adapt and 3) MCC

activation peak for AIDEs, once subject learned their meaning, is the same as in FB in exploration phase. This study suggests the AMG and MCC do present a specific functional connectivity interplay that allow us to properly adapt to our changing environment. AMG first detects salient information from the environment and constantly informs the MCC through a bottom-up pathway. When the MCC identifies a particular event that requires BA, it exerts a top-down control onto AMG to optimize BA. As such, our data demonstrate the critical involvement of a dynamic functional interplay within the AMG-MCC network in behavioural adaptation.

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Poster

488. Cortical Control of Decision Making in Primates

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Program #/Poster #: 488.16

Topic: H.03. Decision Making

Title: Relationship between frontal theta/beta ratio and performance on the ball risk test in middle-aged adults

Authors: F. ALCARAZ-MENDOZA¹, *C. VEGA-MICHEL², E. CAMACHO GUTIERREZ³; ¹Clin. and Res., Inst. de Neuropsicología y Neurociencia Aplicada (INNA), Guadalajara, Mexico; ²ITESO, A. C., Tlaquepaque, Mexico; ³Dept. de Psicología, ITESO, Tlaquepaque, Mexico

Abstract: Decision-making depends on the degree of development of the executive functions, among other factors, and are considered as the cognitive capacities that allow the individual to achieve a goal. There is wide evidence of a relationship between qEEG analysis and the state of conservation of executive functions in adults, however, the brain mechanisms underlying decision-making under risk-benefit conditions remain unclear. A sample of healthy male and female volunteer participants (n=60) age 30 to 55 years responded to the Balloon Risk Test (BART) using an adapted version of Lejuez et al. (2002) and analysis of their resting qEEG was performed, from which the theta/beta ratio of frontal derivations (Fp1, Fp2 and Fz) was calculated. The results show that the higher theta/beta ratio is related to a reduction in the task execution effectiveness determined by the score/money obtained in the task and a riskier decision identified in the response variability. Linear regression analyses were performed from which a predictive relationship was identified between the electrophysiological data and the variables derived from execution of the BART test. The findings are discussed on the basis that the theta/beta ratio has been associated with variations in cortical metabolic consumption and executive control, elements that appear to underlie the variability of the results in our research.

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Poster

488. Cortical Control of Decision Making in Primates

Location: SDCC Halls B-H

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Program #/Poster #: 488.17

Topic: H.03. Decision Making

Support: MnDRIVE Brain Conditions
University of Minnesota Medical Discovery Team on Addiction

Title: Encoding of naturalistic reward and threat in a prefrontal-limbic network in human intracranial electrophysiology

Authors: *B. VAIL¹, S. D. KÖNIG¹, S. B. YOO⁴, B. Y. HAYDEN², A. B. HERMAN¹, D. P. DARROW³;

¹Dept. of Psychiatry and Behavioral Sci., ²Dept. of Neurosci., ³Dept. of Neurosurg., Univ. of Minnesota, Minneapolis, MN; ⁴Dept. of Biomed. Engin., Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Reward pursuit and threat avoidance comprise fundamental building blocks of behavior. The orbitofrontal cortex (OFC), amygdala, and anterior cingulate cortex (ACC) have all been implicated in networks differentiating reward and threat, but these are rarely assessed in naturalistic tasks or using invasive recording in humans. The objective of our study was to determine whether activity in these areas differs between dynamic states of reward pursuit and threat avoidance in humans. Electrophysiology data were recorded from electrodes implanted in the OFC, amygdala, and ACC of epilepsy patients while they performed a naturalistic pursuit/avoidance task based on the “Pacman” video game. In one condition they had to pursue “prey”, and in another they had to both pursue “prey” and avoid “predators”. For each condition, wavelet convolution was performed to obtain induced power at frequencies from 2 to 150 Hz over a one second window from 500 ms before the appearance of predators and prey to 1000 ms after this event. We then subjected each time-frequency (TF) point in this window to a linear mixed effects model with induced power as a function of predator/prey condition, and with subjects and channels as random effects. We corrected for multiple comparisons within brain areas using permutation testing, and between areas with a Bonferroni correction. Clusters of adjacent TF points with a corrected p value of $< .05$ were then treated as significant differences between predator and prey conditions. In OFC (5 subjects, 41 channels), theta power (4-8 Hz) was elevated in the predator condition compared to the prey condition at approximately 365-625 ms after the appearance of prey/predators, and again at 650-1000 ms; delta power (2-4 Hz) was elevated at 565-1000 ms, and beta (12-30 Hz) and gamma power (30-150 Hz) were reduced at 775-885 ms. In the amygdala (5 subjects, 32 channels), delta and beta power were elevated in the predator condition at approximately 625-895 ms and 685-800 ms, respectively. In ACC (4 subjects, 18 channels), alpha (8-12 Hz) and beta power were reduced in the predator condition at 200-270 ms, as were beta and gamma power at 330-450 ms and gamma power again at 770-815 ms. Theta and delta power in ACC were increased in the predator condition at 585-810 ms and

845-1000 ms, respectively. The multiple clusters of significantly different activity between the prey and predator conditions in the Pacman task suggest that the OFC, amygdala, and ACC all participate in differentiating between reward pursuit and threat avoidance under dynamic, naturalistic conditions. The naturalistic task and human intracranial data give these results an unprecedented level of ecological validity.

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Poster

488. Cortical Control of Decision Making in Primates

Location: SDCC Halls B-H

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Program #/Poster #: 488.18

Topic: H.04. Executive Functions

Support: This work was supported by Scientific and Technological Research Council of Turkey (TÜBİTAK) grant number 120K924

Title: Attentional and Working Memory Demands Activate FrontoParietal Regions less during Longer Tasks

Authors: ***I. ÇİFTÇİ**^{1,2}, A. A. FAROOQUI^{3,4};

¹Natl. Magnetic Resonance Res. Ctr., Ankara, Turkey; ³Psychology, ²Bilkent Univ., Ankara, Turkey; ⁴Natl. Magnetic Resonance Res. Centre, Ankara, Turkey, Ankara, Turkey

Abstract: We hierarchically execute extended tasks as single entities. We prepare breakfast, write emails, and not individually execute their many components. Such execution occurs via programs related to the overarching task that subsume the entire task execution.

Since these programs are related to the entire task, longer tasks require larger programs. These programs seem to have a cognitive load, hence executing longer tasks as one unit is more demanding than same but shorter tasks. Further, deactivation in many brain regions (including the default mode regions) is greater during longer tasks with larger programs (Farooqui & Manly, 2018), e.g., attentional tasks that are expected to last 40 s elicit greater deactivation than identical tasks expected to last 20 s.

A set of fronto-parietal regions (also called Multiple Demands or MD regions) famously activates in response to control demands like increased attention, working memory, response inhibition etc. Since these control demands typically occur during some overarching extended task, related control processes are likely to be instantiated via the related to the overarching task. We investigated if the load related to the overarching program is neurocognitively very different from the load related to control processes like attention and working memory. If this is the case, then the increased load of the overarching program will affect MD regions' activity very differently compared to the effect of increased attentional or working memory load which are well-known activators of these regions.

Participants (n=38) either executed short tasks made of 9 3-back trials or long tasks made of 9-18 3-back trials. We found that identical 3-back trials elicited lower MD region activity when they occurred in the context of long compared to short tasks.

The load of a larger program related to a longer task thus affected MD regions in the opposite direction compared to increased attentional/working memory demands, suggesting that these two kinds of demands may be categorically different.

Disclosures: **I. Çiftçi:** None. **A.A. Farooqui:** None.

Poster

488. Cortical Control of Decision Making in Primates

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Program #/Poster #: 488.19

Topic: H.04. Executive Functions

Support: This work is supported by The Scientific and Technological Research Council of Turkey, grant no: 120K924

Title: Is Task Positive Activity in Multiple Demands Regions Primarily a Goal Completion Activity?

Authors: G. ŞENGİL¹, I. ÇİFTÇİ³, *A. FAROOQUI²;

¹Neurosci. Grad. Program, ²Bilkent Univ., Ankara, Turkey; ³Ipek Çiftçi, Ankara, Turkey

Abstract: Numerous studies have shown activation of a defined set of fronto-parietal regions during all kinds of task blocks. These have been termed as multiple-demand (MD) or task-positive regions. Various accounts explain such activity as relating to attention, cognitive control, working memory (WM) etc. - issues that will be in-play during any kind of task. We have previously demonstrated that these regions do not activate in response to attention, WM or general control demands. When delinked from goal completion, attention deactivated these regions. Why then have other studies found these regions to activate during all kinds of tasks? One possibility is that the basic unit of activity in most experiments is a trial. A trial involves stimulus presentation to which a response is to be made according to some rule. Participants' aim is to make the right response. Every time that happens goal, albeit a lower-level one, gets completed. It is plausible that MD activation seen during various experimental tasks reflects not attention, WM or cognitive control but goal-completions inherent in trials. If this is the case, then task blocks with the high control demands but not consisting of trials will not elicit MD activity. We had two groups of participants (30 each). Group 1 executed extended tasks made of nine n-back trials (n = 2-3). At each trial participants responded if the presented picture was the same or different compared to that seen n trials earlier. Group 2 participants executed tasks that involved sequential presentation of nine pictures. They had to monitor these pictures and covertly note when the picture identity repeated after n trials. At the end of the task, they keyed in the number of repeats they had noted. Note that while both groups executed tasks made of 9 steps and made

identical decisions at each step, the steps of group 1 were *trials* but those of group 2 were not. We found that early trials of Group 2's task deactivated MD regions while that of Group 1 activated them. Later trials in both groups activated these regions likely due to approach to task completion. Ubiquitous task positive activity in MD regions during many experiment tasks may thus be due to them consisting of trials. The goal-completion inherent in a trial and not attention, WM or control demands is the cause of this activity.

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Poster

488. Cortical Control of Decision Making in Primates

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Program #/Poster #: 488.20

Topic: H.04. Executive Functions

Title: Reasoning improvements after cognitive control: Frontoparietal network under phasic inhibition through the hyperdirect pathway

Authors: *C. DEWEY;
Univ. of Arizona, Tucson, AZ

Abstract: Existing models propose that cognitive control improves performance on conflict reasoning tasks (base rate tasks, belief bias tasks, and other heuristics-and-biases tasks) by selectively inhibiting incorrect responses. Cognitive control in reasoning tasks is associated with the hyperdirect pathway: the rIFG projects to the STN, which then delivers global inhibition through the thalamus back to frontal and parietal cortex (the specific regions in these areas that function in reasoning depend on the nature of the task). But STN projections are so diffuse that they can't discriminate between conflicting responses: they are only capable of global inhibition. No evidence indicates that cognitive control implements response inhibition via the indirect pathway (that is, in reasoning tasks), which could selectively inhibit incorrect responses. This raises the question: how does global inhibition through the hyperdirect pathway promote the selection of correct responses in the frontoparietal network? In this study, I develop a model of cognitive control that distinguishes between two effects of feedback from the STN. The first effect is already specified in current models of cognitive control: global inhibition. This is insufficient to cause reasoning improvement: without further changes, it delays the selection of an incorrect response. The second effect is novel to my model: frontoparietal synchronization. After all, global inhibition is delivered from the STN, which is known to respond to reasoning conflict with increased power in the theta-band: I argue that theta-band activity (θA) in the STN entrains θA in the frontal and parietal cortex, which synchronizes the frontoparietal network. Task representations are associated with parietal areas and task responses are associated with frontal areas, so frontoparietal synchronization may increase interactions between these representations. In particular, my model predicts that this increases negative feedback (conflict) between parietal task representations and incorrect frontal responses and positive feedback

between parietal task representations and correct frontal responses, which promotes the selection of correct responses in frontal cortex in a drift-diffusion model of decision. Behavioral evidence confirms this prediction: task formats that make the relationship between the salient responses and the task properties more salient are associated with reasoning improvements. Thus, the hyperdirect pathway functions to delay decision-making through global inhibition until it can synchronize the frontoparietal network, increasing sensitivity to conflict and improving reasoning.

Disclosures: C. Dewey: None.

Poster

488. Cortical Control of Decision Making in Primates

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Program #/Poster #: 488.21

Topic: G.04. Emotion

Support: NIH Grant R03-DA046733 (DVS)

Title: Bargaining Decisions Are Associated with Enhanced Effective Connectivity Between the Temporoparietal Junction and the Medial Prefrontal Cortex

Authors: *D. SAZHIN¹, J. B. DENNISON¹, J. B. WYNGAARDEN¹, A. VAFIADIS¹, O. ZAFF¹, N. A. KAHN¹, M. COLLINS¹, I. KOHLI¹, D. S. FARERI², M. MCCLOSKEY¹, J. M. JARCHO¹, D. V. SMITH¹;

¹Temple Univ., Philadelphia, PA; ²Psychology, Adelphi Univ., Garden City, NY

Abstract: Study Objective: Bargaining situations, such as when negotiating a salary, require considering another person's point of view while maximizing one's rewards. Previous studies indicated that bargaining decisions are driven by the threat of rejection in the Ultimatum (UG) vs. Dictator games (DG), which is associated with increased dorsolateral prefrontal cortex (dlPFC) and ventromedial prefrontal cortex (vmPFC) activation (Spitzer et al., 2007). However, it is unclear how responses in the vmPFC and dlPFC during bargaining vary depending on reward sensitivity. Thus, we used task-based fMRI to examine the neural correlates of bargaining choices among individuals who are hyposensitive and hypersensitive to rewards.

Methods: Participants (N = 60) completed the Sensitivity to Punishment (SP)/Sensitivity to Reward (SR) Questionnaire (Torrubia et al., 2001) and the behavioral inhibition system/behavioral activation system scales (BIS-BAS) (Carver and White, 1994). They underwent fMRI while performing the UG (Güth et al., 1982) and DG (Kahneman et al., 1986) as a proposer. In the DG, a participant unilaterally decides how to split an endowment with another person (recipient), whereas in the UG, the recipient can reject the offer. Participants were given \$15-\$25 and proposed a high vs. low split. Offers were 6, 19, 32, or 45 percent of the endowment. After motion and behavioral exclusions, N=54 subjects had usable neuroimaging results. Whole-brain results were thresholded in FSL with a cluster-defining threshold of $Z > 3.1$, correcting across

the whole brain (FWE < 0.05).

Results: Using a mixed effects model, we found that participants made more selfish offers in the DG vs. the UG (B = -0.43, SE = 0.015, t = -27.95, p < .001). Following our pre-registration (<https://aspredicted.org/55gd8.pdf>), Region of Interest analyses during the endowment phase of the DG demonstrated trends towards elevated vmPFC activation as the offers proposed increased $t(53) = 1.99$, $p = .0523$. Second, we found enhanced effective connectivity in the UG compared to the DG between the ventral striatum and both the temporoparietal junction $t(53) = -3.11$, $p = 0.013$ and the medial prefrontal cortex $t(53) = -2.04$, $p = 0.046$. Contrary to our hypotheses, neither of these effects were associated with reward sensitivity in our preliminary analyses. Conclusions: Overall, our results suggest that connectivity between reward and social brain regions is enhanced by strategic bargaining behavior among individuals who vary in reward sensitivity. Future analyses will explore relations between the interaction of substance use and reward sensitivity in bargaining decisions.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

Support: P50MH119569
R01MH123661

Title: Explore-exploit state governs the spatial configuration of touch actions in a mouse bandit decision making task

Authors: *D. MUELLER¹, E. GIGLIO², C. CHEN², N. M. GRISSOM¹;

¹Dept. of Psychology, ²Univ. of Minnesota, Minneapolis, MN

Abstract: In bandit decision making tasks, the challenge of sampling between options versus settling on a currently best option is better known as the explore-exploit tradeoff. Across species, there is substantial evidence that explore and exploit can be defined as neurobehavioral states using a hidden markov model (HMM) approach. Using a restless bandit task, in which the reward probability of each choice changes randomly and independently across trials, we see that animals enter self-initiated periods of exploration and exit these to begin exploiting an option. In mice, the use of touchscreen chambers allows us to record precise locations for each mouse touch, allowing us to consider detailed information about how decisions translate into physical motion. Transitioning between explore and exploit states could be considered as an online change in cognitive flexibility, which may be reflected in motor and behavioral flexibility. We

took advantage of the data on touch locations to test whether individual trials labeled as exploit by our HMM are accompanied by more stereotyped motor behaviors in choice selection than the same choices during explore states. Thirty-two 129/b6j F1 mice (16 male and 16 female) were tested on restless bandit schedules. We find that successive touches to the same choice are further apart while an animal is in an explore state than in an exploit state, suggesting greater motor stereotypy when exploiting an option. Male mice tended to have a wider range of nosepoke coordinates than female mice do across states, suggesting different levels of coordination between motor and cognitive systems across sexes. This novel analysis has the potential to allow all labs using touchscreens to investigate how stereotyped motor behaviors may be captured in response data and reflect hidden contributions to decision flexibility.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Title: Dopamine and norepinephrine signaling differentially mediate the exploration-exploitation tradeoff

Authors: *C. CHEN¹, E. KNEP², B. A. EBITZ⁴, N. M. GRISSOM³;
²Psychology, ³Dept. of Psychology, ¹Univ. of Minnesota, Minneapolis, MN; ⁴Neurosciences, Univ. de Montréal, Montréal, QC

Abstract: In an uncertain world, we balance two goals: exploiting rewarding options when they are available, and exploring potentially better alternatives. One neuromodulatory system that has been implicated in mediating the transition between exploration and exploitation is the catecholamine system, in particular, norepinephrine (NE) and dopamine (DA). Although both molecules have been implicated in decision making, their contributions have not been directly compared. When each neuromodulatory system is examined in isolation, they have been assigned similar roles in the latent cognitive processes that mediate exploration and exploitation. To understand the differences and overlaps of the role of these two catecholamine systems in regulating exploration and exploitation, a direct comparison using the same dynamic decision making task is needed. Here, we ran mice in a restless two-armed bandit task and systemically administered a NE beta-receptor antagonist (propranolol), NE beta-receptor agonist (isoproterenol), a nonselective DA receptor antagonist (flupenthixol), and a nonselective DA receptor agonist (apomorphine) and examined changes in exploration. We found that modulating NE and DA receptor function had opposing effects on exploration - decreasing NE beta receptor

activity or increasing DA receptor activity decreased exploration and resulted in stickier behaviors. Fitting a reinforcement learning model revealed that changes in exploration through manipulating NE and DA were due to changes in different latent processes. Together, these findings suggested that the mechanisms that govern the transition between exploration and exploitation are sensitive to changes in both catecholamine functions and revealed differential roles for NE and DA in regulating exploration.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

Support: NIH Grant R01NS118463

Title: Neural substrate of eligibility trace mediating temporal credit assignment with variable feedback delay

Authors: *P. LIU, S. MURRAY, H. SEO;
Psychiatry, Yale Univ., New Haven, CT

Abstract: Reinforcement learning (RL) theory postulates that an agent can learn the desirable course of actions through trial and error by retrospectively updating the value of a causative action by its temporally delayed outcome. Memory trace of a chosen action, namely eligibility trace (ET) is essential for this process known as temporal credit assignment (TCA) by bridging the temporal gap between the action and corresponding outcome. ET is often modeled as decaying exponentially over time. However, it is unclear how the brain might adjust the time constant of exponential decay when the outcome follows with variable delay as in real life. To investigate neural ET mediating TCA with variable feedback delay, we designed a behavioral task, in which animals played a modified matching pennies game against the computerized opponent, and the interval between choice and feedback (CFI) as well as inter-trial-interval (ITI) independently and randomly varied between two distinct values- 1s and 5s on a trial basis. To facilitate trial-by-trial analysis of the behavioral and neural effect of CFI and ITI, reward probability of each choice target was determined by first order Markov process, requiring animals to maintain their probability of Win-Stay-Lose-Switch around 2/3 to maximize reward. Two rhesus monkeys were trained for the task. We found that CFI consistently reduced the probability of repeating the same choice after reward (Win-Stay) without affecting the probability of switching after no-reward (Lose-Switch). ITI reduced both Win-Stay and Lose-Switch. Behavioral modeling under RL framework suggested that the effect of variable CFI might be partially explained by the decay of choice value. To understand underlying neural mechanisms, we decoded animal's choice (memory) with logistic regression classifier during

CFI and ITI from the population activity recorded from dorsomedial prefrontal cortex with Neuropixels probe. Immediately following the choice, the signal rapidly decayed within 1s and the time course of decay was independent of CFI. However, we found that animal's choice could be decoded following feedback and around the decision of the subsequent trial with the decoding accuracy being modulated by feedback, indicating the reactivation of choice memory after fast decay immediately following the choice. The reactivation was flexible in timing, and robust across variable CFI. These results suggest that the reactivation dynamics of choice memory and its modulation by feedback might serve as neural substrate for eligibility trace and for temporal credit assignment with variable feedback delay.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

Support: NIH R01NS118463

Title: Neural basis of temporal credit assignment during reinforcement learning

Authors: *S. K. MURRAY¹, P. LIU¹, D. LEE², H. SEO^{3,4};

¹Interdepartmental Neurosci. Program, Yale Univ., New Haven, CT; ²Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD; ³Dept. of Psychiatry, ⁴Dept. of Neurosci., Yale Sch. of Med., New Haven, CT

Abstract: Humans and other animals learn about the environment through trial and error by updating estimates of the values of actions with reward prediction errors. Previous work examining the neural basis of reinforcement learning has focused on contexts with unambiguous action-outcome pairings. However, in reality, actions are often temporally separated from the outcomes they cause, with irrelevant actions performed during the delay. In these circumstances, animals must rely on knowledge of the environment to correctly update action values. This challenge is referred to as the temporal credit assignment (TCA) problem. Despite the importance of TCA in learning, the brain mechanisms underlying TCA are not well understood. We developed a novel lagged bandits paradigm to study TCA. We trained a pair of rhesus monkeys to learn about two independent sets of targets ('bandits') whose choices and outcomes were interleaved. On each trial, the animals made a choice and observed an outcome. Crucially, the outcome of a choice from one bandit was not delivered on that same trial, but rather on the subsequent trial following a choice from the other bandit. To maximize reward, the animals had to utilize knowledge of the task structure to assign credit for a given outcome to a choice remembered from the previous trial, rather than the choice that directly preceded the outcome. One bandit had a spatial contingency, where either the left or right choice was highly rewarded

($p=0.85$; $p=0.15$ for low value target) and underwent frequent, performance-dependent reversals. The other had an object-based contingency, where only one of two colors was rewarded probabilistically ($p=0.5$). The two colors were randomized between the top and bottom spatial positions across trials, and the rewarded target color was fixed across sessions. Using logistic regression, we found the animals applied knowledge of the task structure. When selecting from the spatial bandit, they predominantly used a win-stay lose-switch strategy based on the conjunction between the most recent spatial bandit choice and the spatial bandit outcome delivered one trial later (t-tests on regression coefficients; all $p<0.0002$; $n=58$ sessions monkey C; $n=47$ monkey K). In the same sessions, both animals consistently chose the high value target from the object bandit (all probabilities $>78\%$). We performed neural recordings in dorsomedial and dorsolateral prefrontal cortex and striatum during the task using a combination of single electrodes and linear probes. Ongoing analysis shows that neurons in these regions carry information necessary for correct TCA and represent information about previous choices, outcomes, and their conjunctions.

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Poster

489. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 489.05

Topic: H.03. Decision Making

Title: Rewards Elicit Choice Reactivation For Credit Assignment

Authors: *C. D. CARRASCO, Y. ZHANG, S. J. LUCK, E. D. BOORMAN;
Ctr. for Mind and Brain, Univ. of California, Davis, Davis, CA

Abstract: The process of identifying what set of choices leads to an outcome, known as credit assignment, can be formalized as occurring in a model-free or a model-based manner. In the model-free case, reward is thought to be tied back to the most recent choices through eligibility traces that weigh these actions with an exponential decay over time. In the model-based case, when the choice and outcome are separated by a sufficient delay, it is theorized that a reactivation of a causal choice's representation triggered by an outcome is needed for a given choice-outcome association to form through Hebbian plasticity. However, if and when this reactivation occurs during learning is still unknown. To test for reactivation of neural representations associating outcomes with choices separated in time, we had participants perform a decision-making task where they had to gamble between two stimulus options (faces and houses) which predicted rewards with slowly drifting and independent probabilities. For each of these stimuli participants received feedback as to whether their choice led to reward and if the unchosen stimulus would have led to reward. Using electroencephalography, we generated event related potentials (ERPs) while participants performed this task that were time locked onto stimulus presentation, choice, and feedback portions of the task. We then used these ERPs to

train a series of support vector machines to test if we could identify a reactivation of neural representations tied back to a specific stimulus choice. Results (N= 21) identify a neural reactivation of the choice identity, in the alpha band of the data, specifically at the time feedback is delivered, to form associations between outcomes and stimuli ($p < 0.05$, cluster-based permutation) that was irrespective of choice. Additionally, we find preliminary evidence that these representations were the same as sensory representations measured at stimulus presentation. Our results suggest that model-based credit assignment indeed elicits a reactivation of neural representations to form associations between choices and delayed outcomes at times of feedback.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Program #/Poster #: 489.06

Topic: H.03. Decision Making

Support: ONR MURI N00014-16-1-2832

Title: Evaluating a rodent model of generalization in an evidence accumulation task

Authors: *Q. DO¹, M. E. HASSELMO², B. B. SCOTT²;

¹Grad. Program for Neuroscience, Ctr. for Systems Neurosci., ²Psychological & Brain Sciences, Ctr. for Systems Neurosci., Boston Univ., Boston, MA

Abstract: Humans are capable of generalizing using the similarity between physical features of the stimuli as well as using the abstract relationships between stimuli. The neural mechanisms underlying generalization are unclear, and future discoveries would be facilitated by studies in animal models. Here we evaluate a rat model of generalization using a pulse-based evidence accumulation task. Recent work demonstrates that rats can be trained on a perceptual decision-making task in which the rule is to accumulate up to 10 identical pulses of light presented to both the left and right visual hemifields over a fixed period of time, and to decide on the side with the greater number of pulses. It is unclear whether rats performed the task by memorizing all the stimulus-action pairings or by generalizing. We trained rats (n=16) on a similar task using a restricted number of light pulses (i.e. training set) to ask whether rats generalize in the presence of novel stimuli (i.e. probe set). Generalization was evaluated using a 95% binomial confidence interval of rats' performance on probe trials (3 vs 7, 4 vs 6) that have a low probability of occurrence and probabilistic reward. We found that rats (14 out of 16) performed above chance (mean accuracy = 0.65, p-value < 2.2e-16, alternative hypothesis: true probability is not equal to 0.5, 95% confidence interval: 0.62-0.67) on trials with numbers of light pulses they had never encountered. Furthermore, we conducted a regression analysis on the choice data and found that rat's performance on the task including the probe trials was best explained by an evidence

accumulation strategy. These results suggest that rats with limited exposure to sensory input can generalize to novel stimuli, and introduce a useful framework to uncover the neural mechanisms underlying generalization.

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Poster

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Program #/Poster #: 489.07

Topic: H.03. Decision Making

Support: NSF GRFP Grant No. DGE-1656466

Title: Comparing online and offline hippocampal sequences during model-based planning

Authors: *S. C. VENDITTO¹, K. J. MILLER², N. D. DAW¹, C. D. BRODY^{1,3};

¹Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; ²Univ. Col. London, London, United Kingdom; ³Howard Hughes Med. Inst., Princeton, NJ

Abstract: Humans and animals construct internal models of the world around them and use these models to guide behavior. Such model-based cognition is often referred to as “planning”, and its neural mechanisms remain poorly understood. Planning has been proposed to depend on hippocampal sequences, in which place cells “sweep out” non-local trajectories through space (Mattar et al., 2018, Pezzulo et al., 2019). Sequences of one type (“online”) are associated with the theta rhythm and typically occur during active behavior, while sequences of another type (“offline”) are associated with sharp wave ripples and typically occur when the animal is at rest. Both online and offline sequences have been suggested to play a role in planning; however, evidence supporting their role, especially offline sequences, has been conflicting (Xu et al., 2019; Gillespie et al., 2021). Here, we attempt to address these conflicting results by presenting electrophysiological recordings using high-density Neuropixels 2.0 probes in the dorsal hippocampus while rats perform a multi-step planning task (Miller et al 2017). We decode both online and offline sequences across trials and relate their representations to the rats’ behavior throughout the task, and we hypothesize what role, if any, both sequence types may play to support planning.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Support: Australian Research Council (DP200102445)
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Title: Lipopolysaccharide induced neuroinflammation in the dorsal hippocampus accelerates goal-directed action in female mice, facilitates Pavlovian approach in male mice

Authors: *K. GANESAN¹, L. A. BRADFIELD²;
²Fac. of Science, Sch. of Life Sci., ¹Univ. of Technol. Sydney, Sydney, Australia

Abstract: Lipopolysaccharide induced neuroinflammation in the dorsal hippocampus accelerates goal-directed action in female mice, facilitates Pavlovian approach in male mice. Kiruthika Ganesan, Laura A Bradfield*School of Life Sciences, Faculty of Science, University of Technology Sydney, New South Wales, 2007, Australia*Corresponding Author Hippocampal neuroinflammation plays a crucial role in neurodegenerative and neuropsychiatric disorders. Goal directed action and other executive functions are also impaired in individuals with these disorders. Whether neuroinflammation is the cause for these cognitive-behavioural deficits is unclear. The dorsal hippocampus has been shown to play a temporally transient role in goal directed action in rodents. Thus, we here tested the hypothesis that hippocampal neuroinflammation impairs goal-directed action using mice (8 -10 weeks). In Experiment 1, female C57BL/6Jausb mice received bilateral micro-injections of 4µg/µl Lipopolysaccharide (n=12), or Saline (n=10) into the dorsal hippocampus to induce neuroinflammation. Mice were trained to press two levers – left lever for sucrose and right lever for pellets (counterbalanced) for 4 days. We then tested devaluation performance by devaluing pellets or sucrose by pre-feeding it to satiety. Next, mice were presented with two levers in the choice extinction test (no outcomes were delivered on this test). Sham animals were not yet able to demonstrate goal-directed action as they pressed each lever equally, whereas group LPS were goal-directed because they selectively pressed the lever associated with the valued outcome. After 4 more days of lever press training (8 days total) mice were again devaluation tested, and this time both groups were goal-directed (i.e. Valued>Devalued). Experiment 2 was conducted identically except it used male C57/6Jausb mice (LPS, n=11; Saline, n=13). This time, goal-directed action was intact (Valued>Devalued) for all mice on both tests, however group LPS demonstrated a clear and persistent increase in Pavlovian approach behaviour (i.e. food magazine entries) that was sustained across all days of training and test. Taken together, these results demonstrate clear sex differences in the cognitive/behavioural outcomes that result from dorsal hippocampal neuroinflammation. This could be indicative of the gender differences observed in humans with similar neuropathological features, such as Alzheimer's disease or depression which is twice as prevalent in females than males. Statistics: Differences between means were assessed using two-way analysis of variance (ANOVA)

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Poster

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Topic: H.03. Decision Making

Support: Australian Research Council (DP200102445)

Title: Lipopolysaccharide-induced neuroinflammation in the posterior dorsomedial striatum facilitates goal-directed action

Authors: *A. R. ABIERO, L. A. BRADFIELD;
Sch. of Life Sciences, Fac. of Sci., Univ. of Technol. Sydney, Sydney, Australia

Abstract: Disorders of compulsion, such as substance use disorder (SUD), are characterized by loss of cognitive control. Studies have identified the putative neural circuits of compulsive reward-seeking but have failed to identify the endogenous mechanisms that drives their dysfunction. Here we aimed to establish a direct, causal link between striatal neuroinflammation and impaired goal-directed control. In Experiment 1, male and female (n=14-18) Long Evans rats aged 8-15 weeks received micro-injections in the posterior dorsomedial striatum (pDMS) of either saline or the endotoxin lipopolysaccharide (LPS) (5mg/ml) to induce neuroinflammation, then trained on Pavlovian-instrumental transfer and outcome devaluation tasks. Rats were first trained to associate two unique auditory cues with pellets and sucrose (counterbalanced), then trained to press left and right levers for pellet and sucrose outcomes respectively (counterbalanced). Transfer testing consisted of the cues and levers being presented together for the first time in the absence of any outcomes. When animals were partially sated and control effects were small, transfer and devaluation was enhanced in group LPS. Specifically, during transfer, group Sham responded equally on both levers whereas group LPS responded selectively on the lever that predicted the same outcome as the current cue (Same>Different). During devaluation testing - following specific satiety - both groups responded more on the valued relative to the devalued lever, but this difference was larger for group LPS. Experiment 2 tested whether pDMS neuroinflammation increases motivation generally, or goal-directed action specifically. Rats pressed a single lever for sucrose on an interval schedule over 15 sessions. We then tested when animals reached a 'breakpoint' (i.e. 5 min of no lever pressing) using a progressive ratio design in which animals initially received a sucrose reward for a single lever press, then for 5 lever presses, then n+5 lever presses until breakpoint. When tested, animals in group LPS reached consistently higher breakpoints than group Sham. Upon devaluation testing, group Sham demonstrated evidence of habits (Valued=Devalued) and group LPS demonstrated intact goal-directed actions (Valued>Devalued). Immunohistochemistry was performed to confirm neuroinflammation and to measure any neuronal death. Together, these results suggest that LPS-induced neuroinflammation in pDMS increases both motivation and goal-directed action, akin to a person who compulsively seeks rewards under low motivation conditions (e.g. a person with SUD who drinks alcohol whilst hungover).

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

Support: NSF Grant BCS1844144

Title: Dissociable roles and sex differences in the contributions of anterior cingulate cortex, but not basolateral amygdala, to information bias

Authors: *V. V. GONZALEZ, S. ASHIKYAN, A. P. BLAISDELL, A. IZQUIERDO;
UCLA, Los Angeles, CA

Abstract: Information has been claimed to be reinforcing such that animals prefer a lean but informative alternative over a richer but uninformative option, even when the former is not instrumental (i.e., it cannot be used to change the outcome). Most studies have focused on better understanding the behavioral limits of this phenomenon, yet there are few experiments directed at identifying the neural substrates of the preference for information. Animals prefer the option they value the most after integrating all the features of the options, and perhaps also including information value. This process may rely on corticoamygdalar circuits. In the present experiments, Long-Evans rats (n=37, 23 females, 14 males) were prepared with either bilateral inhibitory hM4Di DREADDs on a CaMKIIa promoter or administered an eGFP virus in basolateral amygdala (BLA) or anterior cingulate cortex (ACC). Rats were administered either clozapine-N-oxide (CNO) or vehicle (VEH) solution (3.0 mg/kg, i.p.) 10 min prior to behavioral testing. We first evaluated the role of BLA and ACC using a within-subject counterbalanced design for drug administration. Rats chose between two alternatives by pressing the right or left lever, in which choice of one (Info option) resulted in 20% of the trials followed by a tone (S+) that lasted 60s and ended with a sucrose pellet, and where the other 80% of the trials followed another tone (S-) lasting 60s but never resulting in reward. Choice of the other lever (No-Info option) resulted in a third 60-s tone (S3) followed by reward on 50% of the trials. Then, we trained a new set of tones to be S+, S- and S3 to assess the role of ACC and BLA in the acquisition of new Info and No-Info cues in preference, with drug administered in a between-subject design. We analyzed preference for the Info option from baseline (no injection) to drug (CNO or VEH): the variable "Condition". For ACC rats, a mixed ANOVA on Steady-State performance resulted in a significant effect of Condition ($p = .006$) and Sex ($p = .014$). Significant interactions of Condition x Virus ($p = .003$) and Sex x Virus ($p = .042$) were found. Post-hoc Holm analysis revealed that hM4Di females reversed their baseline preference when ACC was inhibited. The same analysis performed on BLA animal data did not result in an effect of BLA inhibition on performance (no effect of Condition [$p = .158$], Sex [$p = .745$], Virus [$p = .267$] nor interactions [$p > .175$]). For New Cues, ACC and BLA inhibition had no effect on preference, which might be explained by the value of options remaining unchanged. Ongoing experiments are evaluating the role of orbitofrontal cortex, and other variables that might affect information preference such as delays and effort.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

Support: NIH Grant 1 R01 NS111470

Title: Complex cue and delay period representations in the mediodorsal thalamus during a decision making task in mice

Authors: *N. KATYARE, D. JAEGER;
Emory Univ., Atlanta, GA

Abstract: Mediodorsal nucleus of the thalamus (MD) forms an important hub in thalamocortical computations with roles related to attention, working memory, and decision making; mainly via its interactions with the prefrontal cortex. Neurons in MD have been previously reported to display activity related to sensory cues and task delay period, however, the intricacies of such responses have not received due attention. We recorded extracellular neuronal activity in MD over weeks in head-fixed mice chronically implanted with silicon probes (Cambridge Neurotech), while they performed a two-alternative forced choice (2AFC) lick decision making task. We observed MD neuronal responses to be modulated by several task variables. We observed most neurons increasing or decreasing their activity during the task with varying peak times and durations ranging from milliseconds to seconds. A few neurons also displayed multiple activity peaks. Activity changes were observed across several task phases including cue onset and offset, delay period, and reward period. A few neurons displayed changes in activity even before the cue onset. Although most neurons responded to the cue, a few neurons also showed modulation by lick-related motor activity. Activity profile was also observed to differ between the 2 types of trials and between correct Vs. incorrect side trials based on the decision licks. We also observed these representations to be stable over weeks in neurons whose activity could be successfully traced over this period. Analyses of simultaneously recorded local field potentials revealed changes in broadband power mainly during the cue delivery. We thus report that the decision making task is represented in a complex manner in MD neuronal network, possibly representing a multiplexed role of sensory, motor, and reward representations.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Title: Defining frontal-basal ganglia circuit dynamics in rats during cost-benefit decisions

Authors: *O. HAERMSON¹, I. GRENNAN², B. PERRY², R. TOTH², S. G. MANOHAR¹, M. E. WALTON¹, A. SHAROTT²;

¹Dept. of Exptl. Psychology, ²Nuffield Dept. of Clin. Neurosciences, Univ. of Oxford, Oxford, United Kingdom

Abstract: Adaptive value-guided decision-making often requires weighing-up of costs and benefits of pursuing an available opportunity. While several brain areas, particularly in frontal-striatal circuits, have been reported to be important for this behaviour, little is currently known regarding how the decision variables are represented and evolve on the single cell and population levels *across* these circuits within the confines of a single behavioural paradigm. We developed a novel rodent cost-benefit “accept-reject” task, in which food-restricted rats choose whether or not to run to the end of a corridor to collect sucrose pellets based on the prospective reward (4 different levels, varied trial-by-trial, signalled by auditory cues) and effort cost (3 different levels, varied over blocks of trials, implemented with barriers placed in the corridor that needed to be scaled to reach the reward magazine). Behavioural data (n=12 rats, average 1119 trials per animal) demonstrate a positive effect of reward and a negative influence of effort on the likelihood of accepting an offer, without an interaction between the two. Outcome devaluation strongly reduces acceptance of any offer. A subset of rats (n=5) were also implanted with a bespoke driveable micro-electrode array targeting the anterior cingulate cortex (ACC), medial orbitofrontal cortex (mOFC), dorsomedial striatum (DMS), ventral pallidum (VP), and subthalamic nucleus (STN) and recordings were made as rats performed the task. Preliminary analysis of electrophysiology data (n=4 rats; average 119 cells per region per animal) suggests that individual neurons in the 3 targeted basal ganglia areas (DMS, VP and STN) encode reward and/or effort with high fidelity during cue presentation. By contrast, neurons in the two cortical areas (ACC and mOFC) display tuning to these variables across multiple task points. Intriguingly, reward and effort information can reliably be decoded from the activity of the whole network of targeted regions. These results promote the idea that decision variables emerge from collective frontal-striatal circuit dynamics.

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Poster

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Support: NIH Grant MH068073

Title: Looking at dopamine release and direct and indirect pathway activity during inhibitory learning.

Authors: *C. UPRETI¹, V. WINIGER¹, B. J. DE CORTE¹, P. D. BALSAM², E. H. SIMPSON¹;

¹Columbia Univ., New York, NY; ²Barnard Coll Columbia Univ., New York, NY

Abstract: Adaptive behavior requires an animal to know both when to respond and when not to respond to environmental cues. The failure to inhibit inappropriate behavior is characteristic of several psychiatric conditions including impulse disorders, psychotic disorders and ADHD. Research has predominantly focused on investigating mechanism that underlie positive contingencies. But in the natural world, animals must also pay attention and learn (equally well) about stimuli that predict the absence of events and thus inhibit unnecessary responses. To understand how animals learn about when rewards can and cannot be earned, we employed a behavioral paradigm in which mice are randomly rewarded for lever pressing every 20s on average, except when an 80s tone is presented. During the tone, no rewards can be earned. After very few trials, at tone onset dopamine (DA) level sharply decreases, remains low throughout the tone and rapidly rebounds at tone offset (Kalmbach et al., 2022). Therefore, dopamine tone reflects reward availability state and rapid dopamine transients encode transitions between reward states, but what is the role, if any, of this dopamine signal in learning? We hypothesize that DA encoding of the negative contingency serves to tune the activity of the output GABAergic Spiny Projection Neurons (SPNs) from the NAc, thereby resulting in conditioned behavior when reward is available and inhibition when it is not. To test this, D1R-Cre and A2A-Cre animals were injected in the NAc with the green dopamine sensor dLight1.2 and a cre-dependent red calcium sensor jRGECO1b for simultaneous dual channel fiber photometry in behaving mice. Mice were trained to earn milk rewards on average once every 20s. Once lever pressing was consistent, sessions continued with the addition of 80s tones (S⁻) of fixed duration during which rewards could not be earned. Mice learned the task, as expressed by a decrease in the ratio of lever pressing during the tone compared to the intertrial intervals (ITIs). Both the D1-Cre and A2A-Cre animals showed the same signature DA signals previously observed in WT mice. Simultaneous recording of calcium activity in the NAc D1 or D2 (A2A-Cre) SPNs revealed an increase in activity during S⁻ tone onset as seen by a positive deflection for the jRGECO signal. This translates to an increase in the output GABAergic tone from the ventral striatum during the negative contingency. Since D2 and D1 SPN's respond differently to dopamine and project to different nodes in the basal ganglia circuit (via the direct or indirect pathway), ongoing experiments will determine the specific roles of D1 and D2 receptor subtypes in behavioral adaptation to reward availability states.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

Support: NIH Grant R01- MH123679

Title: Evaluation of thalamic and prefrontal activity in perceptual-based decision making

Authors: *I. IBARRA-LECUE^{1,2}, K. ESPINOZA¹, L. B. JOHNSTON^{1,2}, A. Z. HARRIS^{1,2}, S. HAEGENS^{1,2,3};

¹Psychiatry, Columbia Univ., New York, NY; ²New York State Psychiatric Inst., New York, NY; ³Donders Inst. For Brain, Cognition & Behaviour, Radboud Univ., Nijmegen, Netherlands

Abstract: Behavioral choices often require making decisions based on sensory input from the environment. However, how the brain sets up the functional neural architecture to support perception-based decision making is currently unknown. Evidence from our lab and others suggests that thalamo-cortical circuitry is crucial for decision making and spatial working memory. Moreover, we have previously shown based on both human and non-human primate studies that cortical oscillatory activity, particularly in the beta range (15-30 Hz), encode task-relevant sensory information, suggesting that these oscillations reflect the stimulus evaluation during decision-making. However, the neural elements that produce this oscillatory activity remain unknown. In this study, we implanted chronic electrodes to record local field potentials (LFP) from medio-dorsal thalamus (MD) and medial prefrontal cortex (mPFC), and single unit activity from mPFC as mice performed a sensory discrimination task, where they have to successfully associate two different auditory cues with two opposite arms of a T-maze (n=4, 1 female, C57BL/6 strain). We observe that by session 18 (16-40 trials/session) all mice reach stable performance (>60% success rate over 3 consecutive days). To assess whether beta oscillations reflect the information being processed, we analyze the LFP power in MD and mPFC regions, the LFP synchrony between them, and phase-locking of mPFC single units in the delay period between the auditory cue and the decision-making period (vs. inter-trial interval) in correct and incorrect trials. These results will provide key insight into the role of beta oscillations in decision making.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Program #/Poster #: 489.15

Topic: H.03. Decision Making

Title: Flexible integration of evidence in rat parietal cortex for perceptual decisions

Authors: *P. GANUPURU, A. GOLDRING, T. STEVENSON, T. HANKS;
UC Davis, UC Davis, Davis, CA

Abstract: A crucial component of successful decision making is determining the optimal timescale over which to evaluate evidence. For example, when detecting transient changes in the environment, it may be best to only evaluate the most recent evidence while discounting older evidence. However, it is unclear how this adjustment in timescale is achieved in the brain with respect to how populations of neurons processing evidence adjust their dynamics. To address this question, we used Neuropixel probes to record spiking activity from neurons in the posterior parietal cortex (PPC) of adult male rats performing an auditory change detection task in which rats must evaluate sensory evidence over short timescales while down-weighting earlier evidence. We found that PPC neurons modulated their activity by the strength of evidence leading to decisions and were selective for the rats' choices (i.e. go vs no go). In addition, responses of neurons to individual units of evidence were transient, such that the effect of the evidence on activity tapered off over a timescale in line with the subject's behavioral performance. Finally, we reversibly inactivated PPC of rats with muscimol and identified a causal role for PPC in auditory change detection, such that PPC inactivation enlarged the timescale over which rats evaluated evidence. Together, our results suggest PPC contributes to free-response decisions by controlling the timescale over which evidence is evaluated.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Title: How humans and rats adapt to task statistics in an auditory categorization paradigm

Authors: *E. MENICHINI¹, V. PEDROSA¹, L. ZHOU², V. PLATTNER¹, R. LOW¹, P. LATHAM², A. AKRAMI¹;
¹Sainsbury Wellcome Ctr., ²Gatsby Computat. Neurosci. Unit, Univ. Col. London, London, United Kingdom

Abstract: Traditionally, improved performance is a key hallmark of learning. However, even if the process of learning may eventually lead to near-perfect performance, the learning trajectory depends on many factors, such as trial order or how the task is represented. In addition, a myriad number of biases affect human and animal performance, for instance serial, choice or sensory history biases in perceptual decision-making tasks. Here, we investigate how humans and rats optimally adapt their performance and choice strategy to the underlying sensory statistics in a sound categorization paradigm. In this 2-alternative forced-choice (2AFC) task, humans and rats categorize auditory stimuli based on an arbitrary perceptual boundary. Utilizing parametric and non-parametric methods we quantify how previous experience (sensory priors, choice and outcome history) informs their internal models during decision-making. Rats and humans show different learning patterns, with humans adapting policy updates at a stimulus-specific level. We show that models based only on feedback-dependent learning, including those incorporating statistical decision confidence, are not sufficient to explain the observed trial-to-trial learning behaviour. Instead, we identify a stimulus-dependent repulsion effect that works in tandem with a feedback-dependent component.

We further identify neuronal representations involved in learning and adapting to sensory statistics by performing large scale electrophysiological recordings in rat brains over the time course of rule acquisition and switching between distinct statistical contexts.

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Poster

489. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 489.17

Topic: H.03. Decision Making

Support: NIH 5T32MH020002-20
5R01DC018055-02

Title: Rapid expectation-driven sensory changes guide categorical vocal perception

Authors: *T. SAINBURG¹, T. GENTNER², T. MCPHERSON³, E. M. ARNEODO⁴;
¹Harvard Med. Sch., Harvard Univ., Cambridge, MA; ²UC San Diego, Univ. Of California San Diego Neurosciences Grad. Program, La Jolla, CA; ³Univ. fo California San Diego, UCSD Dept. of Neurosciences, La Jolla, CA; ⁴UCSD, La Jolla, CA

Abstract: Vocal communication in both songbirds and humans relies on categorical perception of smoothly varying acoustic spaces. Vocal perception can be biased by expectation and context, but the mechanisms of this bias are not well understood. We developed a behavioral task in which songbirds, European starlings, are trained to classify smoothly varying song syllables in the context of predictive syllable sequences. We find that syllable-sequence predictability biases perceptual categorization following a Bayesian model of probabilistic information integration. We then recorded from populations of neurons in the auditory forebrain while birds actively categorized song syllables, observing large proportions of neurons that track the smoothly varying natural feature space of syllable categories. We observe that predictive information in the syllable sequences dynamically modulates sensory neural representations. These results support a Bayesian model of perception where predictive information acts to dynamically reallocate sensory neural resources, sharpening acuity (i.e. the likelihood) in high-probability regions of stimulus space.

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Poster

489. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 489.18

Topic: H.03. Decision Making

Support: PAPIIT Grant IN232120

Title: Contribution of CB2r in the anterior cingulate cortex on decision-making of rats with induced Binge-type behavior

Authors: *F. CORTÉS SALAZAR¹, N. F. ROMERO-OCHOA², J. O. SUÁREZ-ORTIZ², E. M. HERNANDEZ-MORALES², J. C. JIMÉNEZ-MEJÍA³, J. C. RODRIGUEZ-AGUILAR², A. MARTINEZ-LOPEZ², J. M. MANCILLA-DÍAZ², R. E. ESCARTIN-PÉREZ²;

²Neurobio. feeding Lab., ³Neuropharmacology of addictions, ¹Univ. Nacional Autonoma de Mexico, Facultad De Estudios Superiores Iztacala, Mexico

Abstract: The research on CB2 receptors (CB2r) in the central nervous system is relatively recent, however, there is experimental evidence that suggests a possible functional role of CB2r in the modulation of the cognitive processing of hedonic properties of palatable food. Animal models of Binge-eating suggest that impulsivity might drive Binge-like behavior induced by experimental protocols in rodents. Consequently, it is pertinent to ask whether the CB2r expressed in brain areas associated with cognitive processing like the anterior cingulate cortex (ACC), contributes to the modulation of impulsive behavior in an animal Binge-type eating model. This research aimed to evaluate the role of the CB2r in the ACC on the impulsive behavior of rats with combined feeding in an effort-based and a delay-based decision-making

task. The combined feeding protocol consisted in feed male Wistar rats (n= 7) with a standard diet and a high-fat diet with restricted access. Control and experimental groups had *ad libitum* access to tap water and a standard diet, while the experimental group had access to a high-fat diet three times per week, this protocol induced binge-type behavior in 21 days. Microinjection cannulas were stereotaxically implanted unilaterally in the ACC, and subjects received intra-ACC injections of vehicle (0.9% saline, 0.5% DMSO), GW405833 (0.25 µg, CB2r agonist), AM630 (1 µg, CB2r antagonist), or both. In the effort-based task, the left lever delivered one drop of sucrose solution (2%) with a ratio of, while the right lever with a ratio of delivered five drops. In the delay-based task, the left lever delivered one drop immediately, meanwhile, the right lever delivered five drops with an increasing delay (1, 3, 6, 10, and 60s). We measured each lever's total responses, breakpoints, latencies, and preferences. In the effort-based task, we observed a trend to increase the lever presses and the breakpoints in the experimental group, caused by the activation of CB2r in the ACC. The delay-based task failed to induce a preference for the big reward in both groups. Our results are consistent with the hypothesis that activation of CB2r in the ACC may affect decision-making processes and increase impulsive responses in rats with binge-type eating.

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Poster

489. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 489.19

Topic: H.03. Decision Making

Title: Impact of cerebellar manipulation on behavioral flexibility

Authors: S. VANDER DUSSEN¹, A. F. WANG³, J. J. ALGA-SHERIFF³, N. G. HARRIS², *P. J. MATHEWS⁴;

²Neurosurg., ¹David Geffen Sch. of Med. at UCLA, Los Angeles, CA; ³UCLA, Los Angeles, CA; ⁴Neurol., Lundquist Inst. at Harbor-UCLA, Torrance, CA

Abstract: The ability to adaptively shift a previously learned behavior in response to changes in environmental context is an important feature of learning and memory. Several challenging neuropsychiatric disorders, including autism spectrum and schizophrenia, are associated with deficits in this type of behavioral flexibility. It is generally thought that forebrain regions involved in goal-directed behavior, like the prefrontal cortex and basal ganglia, mediate these adaptive behavioral shifts. Here, we provide novel data that indicates the cerebellum plays a role in controlling behavioral flexibility that is in line with other recent studies. In a goal-directed learning paradigm that probes a mouse's ability to adaptively shift previously learned associative behaviors, we found that mice whose cerebellar cortex was experimentally disrupted took

significantly longer to adaptively shift prior associations. Specifically, head-fixed mice were trained in a 2-cue (odor) reward learning task where on each trial they were presented one of two possible cues (1s), only one of which predicted delivery of a liquid reward. The mouse's ability to discriminate between the two cues was assessed based on whether the mouse licked in anticipation of the reward during a delay period after cue presentation. Upon sufficient cue-reward learning (>80% correct), behavioral flexibility in the mice was tested by measuring behavioral responses after reversing the cue-reward relationship. In mice whose cerebellar Crus I subregion was chemogenetically altered to suppress Purkinje neuron activity by the agonist Clozapine N-Oxide (CNO), there was essentially no adaptive shift in learning (day one anticipatory licks on $18 \pm 3\%$ of reward and $41 \pm 6\%$ of no reward trials, $n=6$). In contrast, CNO-treated control mice quickly learned the new cue-reward relationship (licks on $42 \pm 6\%$ of reward and $37 \pm 1\%$ of no reward trials, $n=7$). Control trials at the start of the first reversal learning day, in which the cue-reward relationship was not reversed, indicates these results are not due to a physical inability to respond (licks on $82 \pm 3\%$ of reward and $24 \pm 3\%$ of no reward trials, $n=6$). In addition to a decrease in the proportion of correct responses for over three days of reversal training, the time to the peak of the anticipatory licking during the delay period was longer in the experimental vs. control mice (1.7 ± 0.1 ms vs. 0.9 ± 0.4 ms). This novel paradigm is now being exploited to examine the neural activity and network dynamics using dense-electrode arrays and neuroimaging methods (e.g., functional ultrasound). Overall, these results support the idea that the cerebellum plays a role in behavioral flexibility.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Program #/Poster #: 489.20

Topic: H.03. Decision Making

Support: NIH RL5GM118975

Title: Male and female rats use different foraging strategies on a spatial patch-leaving task

Authors: M. GARCIA¹, S. GUPTA², *A. M. WIKENHEISER²;

¹Neurosci., UCSD Dept. of Neurosciences, La Jolla, CA; ²Psychology, UCLA, Los Angeles, CA

Abstract: Foraging efficiently is crucial for survival in most species. One key foraging problem is deciding when to abandon a diminishing resource. As foragers exploit a food patch, their rate of food intake naturally diminishes over time. At some point it becomes advantageous to seek out a replete patch elsewhere, but travelling to a new location entails costs in time spent travelling and energy spent moving. Adaptive foraging strategies balance these factors to determine the patch-leaving time that maximizes the overall rate of food intake. We developed a

spatial task to test this sort of foraging decision in rats. Rats ($n = 10$; 5 female) searched for food pellets scattered randomly within two open-field arenas that comprised food patches. The patches were connected to one another by a corridor, and computer-controlled doors in the corridor allowed us to impose a travel time delay (0, 30, or 60 s) when rats switched between foraging patches. Within each patch, food pellets were delivered following a depleting gain function (fast, medium, or slow depletion) that remained fixed for the duration of a behavioral session. Switching between locations reset the patch to its maximum possible rate. Rats were tested on all possible combinations of travel times and gain functions in pseudorandom order. We used numerical simulations to determine the rate-maximizing patch residence times for all testing conditions. Both male and female rats chose foraging strategies that were qualitatively consistent with the predictions of rate maximization: rats remained longer in patches with slower depletion rates, and remained longer in *all* patches as travel time increased. However, rats tended to “overstay” in patches, choosing residence times longer than those prescribed by rate maximization. Though all rats overstayed patches, this tendency was significantly stronger in male rats. Across all testing conditions, female rats remained in patches for shorter durations, switched between patches more frequently, and earned significantly more food pellets per session than male rats. These data are interpreted in light of previously-reported sex differences in rats’ exploration of open-field environments.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

Support: NIH Grant R01 DA04787
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Title: Higher long-term global reward improves consistency of reward-dependent choice strategies in mice and monkeys

Authors: *J. WOO¹, V. D. COSTA³, B. A. BARI⁴, J. Y. COHEN⁵, A. SOLTANI²;
¹Dartmouth Col., ²Psychological and Brain Sci., Dartmouth Col., Hanover, NH; ³Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Beaverton, OR; ⁴Sch. of Med., Baltimore, MD; ⁵Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Local effects of reward feedback on learning and decision-making are often investigated by computing quantities such as win-stay or lose-switch probabilities or by fitting choice behavior using reinforcement learning (RL) models, all of which consider response or adjustment to individual reward feedback. However, how reward history on a longer timescale affects animal’s choice behavior is relatively less known mainly because of the difficulty of

measuring such effects using reinforcement learning models. To address this question, we re-analyzed choice behavior of mice (n=16) and monkeys (n=4) performing two dynamic foraging tasks. We extended our previous metrics based on information entropy (Trepka et al., *Nat Comm* 2021) to incorporate reward history spanning more than one trial. Specifically, we defined the entropy of global and local reward-dependent strategies (EGLORDS) equal to the conditional entropy of stay/switch strategy given the immediate reward feedback as well as global reward state (GRS) based on the history of reinforcement. Lower EGLORDS values correspond to decreased uncertainty and thus more consistency in the utilized strategy. To estimate the timescale on which animals integrate past reward, we fitted RL models with additional components that modulates choice behavior by expected reward. We used the estimated timescales from the best RL model to infer the global reward state and compute EGLORDS in each block of trials. We found consistent effects of global expected reward across both species: when animals were earning more rewards than expected (high GRS), their choice strategy became significantly more consistent in both rewarded and unrewarded trials as reflected in lower EGLORDS ($p < .001$; Wilcoxon signed rank test). Additionally, we found that reaction time (RT) was significantly shorter when animals were in the high GRS (mice: $p < .001$ for rewarded/unrewarded trials; monkeys: $p < .001$ for rewarded trials; one-sample T-test). Further analyses using GLM confirmed that expected reward is a significant predictor of RT even when immediate reward is accounted for: higher expected rewards accompanied shorter RT in both mice ($p < .001$) and monkeys ($p < .001$; one-sample T-test). These findings show that in addition to immediate reward feedback, global reward state has a significant impact on choice strategy, perhaps via modulations of motivation and thus, elucidate the role of motivation in choice behavior.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Title: A piece-wise linear recurrent neural network identifies generalizable dynamics from neural measurements during decision-making in rodents

Authors: ***W. H. BARNETT**¹, **D. DURSTEWITZ**², **A. S. KUZNETSOV**³, **C. C. LAPISH**⁴;
¹IUPUI, Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN; ²Central Inst. Mental Hlth. & Bernstein-Center For Computat. Neurosci., Mannheim, Germany; ³Mathematical Sci. and Ctr. for Mathematical Biosci., Purdue Univ., Indianapolis, IN; ⁴Indiana University-Purdue Univ. Indianapolis, Indianapolis, Indianapolis, IN

Abstract: Understanding the neural dynamics that underlie impulsive decision making is a major challenge in computational neuroscience. Piecewise linear recurrent neural networks (PLRNNs) are capable of generating remarkably accurate neural activity patterns when inferred directly from data sets measured from the brain. However, the extent to which a PLRNN can be constructed that infers latent dynamics that are capable of generalizing to multiple different and independent subjects and datasets is an open question. If successful, this will be a powerful tool to better understand the neural dynamics that underlie decision-making and how they are altered in impulsive decisions. Here we investigate generalization of a PLRNN model of neural dynamics during a task designed to assess impulsive decision making task in Wistar rats. An adjusting amount delay discounting task was used where animals chose between large delayed vs. small immediate rewards, which is a common test of cognitive impulsivity that can be implemented in several species, including humans. In vivo neural recordings were performed in the medial prefrontal cortex of awake behaving animals resulting in a data set that consists of more than 4,000 single units that were acquired from 55 neural recording sessions across 9 different rats. The PLRNN model consists of two modules: a latent model of neural dynamics and an observation model that maps the latent variables onto the observed spike trains. A global latent model is shared across all 55 datasets, and a unique instance of the observation model transforms the global latent model to reproduce the single unit firing rates for each dataset. We use stochastic gradient descent (SGD) for training the model. SGD-based methods come with lower computational costs than the expectation-maximization algorithms that have been used most commonly so far to train PLRNNs. After training, PLRNN model generalized with an out-of-sample correlation of 0.85 to observed neural firing rate data. Inferring PLRNNs that reproduce neural dynamics in different types of decision-making will be critical to determine how the brain implements the computations that determine behavior. This approach will be informative in the investigation of the neuro-dynamical mechanism involved in impulsivity or neuropsychiatric impairment of impulse control and may be instrumental in the development of treatments or diagnoses that would otherwise be impossible from the simple assessment of neurophysiology.

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Poster

489. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 489.23

Topic: H.03. Decision Making

Title: Effort- and reward- decision making in initiative apathy - An electrophysiological study

Authors: *G. LAFOND BRINA^{1,2}, B.-T. PHAM², A. BONNEFOND^{1,2};

¹Univ. De Strasbourg, Strasbourg, France; ²U1114, INSERM, Strasbourg, France

Abstract: Apathy is a disabling symptom prevalent both in neuropsychiatric pathologies and in the healthy population. Even if more and more clinical and fMRI studies support the existence of three forms of apathy (executive, emotional, initiative), the knowledge of the impaired mechanisms, which could specifically underlie each of them, is still very limited. The aim of the present study is to specify the cognitive and neuronal mechanisms of initiative apathy. This form could be specifically associated with deficits at the interplay between motivation and cognition, underpinned by the anterior cingulate cortex, the main structure for effort- and reward-related decision making. Based on the subscores of the Dimensional Apathy Scale, two groups of 20 subjects were formed: one with a specific subclinical form of initiative apathy and one with no apathy. All the subjects were matched for age (21±2 years) and sex (40% men). They completed an effort- and reward-based decision-making (ERDM) task, combined with an electrophysiological recording. In this task, subjects must first choose between 2 options, mixing the 2 levels of effort (easy - difficult) and the 2 levels of motivation (low - high reward). Then, a sequence of 5 trials is presented. For each, subjects must decide if the number presented is odd/even or if it is higher/lower than 5. In the easy condition the 5 trials require the same activity, a switch between the two activities is required in the difficult condition. At the end of the 5 trials, a performance feedback is given. In case of positive feedback, subjects win a low or high reward, according to their choice. ANOVA performed on behavioral measures showed that subjects with initiative apathy made less effortful choices than controls, whatever the potential reward ($p < .06$) and the last obtained reward ($p < .001$). In case of a difficult option chosen, initiative group had a lower efficiency (number of accurate responses / mean of RT) than controls ($p < .05$). ANOVA performed on theta (4-8Hz) power on medial prefrontal cortex (mPFC) electrodes revealed a tendency to an effort x motivation x group interaction ($p < .10$). Only control group shows a higher mPFC theta power in the difficult-high reward condition in comparison to the three others, suggesting a greater effort for this specific condition. No such modulation has been evidenced in initiative group. Overall, these preliminary results suggest, in subjects with an initiative form of apathy, a deficit in “cost-benefit” computation. Subjects with initiative apathy are not able to modulate their cognitive effort as a function of difficulty and whatever the potential benefit. This may explain their flight from difficult choices.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Support: HBHL Doctoral Fellowship
FRQNT Doctoral Fellowship
NSERC Grant

Title: Pain now! The influence inter-temporal delay on choices about pain

Authors: ***T. BERMAN**, E. ROCHELET, M. ROY;
McGill Univ., Montreal, QC, Canada

Abstract: Accepting pain is counterintuitive, yet individuals willfully accept immediate discomfort to gain long-term benefits. It is imperative to understand how we make decisions about pain across time. Biases in inter-temporal decisions about pain cause people to experience more pain than required. Our study sought to investigate how inter-temporal choices for pain are made, the brain mechanisms underlying these decisions, and what causes these differences. Sixty healthy adults were recruited for this study. First, participants underwent a sensory calibration procedure to assess pain tolerance using electrical stimulation. Following this, they performed an inter-temporal choice task in an MRI, wherein they selected between two choices which differed in pain intensity (i.e., 60 - 90% of pain tolerance) and delay (i.e., 15s, 30s, 1-hour, and 1-month). Importantly, they were always choosing between immediate and delayed pain. We examined how pain offer level, delay, and interindividual differences predict participants choosing pain now. To accomplish this, we estimated a multilevel linear regression and found a main effect of delay, which indicates that people prefer pain now - rather than later - and that this is proportional to the delay of the later choice. Significant interactions between delay and pain levels indicated that people are willing to accept more pain now - rather than later - with increasing delay, suggesting that people have an aversion to delay. Fear of minor pain and trait anxiety were associated with a preference towards pain now, while agreeableness was linked to a preference for delayed pain. Analyses revealed clusters of neural activation in the parietal lobule, posterior cingulate cortex, and precuneus while thinking about temporal delay; additionally, the degree of activation in these regions is proportional to the delay and these regions are involved in projecting yourself into the future and mental simulations. Dorsomedial prefrontal cortex activation was proportional to the pain - regardless of delay - and has been previously reported to be related to the evaluative component of pain independent of nociception. Overall, our findings suggest that people would rather accept immediate pain than wait a longer period for less pain. The aversive nature of anticipating pain plays a larger role when waiting for longer, than shorter, delays. Neural activation patterns suggest that there are two sets of regions for delay and pain value and the psychological aspects of pain are being fixated on more than the pain itself. This study has important implications for interventions aimed to reduce detrimental biases that lead to added suffering.

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Poster

489. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

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Program #/Poster #: 489.25

Title: WITHDRAWN

Poster

489. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 489.26

Topic: H.03. Decision Making

Support: Simons Collaboration on the Global Brain 543005
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Title: Neural dynamics within and across multiple brain regions in a delayed-response task

Authors: *Y. LIU¹, S. CHEN³, Z. WANG², B. KANG¹, H. HOU⁴, L. D. LIU⁵, N. LI⁵, K. SVOBODA⁶, S. DRUCKMANN¹;

²Dept. of Applied Physics, ¹Stanford Univ., Stanford, CA; ³Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA; ⁴Janelia Res. Campus, Ashburn, VA; ⁵Neurosci., Baylor Col. of Med., Houston, TX; ⁶Allen Inst. for Neural Dynamics, Seattle, WA

Abstract: Persistent neural activity encoding short-term memory (STM) is observed in many brain regions, but how they interact to maintain STM is unclear. We used up to 5 Neuropixels probes to simultaneously record the activity of hundreds of neurons across multiple brain areas during a memory-guided delayed response task in mice. Anterior lateral motor cortex (ALM; part of M2) is an essential hub for the planning and execution of memory-guided directional licking. We targeted regions that form multi-regional networks with ALM, including nuclei in thalamus, basal ganglia, midbrain, pons, medulla, cerebellum, etc (see Susu Chen et al SfN 2022). We used reconstructed single ALM neurons from the MouseLight dataset to identify ALM projection zones for targeting recordings. ALM optogenetic photoinhibition trials were included in experiments to probe its effect on downstream regions. Here, we compare neural coding across brain regions and employ statistical models to understand brain region interactions under STM. We decoded stimulus and choice information in individual brain regions. Although coding was widely distributed, individual areas exhibited distinct dynamics. Stimulus information emerged first in midbrain and thalamus. ALM exhibited a distinct ramp-up of choice information during the memory epoch. Simultaneously recorded brain areas were highly correlated in their choice related information on a single-trial level. We assessed coordination of dynamics across brain regions using various computational approaches. We determined

subspaces that maximize correlation between neural populations (canonical correlation analysis) or variance explained (reduced rank regression). In addition, we introduced the neural encoding subspace model (NESI), which directly analyzed choice-related information by first projecting population activity of each brain area into a choice-related subspace (coding dimension, CD). NESI evaluates between-area interactions by using activities of source areas to predict the residuals after subtracting within-area dynamics in target area. We evaluated these approaches by modeling the interaction of ALM with connected brain regions. Models were fitted with simultaneous recordings and tested with ALM inhibition trials. We found commonalities and differences across different approaches. NESI predicted the effect of ALM inhibition on choice selectivity of downstream regions. We also compared differences in communication strengths between ALM vs projection or non-projection zones. These analyses reveal how brain areas communicate within different subspaces and how that relates to neural encoding of task information.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Program #/Poster #: 489.27

Topic: H.03. Decision Making

Support: Whitehall Foundation Award

Title: Low dose of clonidine improves task performance in a visual evidence accumulation task

Authors: *H. XIA¹, G. KANE², S. LI³, B. B. SCOTT⁴;

¹Boston Univ., ²Ctr. for Systems Neurosci., ⁴Psychological & Brain Sci., ³Boston Univ., Boston, MA

Abstract: Norepinephrine (NE) has been shown to play an important role in sensory processing. However, the precise function of NE in perceptual decision making remains unclear. We utilized a free-response evidence accumulation task in which rats choose a reward port based on a sequence of randomly timed light flashes from two LEDs. Rats are rewarded for orienting to the port associated with a greater probability of pulses of light. Behavior analysis suggests that rats solve this task by integrating light pulses from both sides over time and then choose a side once an internal threshold has been reached. To evaluate the effect of NE in this task, we injected clonidine, an agonist of α 2-adrenergic receptors, into six adult female Long-Evans rats. Rats that were administered with 0.01mg/kg (i.p.) of clonidine increased their accuracy and reaction time (RT) significantly compared with saline condition. To further evaluate the effects of clonidine on accumulation behavior, we fitted data from over 11,000 trials to a drift diffusion model (DDM), which describes two alternative forced choice(2AFC) as a noisy accumulation process

approaching a decision boundary. Rats injected with clonidine showed wider decision boundary separation, indicating they need more evidence to reach decision criteria. Our results are consistent with the adaptive gain theory of locus coeruleus-norepinephrine system, which predicts that as NE levels decrease, evidences that needed to reach decision criteria increases. Due to non-specific effects of clonidine, future study of cell type specific tools would be necessary to reveal the role of NE in decision-making.

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Poster

489. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

Support: NIH Grant R03DA050962
NARSAD Young Investigator Award

Title: Closed-loop virtual reality system utilizing fixed Zebrafish larvae for novel learning behavior studies

Authors: *J. JUTOY¹, J. GARG², D. AGGRAWAL³, P. BANSAL¹, E. JUNG¹;
¹Mechanical and Industrial Engin., ²Biomed. Engin., ³Computer Sci., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Zebrafish (*Danio Rerio*) larvae are ideal animal models for investigating fundamental learning behaviors. Previous works have used virtual reality in fixed and free-swimming adult and larvae Zebrafish to study learning and decision-making behaviors. Furthermore, Zebrafish larvae are optically transparent allowing the measurement of neural activity noninvasively through calcium imaging. Utilizing both virtual reality and noninvasive measurement of neural activity in Zebrafish larvae can elucidate fundamental neuroscience. Expanding on the work done with virtual reality, we developed a closed-looped virtual reality system that provides Zebrafish larvae an avatar within a physical maze. The avatar consists of a Raspberry Pi Car that receives movement inputs from the larvae while simultaneously providing the larvae physical information via visual stimuli through an LCD screen. A 3D printed microfluidic device was developed to keep the larvae in place under a microscope. Computer vision is used to identify and track larvae eye position and is filtered with an algorithm to give movement directions to the avatar. Larvae eye movement was monitored and analyzed in real-time and the decoded signals were sent to the avatar car to traverse a maze. Using the developed system, we have demonstrated the possibility of Zebrafish larvae to interact with the physical environment through virtual reality. We are currently testing forms of visual stimuli that would best elicit response from the larvae to increase the accuracy of the system. Additionally, we are refining the eye tracking algorithm to reduce noise and classify types of eye movements from saccades to

smooth pursuit. This novel system allows for new methods of experimenting on Zebrafish larvae learning and decision-making behaviors noninvasively. We will further develop the system to map neural activity to specific behaviors.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 490.01

Topic: H.05. Working Memory

Title: Is Theta Power Stable Over Time?

Authors: ***B. A. OSTER**, J. N. PABLO, J. SHIRES, M. E. BERRYHILL;
Programs in Cognitive and Brain Sciences, and Integrative Neurosci., Univ. of Nevada, Reno, NV

Abstract: Theta oscillations are associated with alertness, attention, working memory (WM), and cognitive performance. Theta is commonly compared across task conditions and over time. However, part of the value assumed by these types of comparisons is that theta-band activity remains constant within an individual over time. Yet, this assumption deserves testing as there is no extant longitudinal research on within-participant theta consistency over time. Furthermore, if theta is stable over time neuromodulatory approaches (e.g., tDCS/tACS, TMS) designed to improve cognition become more feasible because one theta calibration procedure can be implemented. Without consistent theta the success of these translational approaches is unlikely. To test whether task-related theta remains stable within-subject over time, we tested 15 participants (11 females, $M = 22.53$ years of age) in a change detection WM task across 3 sessions (minimum 48 hours between sessions) while recording EEG (32-channel BioSemi system: 512 Hz sampling rate, whole scalp). Participants were tested at the same time of day to eliminate circadian effects. Data were preprocessed to remove artifacts via interpolation, filtered (0.1Hz - 50Hz), and epoched to isolate WM delay activity. Peak theta amplitude was isolated from frontal EEG-electrodes using wavelet analysis with a precision of 0.1 Hz. The behavioral data revealed improvement across sessions 1-3 (+2.1% in WM accuracy, 220 msec faster in reaction time by Session 3). Almost all participants (13/15) showed consistent theta across all sessions. The modal peak value was 4 Hz, revealing a visual WM corollary to the visual attention literature. A Friedman's test identified no differences in theta across the three sessions ($\chi^2(2)=.00, p = 1.00$). An implication of these findings is that a single calibration per individual should be generally adequate for paradigms pairing neuromodulation with WM. Questions remain regarding the consistency of an individual's theta across WM tasks.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 490.02

Topic: H.05. Working Memory

Support: R01 NS116623
R01 EY014924

Title: Intermittent coding of memoranda by ensembles of prefrontal neurons during working memory

Authors: *M. F. PANICHELLO, D. JONIKAITIS, J. OH, S. ZHU, E. B. TREPKA, T. MOORE;
Neurobio., Howard Hughes Med. Inst. - Stanford Univ., Stanford, CA

Abstract: The neuronal substrate of working memory in the primate prefrontal cortex is currently the subject of much debate. Persistent, memorandum-selective spiking across neuronal populations has long been hypothesized to maintain information in working memory. However, this view has recently been challenged by models in which memories are retained via ‘activity-silent’ mechanisms such as short-term potentiation. Resolving this question is surprisingly difficult because the interpretation of single-trial spiking is ambiguous when recording from small populations of neurons. If neurons stop spiking, has the network entered a silent state, or has activity simply drifted elsewhere in the population? To address this, we used primate Neuropixels probes to simultaneously record from up to hundreds of prefrontal neurons as monkeys performed a spatial working memory task. To resolve the amount of memorandum-selective information maintained in the spiking of these populations, we trained a classifier to predict the cued location based on the instantaneous firing rate of each simultaneously recorded neuron and examined the time course of classifier confidence during the memory delay of single trials. Confidence on single trials was not persistently above chance level during the memory delay, but instead vacillated between high and low-confidence states. Model comparison using Gaussian mixture models confirmed this observation; confidence values were much better described as draws from a 2-component model composed of a high- and low-confidence states compared to draws from a 1-component model. At the level of single neurons, low confidence epochs were driven by low firing rates in neurons that were typically selective for the cued stimulus. Confidence was related to behavior; during the final 300 ms of the memory delay, higher confidence values strongly predicted faster response times on the task. However, confidence and reaction time (RT) were not significantly correlated prior to this, indicating that, in contrast to models of persistent activity, for much of the memory delay the presence or absence of memorandum-selective spiking was not predictive of behavior. Together, these results suggest that local populations of prefrontal neurons display intermittent bouts of memorandum-selective spiking during single trials. These high-confidence epochs are interrupted by low-confidence epochs with weaker stimulus selectivity and lower firing rates.

Future work will explore whether alternative mechanisms, such as short-term plasticity, span these low-confidence epochs.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Program #/Poster #: 490.03

Topic: H.05. Working Memory

Title: Intuitive Virtual Reality Based Frontal-Midline Theta Neurofeedback: A Feasibility Study

Authors: *S. M. GERBER, J. RIDDLE, H. LAGARDE, M. ZHANG, F. FROHLICH;
Univ. of North Carolina at Chapel Hill, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Introduction: Neurofeedback (NF) is a type of biofeedback, which reinforces desirable neural activity patterns by real-time perceptual feedback derived from ongoing brain signals. It is a promising method to target network dysfunction in psychiatric illnesses. Yet, fundamental questions about the rational design of NF treatment have remained unanswered. In particular, engagement and identification of the participant with the NF task plays an important role in the efficacy of NF. We hypothesized that virtual reality (VR) delivered by a head-mounted display could increase immersion and thus identification of the participant with the NF task. Therefore, the aim of this feasibility study is to demonstrate that NF delivered by immersive virtual reality facilitates rapid learning of the NF task, has a high responder rate, and can enhance both the targeted theta oscillation and the associated cognitive function. **Method:** The NF setup consisted of a head-mounted VR display powered by a high-performing gaming desktop, a 128-channel electroencephalography (EEG) system, and a custom C++/C#-based application for real-time EEG data analysis and updating VR display (head mounted display). Participants performed two interleaved tasks in the experiment: a NF task and a n-back working memory task as a control measure. For the NF task, participants were instructed to clean up trash in an ocean environment using only their thoughts, but they were not given specific instructions on how to achieve this. The amount of trash included in the immersive underwater scene was modulated as a function of frontal midline theta oscillation power, estimated from real-time recording of high-density EEG. Afterwards, self-report instruments that measure immersion and side-effects were administered. **Results and Discussion:** The NF was tested on an initial pilot participant (22 years). 30 healthy subjects are scheduled for enrollment. Preliminary results show that the participant was able to increase the power of theta oscillation by $93 \pm 2.8\%$ (across blocks) with initial successful manipulation of the immersive landscape after only 25 seconds. Furthermore, the NF did not evoke any negative side-effects like nausea (1, 4-point Likert scale) and was reported as immersive (4, 7-point Likert scale). **Conclusion:** Overall, using virtual reality-based NF to enhance frontal theta oscillation is feasible. It may be more intuitive than

traditional feedback methods and thus might lead to a high responder rate. Our preliminary observation suggests a great potential for the development of future non-invasive network-based treatments for psychiatric illnesses.

Disclosures: **S.M. Gerber:** None. **J. Riddle:** None. **H. Lagarde:** None. **M. Zhang:** None. **F. Frohlich:** F. Consulting Fees (e.g., advisory boards); F is the lead inventor of IP filed on the topics of noninvasive brain stimulation by UNC, FF is a paid consultant for Electromedical Products International and has received honoraria from the following entities in the last twelve months: Academic Press, Insel Spital, University of Michigan.

Poster

490. Physiological and Neural Mechanisms of Working Memory

Location: SDCC Halls B-H

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Program #/Poster #: 490.04

Topic: H.05. Working Memory

Support: National Science Foundation

Title: Expression of L-type voltage-gated calcium channel Cav_{1.2} and related signaling mechanisms in primate dorsolateral prefrontal cortex layer III microcircuits

Authors: ***D. DATTA**¹, **S. YANG**², **F. M. KRIENEN**⁴, **M. P. JOYCE**³, **E. K.-Y. WOO**⁶, **I. PERONE**², **S. UCHENDU**², **Y. M. MOROZOV**⁷, **J. I. ARELLANO**², **A. DUQUE**⁸, **P. RAKIC**¹⁰, **C. H. VAN DYCK**⁹, **S. MCCARROLL**⁵, **M. WANG**¹¹, **A. F. ARNSTEN**¹²;
¹Yale Univ., Yale Univ., Branford, CT; ²Neurosci., Yale Univ., New Haven, CT; ³Neurosci., Yale Univ., Boston, MA; ⁴Genet., ⁵Dept. of Genet., Harvard Med. Sch., Boston, MA; ⁶Yale Univ. Interdepartmental Neurosci. Program, New Haven, CT; ⁷Dept Neurobiol, ⁸Neurosci., ⁹Psychiatry, Yale Univ. Sch. Med., New Haven, CT; ¹⁰Dept. of Neurosci., Yale Univ. Sch. Med., Dept of Neurosci., New Haven, CT; ¹¹Yale Univ. Sch. of Med., New Haven, CT; ¹²Dept. Neurosci., Yale Med. Sch., New Haven, CT

Abstract: Magnification of calcium signaling near NMDAR synapses in the macaque dorsolateral prefrontal cortex (dlPFC) is needed to sustain information in working memory, but dysregulation of calcium signaling contributes to cognitive disorders. In particular, gain-of-function mutations in the *CACNA1C* gene encoding the L-type voltage-gated calcium channel, Cav_{1.2}, increase risk for mental disorders, and are associated with impaired working memory and reduced connectivity of the dlPFC. However, it is not known why gain-of-function mutations in Cav_{1.2} would impair dlPFC function. In heart, Cav_{1.2} are activated by noradrenergic β₁-adrenoceptor (β₁-AR; *ADRB1*) stimulation during stress, increasing internal calcium release. The current study examined the anatomical localization of Cav_{1.2} and related signaling proteins (β₁-AR, SK3 and calbindin) in the rhesus monkey dlPFC, and related findings to transcriptome signatures in human dlPFC neurons. Previous studies in monkeys showed that layer III dendritic spines contain the molecular machinery for cAMP to magnify internal calcium release near

NMDAR-GluN2B synapses, where moderate levels sustain neuronal firing, but high levels weaken connectivity via opening of nearby K⁺ channels. The current study used immunoelectron microscopy (immunoEM) and found extensive labeling of Cav_{1.2} and β1-AR within dIPFC layer III dendritic spines. Cav_{1.2} and β1-AR in dendritic spines were predominantly localized on the plasma membrane near asymmetric glutamatergic synapses, often in association with the smooth endoplasmic reticulum spine apparatus where they may contribute to the magnification of calcium release. Layer III dIPFC dendritic spine membranes also express SK3 potassium channels in peri- and extrasynaptic locations, whose open state is increased by calcium. Transcriptomic analyses of human dIPFC using single-cell RNA-sequencing (62,426 nuclei pooled across 50 donors) found subpopulations of superficial pyramidal cells with high co-expression of *CACNA1C*, *ADRB1*, *CALB1* (calbindin), *GRIN2B*, *HCN1*, and *KCNN3* (SK3). The transcriptome pattern was recapitulated with protein expression using multi-label immunofluorescence and confocal microscopy in rhesus macaque layer III. As calbindin-expressing dIPFC pyramidal cells are particularly vulnerable to AD tau pathology when calbindin is lost with age, these Cav_{1.2}-enriched neurons may be especially important for generating cognition, but more susceptible to toxic calcium dysregulation and degeneration following stress or genetic insults.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 490.05

Topic: H.05. Working Memory

Title: Frontoparietal bursts of oscillatory neural activity associated with numerical representations and working memory

Authors: ***X. WANG**^{1,2}, **D. HÄHNKE**¹, **S. N. JACOB**¹;

¹Translational Neurotechnology Laboratory, Dept. of Neurosurgery, Klinikum rechts der Isar, Tech. Univ. of Munich, Munich, Germany; ²Grad. Sch. of Systemic Neurosciences, Ludwig-Maximilians Univ., Munich, Germany

Abstract: In working memory tasks, sustained neural activity that represents the memorized information is a frequent finding. However, recent discoveries support an alternative biophysical model consisting of temporally sparse, short-lived oscillatory activity arranged in bursts. The neurophysiological nature and precise role of these bursts for working memory coding are unknown. We recorded spiking activity of well-isolated single units and local field potentials (LFP) in the prefrontal cortex (PFC) and ventral intraparietal area (VIP) of two rhesus monkeys

performing a delayed-match-to-sample task requiring the animals to memorize a visually presented target numerosity (set of dots) and resist an interfering distractor numerosity. With a temporally precise spectral transformation of the LFP, we confirmed that cortical neural activity was arranged in discrete oscillatory bursts that lasted around two cycles. Bursts represented transient active states of the local neural circuit with increased spiking and spike-field coupling. On a more global scale, bursting was temporally synchronized across channels and slowly drifted across the session, especially in VIP. Bursts of different frequency bands displayed distinct spatiotemporal patterns. High gamma (60-90Hz) bursts appeared in particular during the visual presentation. In the memory epochs, bursts in the low gamma (35-60Hz) and beta (15-35Hz) dominated. Bursts of different frequencies were also clustered in distinct cortical locations. Interestingly, the burst rate increased monotonically with sample numerosity, while the spectral features of individual bursts (width, amplitude, inter-band phase-synchrony) were unchanged. Burst rate, but not spectral features, was also changed by altering dopaminergic neuromodulatory tone in PFC using microiontophoretic drug application. Burst rate predicted trial performance as well as neuronal coding strength: the rate of gamma bursts was positively correlated with performance and the recovery of target information after distraction, while the opposite was found for beta bursts. Our results suggest that, although spikes and LFP bursts both represent discrete neural activities, they act on different spatiotemporal scales and have distinct roles in working memory coding. The spectral features of individual bursts may reflect underlying microcircuit architecture; however, unlike spikes, bursts do not carry information about the memorized item and do not differentiate between targets and distractors.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Program #/Poster #: 490.06

Topic: H.05. Working Memory

Support: CRSNG
Fondation CERVO

Title: Improving brain and behavioral working memory abilities using visual rhythmic stimulations

Authors: *R. HOYER¹, J. GINZBURG², M. PICARD¹, C. LABELLE¹, P. ALBOUY¹;
¹CERVO Brain Res. Ctr., Québec, QC, Canada; ²Lyon Neurosci. Res. Ctr., Lyon, France

Abstract: Working memory refers to the cognitive ability to hold and manipulate information and is supported by frontoparietal functions. Brain oscillations reflect the rhythmic activity of neural networks. It has been shown that oscillatory activity at theta rhythm (5 Hz) in the frontoparietal network predicts behavioral working memory performance. Recently, we demonstrated

that frontoparietal activity in the human brain can be selectively entrained by rhythmic sensory stimulations (visual rotation network); these non-invasive stimulations can causally modulate task performance in another modality (auditory working memory network). More particularly, our previous results indicated that visual 3D shapes in rotation, flickering at 5 Hz before each trial i) enhances theta activity in brain areas supporting working memory and therefore ii) enhances behavioral performance. In the present study, we investigate whether the aforementioned supramodal brain entrainment induced by visual rhythmic stimulation can be generalized across sensory modalities. Our hypothesis is that visual rhythmic stimulations can boost working memory performance irrespective of the sensory modality in which the information to be processed is presented. To test this hypothesis, we created a new visual working memory task, which was performed by 20 adults (18-30 years old) while we recorded their brain activity using EEG. Several blocks of the task were performed by the participants (with and without visual rhythmic stimulation, with visual rhythmic stimulation presented before each trial at 1 and 5 Hz). Preliminary results show that theta oscillation in the fronto-parietal network (at the sensor and source levels) positively predicts behavioral performance (d') during retention and manipulation periods. Moreover, visual rhythmic stimulation presented at a theta rate increases both behavioral performance and brain theta oscillatory activity in the fronto-parietal network. This study enlarges the basic understanding of working memory but, more importantly, supports the assumption that rhythmic sensory stimulation can be used to causally enhance working memory during task performance. The present results also highlight that the beneficial effects induced by this new brain stimulation technique can generalize across sensory modalities. If this innovative method seems promising to directly train brain activity, further studies should firstly investigate its long-term positive impact.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

Support: ONR MURI N00014-16-1-2832
The JPB Foundation
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Title: Robust and Brain-Like Working Memory through Short-Term Synaptic Plasticity

Authors: *L. KOZACHKOV¹, J. TAUBER¹, M. LUNDQVIST³, S. L. BRINCAT¹, J.-J. SLOITINE², E. K. MILLER²;

¹MIT, ²MIT, Cambridge, MA; ³Dept. of Clin. Neurosci., Karolinska Inst., Stockholm, Sweden

Abstract: Working memory has long been thought to arise from sustained spiking/ attractor dynamics. However, recent work has suggested that short-term synaptic plasticity (STSP) may help maintain attractor states over gaps in time with little or no spiking. To determine if STSP endows additional functional advantages, we trained artificial recurrent neural networks (RNNs) with and without STSP to perform an object working memory task. We found that RNNs with and without STSP were able to maintain memories over distractors presented in the middle of the memory delay. However, RNNs with STSP showed activity that was similar to that seen in the cortex of a non-human primate (NHP) performing the same task. By contrast, RNNs without STSP showed activity that was less brain-like. Further, RNNs with STSP were more robust to noise and network degradation than RNNs without STSP. These results show that STSP can not only help maintain working memories, it also makes neural networks more robust and brain-like.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

Support: BrainsCAN Accelerator grant R5316A15
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Title: Cortical Microcircuits Underlying Stress-mediated Impairment of Working Memory

Authors: ***A. HASHAD**, D. PALMER, L. M. SAKSIDA, T. J. BUSSEY, W. INOUE;
Univ. of Western Ontario, London, ON, Canada

Abstract: Stress impairs cognition in healthy people and worsens cognitive dysfunction in mental illness. The prefrontal cortex (PFC) is a major target of stress, where stress-induced neurochemical changes are proposed to impair working memory. However, specific molecular and circuit mechanisms remain unsolved partly due to the difficulty in assessing working memory in rodents. Past studies primarily used delay tasks in mazes that are robust to delays (>10 s) longer than what is reported in primates, rendering comparing results difficult. To overcome this, we focused on using the touchscreen operant chambers to establish a rodent working memory task that is sensitive to short delays. First, we used the trial unique non-matching to location (TUNL) task to test working memory in male and female mice. Both sexes displayed working memory impairment that was highly sensitive to short delays (1-5 s). Next, mice were stressed by acutely restraining them for variable durations to alter the stressor magnitude. Interestingly, 1 hour restraint stress enhanced working memory, whereas 4 hours restraint impaired it pointing to U-shape effect of stress on working memory performance.

Further pharmacology experiments revealed a role for the PFC stress mediator corticotropin releasing hormone (CRH) in working memory alterations. In summary, touchscreen operant chambers provide a stress-sensitive rodent working memory task that will help dissect the neuronal circuits involved in stress-mediated cognitive impairments.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

Support: European Commission Horizon 2020 Marie Skłodowska-Curie Individual Fellowship (grant 843158, COMEDM)

Title: A shared neural circuit for maintenance and integration of information over time

Authors: *P. R. MURPHY¹, H. MCDERMOTT¹, K. WIMMER², J. M. ESNAOLA-ACEBES², A. COMPTE³, R. WHELAN¹;

¹Trinity Col. Dublin, Dublin, Ireland; ²Ctr. de Recerca Matemàtica, Bellaterra (Barcelona), Spain; ³Inst. D'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

Abstract: Working memory and decision-making are two essential cognitive functions for which detailed neural circuit models have been developed in recent years. Prominent classes of these models share key features - persistent neural activity through recurrent excitation within feature-selective populations of neurons and inhibition between them - which has led to the suggestion that both maintenance (subserving working memory) and integration (subserving deliberative decision-making) of information over time are implemented within the same neural circuits. Here, we present new lines of convergent evidence that support this idea. Human participants ($N=30$) and *tabula rasa* recurrent neural networks (RNNs) were trained to remember the spatial location of single stimuli for variable delay periods (working memory); and to estimate the average location of sequences of such stimuli (decision-making). The trained RNNs recapitulated the basic circuit configuration and persistent activation patterns of existing, hand-crafted neural circuit models of working memory and decision-making ('ring attractors'). Crucially, a subset of units in these networks that persistently encoded memorandum location during working memory maintenance also encoded the running average of stimulus locations (i.e. the decision variable) during decision formation, pinpointing a shared circuit for both functions. In the participants, EEG data supported the possibility that humans exploit this same shared-circuit solution, in that the patterns of neural activity encoding these key task variables generalized across the two task contexts. Moreover, while human participants successfully adapted across contexts, their behaviour in both was characterized by shared noise and biases

that could not be attributed to sensory encoding or motor production processes and must instead have arisen from a common source in the memory/decision circuit. Lastly, trial-related changes in pupil diameter suggested a mechanism - dynamic modulation by ascending arousal systems - by which the shared circuit can be tuned to produce stable memory states in some contexts, versus labile decision states in others. These findings promote an integrative perspective of working memory and decision-making that builds on existing neurobiological models, and holds promise for understanding disorders of the brain that are characterized by deficits in both functions.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

Support: Simons Center for the Global Brain

Title: Generalizable memory and prediction of multi-object dynamic scenes in the primate brain

Authors: ***N. WATTERS**¹, **J. GABEL**², **M. JAZAYERI**²;
¹MIT, Somerville, MA; ²MIT, Cambridge, MA

Abstract: The primate mind excels at reasoning about physical scenes comprised of objects, flexibly generalizing to novel objects and novel scenes. Object files and graph-structured scene representations have been hypothesized to underlie such generalizable reasoning, yet these cognitive theories remain largely un-tested in the primate brain. To address this shortcoming, we developed a task for non-human primates (NHPs) that requires tracking the identity and position of multiple occluded objects as they move in a visual display. We first verified that animals were able to remember and predict the positions and identities of up to two independently moving objects. Next, we tested the animals on a suite of held-out generalization conditions including (i) novel object identities, (ii) novel combinations of objects, (iii) novel number of objects, and (iv) novel dynamics of objects. In all cases, NHPs were able to generalize, as predicted by cognitive theories of object files and graph-structured scene representations. Evidence from neuroscience implicates several regions involved in inferring and maintaining dynamic representations of visual scenes in memory, including dorsolateral prefrontal cortex (dlPFC), dorsomedial frontal cortex (DMFC), lateral intraparietal cortex (LIP), parietal area 7a, and inferior temporal cortex (IT). Accordingly, we have begun to perform acute recordings from these areas while the animals perform the task. So far, we have recorded simultaneously from DMFC and dlPFC in two NHPs, and have collected thousands of single neurons in these areas. Importantly, we recorded not only during conditions within the animals' training distribution, but also throughout

the generalization conditions. We now plan to record simultaneously from IT, LIP, and 7a to complete a large-scale multi-area dataset to test specific hypotheses about whether the primate brain employs object files and/or graph-structured scene representations when reasoning about multi-object dynamic scenes.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

Support: R01-MH114877
R01-AG063775

Title: Revealing working memory resilience and reactivation under distracting contexts

Authors: *W. WEN¹, D. HAZEL², R. M. REINHART¹;

¹Boston Univ., ²Boston Univ., Boston, MA

Abstract: A crucial function of working memory (WM) storage is maintaining representations when faced with distractions. Previous studies showed that distraction alters the way visual WM information is stored. However, most studies used task-irrelevant distraction and it remains elusive how WM representations are maintained and retrieved when distractor interference arises at the level of shared attentional control processes. Participants in the present study were instructed to memorize two colored bars and reproduce the orientation of the cued bar at the end of each trial. Static dots (neutral condition) or moving dots would be presented during the retention interval. Depending on whether there is a secondary task pertaining to the moving dots, trials are categorized as the passive distraction condition and the interruption condition. The interruption task is to detect a short-lived speed increase. Participants showed worse performance in interruption trials relative to the neutral condition and the distraction condition, with slower and less accurate responses as well as a larger bias towards the distracting dots. Mahalanobis-distance based decoding was performed on scalp electroencephalogram (EEG) data to reveal the orientation-selective representation of bars and dots. Participants with higher dots decoding accuracy during retention interval had lower WM precision. Compared to the distraction condition where dots were voluntarily inhibited, the remaining representation of dots after an interruption task had been completed caused interference to WM reactivation. Source level analysis suggested stronger theta activity at the anterior medial prefrontal cortex (PFC) and lateral PFC during WM reactivation. Taken together, our results illustrate the dynamic representational change of WM items under distracting contexts.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

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Searle Scholars Program

Title: Theta-rhythmic temporal coordination avoids representational conflicts during working memory

Authors: M. ABDELAZIZ, Z. V. REDDING, *I. C. FIEBELKORN;
Dept. of Neurosci., Univ. of Rochester, Rochester, NY

Abstract: A large-scale network of cortical and subcortical structures (i.e., the ‘attention network’) directs the sensory and motor aspects of environmental sampling. Theta-rhythmic neural activity (~4-6 Hz) within the attention network helps to temporally coordinate these potentially competing functions, seemingly alternating between states that promote either sampling (i.e., sensory functions) or shifting (i.e., motor functions). Here, we tested whether such theta-rhythmic coordination of neural activity might be a more general mechanism for avoiding conflicts in the brain. Specifically, we recorded EEG while human participants performed a working memory task, requiring them to simultaneously maintain representations of two items in working memory: item 1 and item 2. We first demonstrate that behavioral performance and the strength of neural activity, following a memory probe, wax and wane as a function of pre-probe theta phase. We next tested the hypothesis that theta-rhythmic neural activity helps to temporally coordinate potentially conflicting item representations. Consistent with this hypothesis, our results provide evidence that the strength of these competing item representations, for items 1 and 2, alternates over time as a function of theta phase. Different pre-probe theta phases were associated with better behavioral performance and stronger neural activity depending on whether the memory probe matched either item 1 or item 2. We propose that different item representations are sequentially refreshed relative to the phase of theta-band activity, perhaps through the periodic strengthening of short-term synaptic changes. Our findings indicate that theta-rhythmic temporal coordination not only helps to avoid *functional* conflicts during environmental sampling but also *representational* conflicts during working memory.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Title: Altered prefrontal-thalamo-hippocampal circuit function during a spatial working memory task in SETD1A-deficient mice

Authors: *M. J. RAILING¹, S. HUPALO², M. V. MYROSHNYCHENKO³, J. A. GOGOS⁴, D. A. KUPFERSCHMIDT⁵, J. A. GORDON⁶;

¹Natl. Inst. of Hlth., Natl. Inst. of Neurolog. Disorders and Stroke, Villa Park, IL; ²NIH/NIMH, ³Natl. Inst. of Hlth. (NIH), Bethesda, MD; ⁴Zuckerman Inst., New York, NY; ⁵NINDS / NIH, NINDS / NIH, Bethesda, MD; ⁶NIMH / NIH, NIMH / NIH, Bethesda, MD

Abstract: Heterozygous null mutations of SETD1A are definitively linked to increased risk for schizophrenia in humans. Prior research has shown that SETD1A haploinsufficient mice exhibit deficits in spatial working memory, a cognitive function often disrupted in schizophrenia. To explore the neurobiological consequences of SETD1A haploinsufficiency, we examined synchrony across circuits necessary for successful working memory performance in rodents, consisting of the medial prefrontal cortex (mPFC), ventral hippocampus (vHPC), dorsal hippocampus (dHPC), and the thalamic nucleus reuniens (Re). Adult male and female *Setd1a*^{+/-} and C57BL/6J wildtype (WT) littermate controls were trained in a delayed non-match-to-sample T-maze task to assess spatial working memory. Local field potentials were recorded from the mPFC, vHPC, dHPC, and Re prior to and during task performance. Experimenters were blinded to genotype. Contrary to a prior report, we observed that *Setd1a*^{+/-} mice acquired and performed the working memory task comparably to WT controls (n=30-38). Female mice performed comparably to male mice. Coherence, a measure of neural synchrony, was assessed prior to training and during performance of the working memory task (n=17-25 across different brain regions). We found that *Setd1a*^{+/-} mice exhibited greater vHPC-mPFC ($p=0.02$) and vHPC-Re ($p=0.01$) gamma synchrony during pre-training maze exploration relative to WT controls. *Setd1a*^{+/-} mice also showed reduced Re-mPFC broadband synchrony ($p<0.05$), and reduced dHPC-mPFC theta synchrony ($p=0.04$), compared to WT mice. During established task performance, *Setd1a*^{+/-} mice showed greater vHPC-mPFC broadband synchrony ($p<0.001$), and greater vHPC-Re gamma synchrony ($p<0.001$), compared to WT mice. Further, *Setd1a*^{+/-} mice displayed reduced Re-mPFC broadband synchrony relative to WT mice ($p<0.001$). No genotype differences were observed in dHPC-mPFC coherence. These coherence signatures were observed irrespective of task phase (sample, delay, choice), delay length (10, 30, 60 sec) and trial outcome. Importantly, *Setd1a*^{+/-} and WT mice showed no differences in oscillatory power in the mPFC, vHPC, dHPC, or Re. These findings implicate disruptions in prefrontal-thalamo-hippocampal synchrony in SETD1A-related genetic predisposition to schizophrenia. Future studies will examine whether SETD1A haploinsufficient mice exhibit deficits in vHPC, dHPC, and Re oscillatory modulation of mPFC neuronal spiking.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 490.14

Topic: H.05. Working Memory

Support: NIH Grant MH095984

Title: Single-pulse TMS affects working memory performance via posterior beta band oscillations

Authors: *J. M. FULVIO¹, S. HAEGENS², B. R. POSTLE³;

¹Psychology, Univ. of Wisconsin-Madison, Madison, WI; ²Dept. of Psychiatry, Div. of Systems Neurosci., Donders Inst. For Brain, Cognition & Behaviour, New York, NY; ³Psychology & Psychiatry, Univ. of Wisconsin-Madison, Madison, WI

Abstract: The double-serial retrocuing (DSR) working memory task begins with presentation of two items, followed by a cue indicating which will be probed at test 1, then, after test 1, a second cue indicating which will be probed at test 2. Although cue 1 can lead to the loss of multivariate evidence for the uncued item, a single pulse of transcranial magnetic stimulation (TMS) reinstates multivariate decodability of this “unprioritized memory item” (UMI) from concurrently measured EEG in the beta band (16-24 Hz; Rose et al., 2016). Additionally, when UMIs are used as lures, TMS increases false alarm rates. Conventional analysis methods cannot elucidate whether the effects are due to modulation of existing oscillations prior to the manipulation or due to de novo evoked responses. To address this, we replicated the TMS-EEG procedures from Rose et al. (2016), delivering single pulses to right IPS2 (Delay 1 only, Delay 2 only, Delay 1 & Delay 2, no-TMS). We then decomposed the EEG data using Spatially distributed PhAse Coupling Extraction with Frequency Specific Phases (SPACE-FSP; van der Meij et al., 2015; 2016; N=12 participants: 5 females, 18-28 years, M = 21.7 years, all right-handed). This approach decomposes the scalp-level signal into discrete coupled oscillators, which remain intermixed with conventional methods. Here, we focus on low frequency components at posterior electrodes. In all, 115 beta-band components were extracted across participants ($M = 9.6$ ($SD = 4.1$) components per subject). Of these, 78 (2-15 per subject, $mode = 9$) showed task-related modulation, 50 sensitive to trial onset, and an overlapping 62 sensitive to prioritization cues. In general, these components were positively related to “load” (i.e., loading with 2 equally prioritized items held in working memory > loading with 1 prioritized memory item (PMI) + 1 UMI > loading with 1 PMI [after cue 2]). Although the loadings for these components were positively correlated with accuracy, this effect was carried by TMS trials, and was specific to posterior beta: it was not observed in posterior components in the alpha band, nor in beta components from central electrodes. TMS had the effect of decreasing loadings relative to no-TMS. Importantly, none of these component loadings were TMS-evoked, but rather modulations of existing components. Thus, the TMS-related “reactivation effect” derives, in part,

from modulation of endogenous beta-band oscillations that support working-memory performance.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Program #/Poster #: 490.15

Topic: H.05. Working Memory

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Title: Non-oscillatory aperiodic activity influences theta power and theta phase estimates

Authors: *Q. VAN ENGEN¹, A. SMITH², B. VOYTEK¹;

¹Cognitive Sci., ²UCSD, San diego, CA

Abstract: Human cortical theta power and phase alignment have been shown to correlate with better working memory (WM) performance. These results stand in contrast to the fact that, in human neocortex, theta oscillations are infrequent and bursty, with much of the neural signal dominated by arrhythmic, aperiodic activity. This aperiodic activity is itself dynamic and task-modulated. Here, we examine whether aperiodic activity dynamics influences theta power and phase estimates, and whether aperiodic activity in the absence of oscillations relates to working memory. To begin, we simulated EEG time-series that contain different levels of aperiodic, and periodic (theta) activity. For analyses, theta power over time was calculated with the Hilbert transform, and phase-locking values were calculated to study phase alignment. There is a main effect of the aperiodic exponent on theta power such that, even in the absence of an oscillation, apparent theta power increases as a function of changes in aperiodic activity alone. In addition, there is an interaction effect between the aperiodic exponent and theta amplitude such that without an increase in theta amplitude, a decrease in the aperiodic exponent decreases estimated theta power. Furthermore, both theta amplitude and aperiodic exponent influence theta phase locking. Thus, aperiodic activity influences both theta power and theta phase estimates. Specifically, a reduction in theta power or theta phase coupling can be driven solely by a change in aperiodic exponent. This simulation experiment shows how other, non-oscillatory components of EEG signals contribute to traditional, putative oscillation results, previously thought to arise from periodic activity alone. Given that aperiodic activity changes due to task-relevant influences (such as working memory), it is possible these aperiodic changes influence theta power and theta phase estimates as calculated with the Hilbert transform and phase-locking analysis. Thus, caution is required when interpreting results from analyses that do not account for aperiodic changes.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

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Title: Striatal Dopamine Boosts Reinforcement Learning, Controlling for Effects on Working Memory and Cognitive Effort

Authors: *A. WESTBROOK¹, R. VAN DEN BOSCH², L. HOFMANS³, D. PAPADOPETRAKI⁴, J. MÄÄTTÄ⁵, M. J. FRANK⁶, R. COOLS⁷;

¹Brown Univ., Providence, RI; ²Donders Inst., Radboud Univ., Nijmegen, Netherlands; ³Univ. of Amsterdam, Amsterdam, Netherlands; ⁴Donders Inst., Nijmegen, Netherlands; ⁵Karolinska Institutet, Solna, Sweden; ⁶Brown Univ., Carney Inst. for Brain Sci., Providence, RI; ⁷Donders Institute, Radboud Univ., Nijmegen, Netherlands

Abstract: Stimulus-response learning can be accomplished entirely via incremental, dopamine-mediated reinforcement learning (RL). Yet, prefrontal cortex-based working memory (WM) may also contribute. Intuitively, WM affords rapid (e.g. one-trial) learning, but is limited in both capacity and the duration over which information can be maintained. WM is also effort-costly, and striatal dopamine signaling can promote willingness to do cognitive effort. Prior studies have failed to distinguish between the effects of dopamine on striatal RL or prefrontal WM contributions when observing that dopaminergic drugs speed learning. In this study, we test the hypotheses that striatal dopamine mediates both the degree to which people rely on costly working memory during stimulus-response learning and also RL speed, after taking into account WM contributions. N = 100 participants were recruited to complete a paradigm isolating WM contributions in a multi-session, double-blind, placebo-controlled, pharmaco-PET study in which we measure baseline dopamine synthesis capacity with [18F]DOPA, and separately manipulate dopamine with methylphenidate, and antagonize D2 receptors with sulpiride. As predicted, we find that striatal dopamine speeds learning. Specifically, both methylphenidate and higher dopamine synthesis capacity enhance learning, while sulpiride decreases accuracy. Computational modeling reveals that higher dopamine synthesis capacity predicts greater reliance on WM versus RL. Meanwhile methylphenidate boosts the rate of RL, controlling for its effects on WM. Consistent with our previous studies, we also find that methylphenidate diminishes effort discounting during reward learning. Finally, we find that accuracy was lower on sulpiride due to both diminished WM contributions to learning and also faster decay of WM contents.

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Poster

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Program #/Poster #: 490.17

Topic: H.05. Working Memory

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Research to Prevent Blindness

Title: Prefrontal dopamine governs entry of visual targets into working memory

Authors: *I. VANEGAS, K. CLARK, B. NOUDOOST;
Ophthalmology & Visual Sci., Univ. of Utah, Salt Lake City, UT

Abstract: Several lines of evidence highlight the role of the Frontal Eye Field (FEF) part of prefrontal cortex in modulating the strength of signals within visual areas. Results in our lab show that FEF sends a strong working memory (WM)-related input to visual cortical areas. Considering the known role of dopamine D1 receptors (D1Rs) in enhancing WM-related persistent activity in prefrontal areas, we tested how D1R manipulation within the FEF alters the selection of WM targets and its neural counterpart in posterior visual area V4. The monkey performed a modified memory guided saccade task in the presence of a distracting stimulus. In this task, the monkey fixates, a peripheral target appears, and the monkey is required to remember the target location throughout a delay. During the delay period, a distracting stimulus is presented at one of several locations. The monkey must maintain the target information, and after the delay period, execute a saccade towards the target location in order to obtain a reward. Targets and distracters are placed around the overlapping response field of simultaneously recorded FEF and V4 areas. Neural activity in V4 and FEF was simultaneously recorded, before and after manipulation of the D1R-mediated activity within the FEF. Before FEF manipulation, the monkey was consistently able to ignore the distracter and correctly select the target location at the end of the delay period. FEF D1R manipulation (via injection of 0.5-1 microliters of D1R antagonist SCH23390) influenced behavioral sensitivity to visual stimuli. Following this manipulation, when the distracter appeared at the location corresponding to the FEF response field at the injection site, the monkey was biased toward selecting the distracter location. These results show that manipulating FEF D1Rs is sufficient to alter the selection of visual targets for entry into WM. Analysis of spiking activity and local field potentials in FEF and V4 reveals which aspects of the activity within these areas, and the communication between them, govern the ability of a visual stimulus to guide WM-dependent behavior.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

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Title: Frontal and parietal TMS perturbs serial biases in color working memory

Authors: *D. CELLIER¹, J. M. SCIMECA², A. KIYONAGA¹;

¹Cognitive Sci., UCSD, La Jolla, CA; ²Helen Wills Neurosci. Inst., UC Berkeley, Berkeley, CA

Abstract: Serial dependence is the phenomenon by which perceptual information from the recent past influences currently held representations, and has been hypothesized to reflect a stabilization of perceptual processing. Such temporal stabilization could be a key component of working memory (WM) processes. Furthermore, though the fronto-parietal network is known to support WM, distinguishing the roles of prefrontal cortical from parietal regions remains an area of active research. One framework proposes that while frontal regions are preferentially involved in future-planning, parietal regions temporarily store and integrate information from the recent past.

Here, we tested the idea that frontal and parietal regions make distinct contributions to serial dependence in WM. In the context of a Registered Report, participants completed a continuous report color WM task, with variable set size, during fMRI scanning. Individual functional activation peaks were used to localize experimental targets for transcranial magnetic stimulation (TMS). On separate days, participants received continuous theta-burst TMS to one of three experimental targets- superior intraparietal sulcus (IPS), inferior IPS, and lateral prefrontal cortex (PFC) - or to a primary sensory cortex (S1) control site. After each TMS session, they completed the WM task. Serial dependence was assessed as the recall error on the current trial, as a function of the feature distance between the current and previous trial WM probe in a 360-degree color space.

Combining data from all TMS sites, we observed attractive serial dependence: participants recalled colors that were skewed slightly towards the previous WM probe, if the current and previous trial colors were nearby in feature space. However, after S1 control TMS, we also observed a repulsive bias when current and previous trial colors were more distinct. This repulsion effect was eliminated after IPS stimulation, but not PFC, consistent with evidence that the IPS maintains recent sensory history. Moreover, the magnitude of attractive serial dependence was increased at higher set sizes, consistent with the idea that it is an adaptive function to stabilize noisy representations. TMS to IPS vs. PFC sites differentially modulated the magnitude and width of serial dependence, suggesting that the regions play distinct roles in the

adaptive integration vs. differentiation between successive stimuli.

On the whole, these data are consistent with the idea that IPS and PFC serve complimentary but distinct roles in WM, and may support the merging of past information, present perception, and future-oriented goals to accomplish tasks.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Program #/Poster #: 490.19

Topic: H.05. Working Memory

Title: A trainable Oscillatory Autoencoder network to model the storage of working memory in nested theta/gamma oscillations

Authors: *D. BISWAS, R. KRISHNAMURTHY, S. V. CHAKRAVARTHY, T. KANAGAMANI;
Dept. of Biotech., IIT Madras, Chennai, India

Abstract: Notwithstanding the debate on the precise definition of working memory (WM) it is generally agreed that WM is distinguishable from long-term memory (LTM) in two respects. Whereas WM is thought to be encoded by persistent neural activity, LTM is encoded in synaptic connections. While WM has a finite capacity, - typically about 7 ± 2 items – LTM appears to have practically unlimited capacity. Furthermore, there is electrophysiological evidence that the persistent neural activity by which WM is implemented, consists of theta nested gamma oscillation. Hippocampal recordings of navigating animals have shown a similar kind of synchronous activity of distinctive cell assemblies encoding the maze. Each of these cell assemblies encodes a particular memory item or a place field and fires synchronously for 10 to 30 ms, typically the time period of the slower gamma cycle. We propose an Oscillatory Autoencoder (OAE) model that stores WM items in the form of neural oscillators. The OAE model is constituted of an Oscillatory Core (OC) that is flanked by a conventional static autoencoder (SAE). The OC consists of three layers of Hopf oscillators oscillating in delta, theta and gamma bands. The ability of the SAE to generate a compressed representation of high dimensional vectors is used to encode serially represented items into a multidimensional timeseries signal, further forward propagated through hierarchically organized OC layers, where the inner layer of slow oscillators adapts to the principal frequency components in delta band, central layer of faster oscillators steps up the slow oscillation into the theta/gamma band and outer layer of slow oscillators further steps down the fast oscillation into delta band. The complex-valued feed-forward weights forward propagating this slow oscillation to the output complex sigmoidal layer of OC through another hidden layer of complex sigmoidal neurons are optimized using supervised learning framework by the same multidimensional signal at the input of OC. The performance of the OC is dependent on the number of alpha-numeric images

presented. The proposed Oscillatory Autoencoder is able to produce the desired sequence at the output with considerable accuracy only when the no of items presented at the input is below 10. The network additionally exhibits attractor dynamics to reproduce the learnt/memorized memory items in the same order as theta sequence.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

Support: 2020M3C1B8081319 (NRF of Korea)

Title: Improved working-memory performance by cross-frequency coupled transcranial alternating current stimulation to central-executive and default-mode networks with a phase lag

Authors: **J.-C. PARK**¹, **E.-S. HONG**¹, **Y. KIM**¹, **J. KWON**¹, **J. SEO**², **H. KIM**², ***B.-K. MIN**¹;
¹Dept. of Brain & Cognitive Engin., ²Inst. for Brain & Cognitive Engin., Korea Univ., Seoul, Korea, Republic of

Abstract: Transcranial current stimulation is a potent neuromodulation technique used to enhance human cognitive function in a non-invasive manner. In this study, we investigated whether a cross-frequency coupled transcranial alternating current (CFC-tAC) stimulation with phase lags improved working-memory performance. Twenty-two healthy participants were recruited for this study. Participants were instructed to perform a modified Sternberg task, in which a combination of letters and digits was presented. The CFC-tAC stimulation was exposed online during the middle of the retention period for 6 secs: one for the 45-degree phase-lag and the other for the 180-degree phase-lag between the central-executive network (CEN) and the default-mode network (DMN). We analyzed the performance accuracy between these two conditions. We observed that the 45-degree phase-lag condition showed significantly enhanced accuracy as compared to the 180-degree phase-lag condition ($t(21) = 3.02$, $p < 0.01$; 45-degree, 68.03%; 180-degree, 62.80%). This finding may suggest that CFC-tACS with a phase difference between CEN and DMN plays a promotive role in facilitating the sustained maintenance of encoded information during the retention period for better working memory performance.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

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Title: Phase-dependent stimulation modulates phase-amplitude coupling in the human hippocampus

Authors: ***Y. SALIMPOUR**¹, N. E. CRONE², U. RUTISHAUSER³, W. S. ANDERSON¹;
¹Neurosurg., Johns Hopkins Sch. of Med., Baltimore, MD; ²Neurol., The Johns Hopkins Hosp., Baltimore, MD; ³Dept. of Neurosurgery, AHSP #6432, Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Neuronal oscillations in different frequency bands have been reported in the human hippocampus. Theta rhythms and gamma oscillations are well-studied neuronal oscillations in the hippocampus. Additionally, theta and gamma interact with each other in the form of cross-frequency coupling (CFC). Phase-amplitude coupling (PAC) is a typically reported form of CFC in the hippocampus in which the phase of theta modulates gamma amplitude. PAC coordinates neuronal activities at specific timescales essential for memory processing in the hippocampus. The level of hippocampal PAC is correlated with memory performance. It predicts memory activity over the course of associative learning, which seems to be essential for the process of memory consolidation and retrieval. Indeed, a causal relationship between artificially induced PAC in the hippocampus and memory reconsolidation has been shown. This raises the question of whether increasing PAC through electrical stimulation can improve memory. Here, we present and evaluate a technique for doing so. Phase-dependent stimulation (PDS) is an emerging neuromodulation technique that delivers electrical current selectively at specific phases of detected rhythmic activity to a target brain location. The PDS technique comprises several sequential blocks, including frequency band optimization within the theta frequency range, autoregressive spectral estimation, estimation of the future signal by autoregressive time-series prediction, and calculation of the instantaneous frequency and phase. In this study, we applied phase-dependent stimulation directly to the anterior and posterior regions of the hippocampus of five invasively monitored epilepsy patients at rest. We found that stimulation targeted to the peak of the hippocampal theta oscillations increased theta-gamma coupling, whereas theta trough stimulation reduced theta-gamma PAC. Taken together, our results show that in the human hippocampus, phase-dependent stimulation is capable of modulating PAC levels, which could alter memory processes and allow applications for the treatment of memory-related neurological disorders associated with abnormal theta-gamma PAC, such as Alzheimer's disease.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

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MOE AcRF Tier 1 Award RS01/19
MOE AcRF Tier 1 Award RG19/21

Title: Aging effects on the working memory circuit in the prefrontal cortex

Authors: H. CHONG, M. HO, X. OUYANG, S. TSONG, ***T. KAMIGAKI**;
Lee Kong Chian Sch. of Med., Nanyang Technological Univ., SINGAPORE, Singapore

Abstract: Executive function is susceptible to aging. Among cognitive aging, the decline of working memory (WM) has the earliest onset and constitutes one of the most essential components. Converging evidence suggests that the prefrontal cortex (PFC) plays central roles in various aspects of executive function including WM and that the malfunction of the PFC could be a critical contributor to cognitive aging. To examine how aging affects the neural processing for WM in the PFC, we trained mice from young adulthood to advanced age with a delayed two-alternative forced-choice task. During the task, mice were presented with a sensory cue, either an auditory, tactile, or bimodal stimulus, and responded by licking left or right after two seconds of the memory delay period. All the age groups were able to acquire the task with bimodal stimuli, showing age-related decrement in the learning rate. After learning, all the groups achieved comparable performance in the tactile task, whereas they displayed an age-dependent decline in the performance in the auditory task, likely due to age-related hearing deterioration. Calcium imaging from the medial PFC (mPFC) demonstrated an age-related decrement in the ratio of memory-coding neurons and in the memory selectivity of individual neurons in all the modalities, which resulted in the reduced decoding accuracy of memory contents in the aged mPFC. Interestingly, the population activity in the young mPFC exhibited similar patterns between the auditory and tactile modalities, offering cross-modal memory representation, whereas the activity patterns got segregated between modalities in the aged mPFC. Furthermore, measurement of spontaneous activity during a resting state revealed a remarkable reduction in the functional connectivity as age increased, particularly among pairs of memory-coding neurons. These results suggest the age-related retardation of WM-related neural processing and reorganization of the WM-relevant circuits in the PFC.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Topic: H.05. Working Memory

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Research to Prevent Blindness

Title: Tracing the Influence of Oscillations on Gating of Signals into Prefrontal Cortex

Authors: *P. COMEAUX¹, L. NURMINEN³, F. FEDERER², A. ANGELUCCI², B. NOUDOOST²;

¹Dept. of Biomed. Engin., ²Dept. of Ophthalmology and Visual Sci., Univ. of Utah, Salt Lake City, UT; ³Col. of Optometry, Univ. of Houston, Houston, TX

Abstract: Working memory (WM) is the cognitive ability that allows an organism to maintain recent information in order to guide behavior. Communication between the sensory areas that process visual information and prefrontal areas that prepare movement plans is pivotal to the information flow during WM tasks. Efficacy of this communication has been shown to depend on the content of WM. Moreover, coherence between prefrontal and posterior visual areas has been shown to correlate with success in WM tasks. We tested whether local oscillations within, and coherent oscillations between, the visual and prefrontal regions determine the efficacy of communication between these areas in order to govern the dynamic gating of information based on the content of WM. We focus on the frontal eye field (FEF) part of the prefrontal cortex and extrastriate area V4, whose reciprocal communications have been shown to be modulated by the content of WM. In order to determine the impact of oscillations on the efficacy of communication, we used optogenetic photostimulation to induce excitation in V4 neurons projecting to the FEF while monitoring local field potentials within both regions, as well as the spiking activity in the FEF. An anterograde virus expressing channelrhodopsin-2 was injected into V4. After allowing viral expression, we performed photostimulation of V4 axon terminals within the FEF. This allowed us to specifically manipulate the V4 input signal into the FEF. In order to assess the impact of cognitive demand on the efficacy of communication and its relationship with oscillations, we used a classical memory guided saccade task. In this task, the animal fixated on a central point and was presented with a peripheral target stimulus. After a delay, the fixation point disappeared, and the animal responded by moving its eyes to the remembered target location to receive a reward. The visual target was presented either within the overlapping V4 and FEF response fields, or in the opposite hemifield. Photostimulation was delivered either during the fixation, visual target presentation, delay, or response epochs on 50% of trials, with the remaining trials serving as control trials. Stimulation efficacy is measured as the photostimulation-induced FEF spike count. We compare how the efficacy varies as a function of task epoch, the content of WM, oscillation phase, and interregional oscillatory coherence. These analyses will show how cortico-cortical communication can be dynamically gated during a cognitive task, and the role of oscillations in this gating.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Title: Spatiotemporal dynamics of insula involvement in verbal working memory

Authors: *A. LLORENS¹, L. BELLIER¹, J. IVANOVIC², P. G. LARSSON², J. J. LIN³, T. ENDESTAD⁴, A.-K. SOLBAKK⁵, R. T. KNIGHT¹;

¹Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA; ²Dept. of Neurosurg., Oslo Univ. Hosp., Oslo, Norway; ³Dept. of Neurol. and Ctr. for Mind and Brain, Univ. of California, Davis, Davis, CA; ⁵Dept. of Psychology, ⁴Univ. of Oslo, Oslo, Norway

Abstract: The insula lies deep within the lateral sulcus and is divided anteriorly and posteriorly by the central insular sulcus. Insular bi-directional connections with cortical, medial temporal, and limbic structures make it an ideal hub for multiple cognitive functions. A neuroanatomical gradient has been reported in the insula with viscerosensory and sensorimotor functions represented more posteriorly, and higher order cognitive functions more anteriorly. The left insula (LI) has been associated with language processing and the right insula (RI) with error processing, memory, and attention. Given its connectivity to key verbal working memory (vWM) structures and its role in cognition, we hypothesized that the insula is a core component of the vWM network. To address this, we investigated the spatiotemporal dynamics of the insula during a vWM task using intracranial EEG (iEEG). We analyzed neural frequency band power modulations and high frequency activity (HFA; 70-150Hz) recorded from 90 iEEG electrodes implanted in the insulae (35 left/55 right) of 12 patients undergoing presurgical evaluation for medication refractory epilepsy. Each trial consisted of an encoding period (5 letters shown in succession), a maintenance period (fixation point), and a probe period (patient answered if probe letter was in the list using the hand ipsilateral to the implantation). We focused on theta (4-8Hz) and beta (13-30Hz) bands, known to underpin different aspects of vWM processing, and HFA, an index of local neuronal activity. We also assessed the relationship between neural activity and manual response time (RT) for correct trials. We found that theta and beta bands were modulated in the RI during encoding and maintenance onset ($p < .05$). At the probe period, we observed 1) differential involvement across both insulae in low frequency bands ($p < .05$) with a theta decrease

correlating with the RT only in the LI ($p < .001$). This interhemispheric asymmetry may be due to an involvement of the LI in the verbal aspect of the task and the RI in the attentional aspect; 2) HFA modulation along the anteroposterior insular axes ($p < .05$) with anterior HFA activity at the probe onset and subsequent posterior HFA activity occurring after the RT ($p < .001$). This gradient supports the role of the anterior insulae in decision making for encoded verbal material during vWM. The prominent post-response posterior insula HFA activity suggests its involvement in higher-order cognitive processes such as response monitoring. The current study implicates the insula in vWM extending from encoding through maintenance and ending in decision making.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 490.25

Topic: H.05. Working Memory

Support: CIHR
NSERC
BranSCAN
NEURONEX

Title: Spike sequences encode naturalistic working memory in primate prefrontal cortex

Authors: M. ROUSSY¹, A. BUSCH¹, M. L. LEAVITT², R. LUNA ALMEIDA⁵, B. W. CORRIGAN³, M. H. MOFRAD¹, R. A. GULLI⁶, A. J. SACHS⁷, J. MINAC¹, L. PALANIYAPPAN⁸, L. E. MULLER⁴, ***J. MARTINEZ-TRUJILLO**⁹;

²Physiol. and Pharmacol., ³Neurosci., ⁴Dept. of Mathematics, ¹Western Univ., London, ON, Canada; ⁵Univ. Autonoma de Chihuahua, Chihuahua, Mexico; ⁶Columbia Univ., New York, NY; ⁷The Ottawa Hosp. Res. Inst., Ottawa, ON, Canada; ⁸Psychiatry, McGill Univ., Montreal, QC, Canada; ⁹Schulich Sch. of Med. and Dentistry, Robarts Institute, Western Univ., London, ON, Canada

Abstract: Working memory (WM) is the ability to remember and manipulate information in the mind for short time periods. Candidate brain mechanisms for encoding working memory include persistent firing of neurons selective for the memorized items, oscillations, and synaptic storage. Here we recorded the activity of neurons in the lateral prefrontal cortex (LPFC) of macaque monkeys using microelectrode arrays during a naturalistic visuospatial working memory task taking place in a virtual environment. Spiking activity showed clear temporal structure, with transient trains of action potentials occurring in individual units. When ordered by peak firing time, these transients formed clear “bands” spanning the duration of the trial. To determine

whether these temporal sequences were related to memory content, we developed a novel computational method to analyze spike sequences across trials. This method, which works by representing individual sequences as complex-valued vectors and performing dimensionality reduction on the resulting correlation matrix, compares the similarity of spike patterns across trials. We found that the sequences were similar within a trial epoch but different between trial epochs: the sequences in the delay period differ from those during the cue and navigation periods, allowing the trial epoch to be decoded from single trial sequences using an unsupervised classifier derived from our computational approach. We then studied the delay period to investigate the relationship between sequence structure and WM content. We found that the sequential activation of single neurons encoded the path to a target location held in WM. Sequences were not found in WM tasks lacking naturalistic spatiotemporal structure, and were not a mere activation of cells with memory fields at different spatial locations, but an abstract representation of the path. The relation between sequences and WM content was less consistent on incorrect trials, during a perception task control. Sequences were disrupted following injections of ketamine at subanesthetic doses (0.25-0.8 mg/kg), which also selectively disrupts WM task performance. We were able to decode WM content with high accuracy using an unsupervised classifier derived from our computational approach. Taken together, these results reveal a striking spatiotemporal organization of spiking activity during WM in LPFC neuronal ensembles. They also suggest that persistent firing in LPFC has a precise temporal structure that allows neuronal ensembles to robustly maintain and manipulate task relevant information in the absence of sensory inputs.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 490.26

Topic: H.05. Working Memory

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Compute Canada (computecanada.ca)
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Title: An algebraic approach to spike time codes relates working memory activity to behaviour

Authors: *A. N. BUSCH^{1,3}, M. ROUSSY^{2,3,4}, F. W. PASINI¹, J. MINÁČ¹, J. C. MARTINEZ-TRUJILLO^{5,4,3}, L. E. MULLER^{1,3};

¹Mathematics, ²Physiol. and Pharmacology, Schulich Sch. of Med. and Dent., Western Univ., London, ON, Canada; ³Brain and Mind Institute, Western Univ., London, ON, Canada; ⁴Robarts Res. Institute, Western Univ., London, ON, Canada; ⁵Western Univ., Schulich Sch. of Med. and Dentistry, Western Univ., London, ON, Canada

Abstract: The brain encodes sensory information, makes decisions, and generates motor outputs through patterns of activity across large groups of neurons. Rapidly advancing neural recording techniques allow for the simultaneous recording of more neurons than ever before. It is now possible to study large neuronal populations on the timescale of behaviour in awake animals performing complex tasks. However, the best way to analyze patterns of spikes from hundreds to thousands of well-isolated single units in cortex remains unclear. This challenge has two underlying causes: (1) meaningful spike patterns across many neurons may be very high-dimensional, and (2) it is unclear how best to apply traditional dimensionality reduction techniques for continuously sampled variables, like principal component analysis (PCA), to detect arbitrarily complex patterns in recordings with hundreds or thousands of simultaneously isolated cells. To address this challenge, we developed a technique for analysis of spike train patterns using complex variables. Initially developed for detecting spatiotemporal patterns in continuous measures of population activity, such as local field potential or optical imaging recordings, this approach represents spikes as complex numbers with unit modulus. This technique provides a straightforward approach for dimensionality reduction on spikes represented as complex-valued vectors, while also providing a natural, algebraic language for spike patterns that can range from very simple, such as reliably repeating sequences, to arbitrarily complex patterns. We then applied this approach to Utah array data from macaques performing a naturalistic working memory (WM) task in virtual reality. Our approach reveals specific patterns of bursting activity that encode WM content during the delay epoch. An unsupervised classifier derived from this computational approach successfully decodes targets held in working memory using spikes only during the delay. This clear relationship between WM content and spikes was diminished on incorrect trials. This relationship also disappears following injections of ketamine at subanesthetic doses, but recovers in conjunction with regained behavioural performance one hour after injection. Taken together, these results reveal a striking spatiotemporal organization of spiking activity during WM in LPFC neuronal ensembles, demonstrating the utility of this algebraic approach to spike times. (AB and MR contributed equally to this work.)

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Poster

490. Physiological and Neural Mechanisms of Working Memory

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Program #/Poster #: 490.27

Topic: H.05. Working Memory

Support: SCGB 543049
SCGB 543045

Title: Flexible multitask computation in recurrent networks utilizes shared dynamical motifs

Authors: *L. N. DRISCOLL¹, K. SHENOY², D. SUSSILLO¹;

¹Electrical Engin., ²Electrical Engin., Neurosurgery, Bioengin., Neurobio., Wu Tsai Inst., Bio-X Inst., HHMI, Stanford Univ., Stanford, CA

Abstract: Flexible computation is a hallmark of intelligent behavior. Yet, little is known about how neural networks contextually reconfigure for different computations. Humans are able to perform a new task without extensive training, presumably through the composition of elementary processes that were previously learned. Cognitive scientists have long hypothesized the possibility of a compositional neural code, where complex neural computations are composed of simpler constituent components; however, the neural substrate underlying this structure remains elusive in biological and artificial neural networks. Here we identified an algorithmic neural substrate for compositional computation through the study of multitasking artificial recurrent neural networks. Using techniques from dynamical systems theory, we show that the dynamical landscapes that implement neural computation do so by mirroring the modular subtask structure of the set of tasks networks were trained to perform. Dynamical motifs such as attractors, decision boundaries and rotations were reused across different task computations. For example, tasks that required memory of a continuous circular variable repurposed the same ring attractor. We show that dynamical motifs are implemented by clusters of neurons and are reused across different contexts, allowing for flexibility and generalization of previously learned computation. Lesioning these clusters resulted in modular effects on network performance: a lesion that destroyed one dynamical motif only minimally perturbed the structure of other dynamical motifs. Finally, dynamical motifs could be reconfigured for fast learning without risk of catastrophic forgetting. After slow initial learning of dynamical motifs, a subsequent faster stage of learning reconfigured motifs to perform novel tasks. As more whole brain imaging studies record neural activity from multiple specialized systems simultaneously, the framework of dynamical motifs will guide questions about specialization and generalization across brain regions. We believe that our framework establishes dynamical motifs as a fundamental unit of computation, intermediate between the neuron and the network.

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Poster

490. Physiological and Neural Mechanisms of Working Memory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 490.28

Topic: H.05. Working Memory

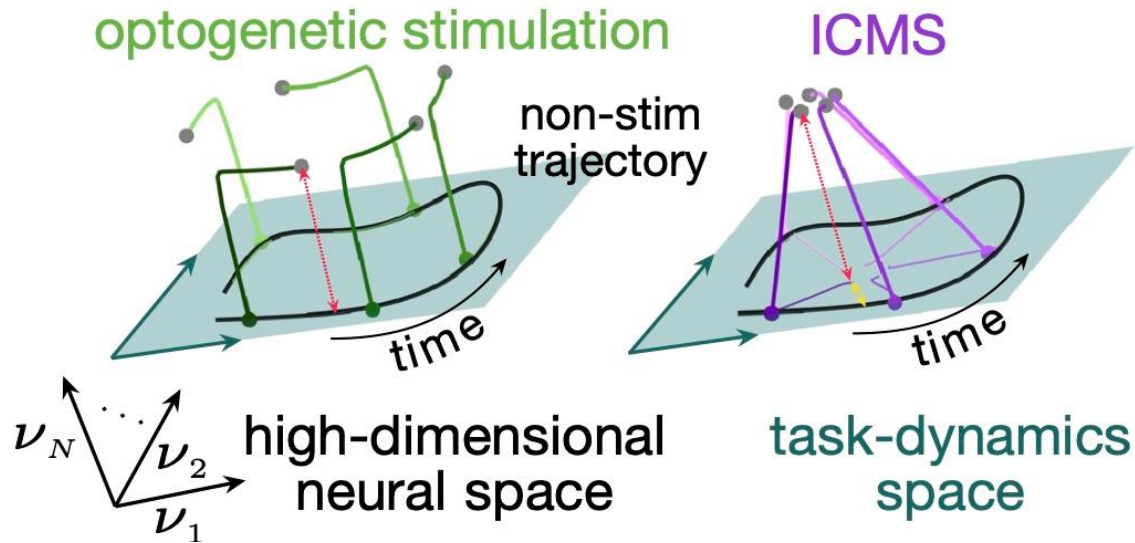
Support: DARPA REPAIR
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Title: Optogenetic and electrical perturbations in motor cortex reveal a neural population mechanism for robust computation

Authors: *D. J. O'SHEA¹, L. DUNCKER², X. SUN³, S. VYAS⁴, K. DEISSEROTH⁵, M. SAHANI⁶, K. V. SHENOY⁷;

¹Electrical Engineering; Wu Tsai Neurosciences Inst., Stanford Univ., San Francisco, CA; ²Wu Tsai Neurosciences Inst., Stanford Univ., Stanford, CA; ³UCSF, San Francisco, CA; ⁴Zuckerman Mind Brain Behavior Inst., Columbia Univ., New York, NY; ⁵Bioengineering, Stanford, Stanford, CA; ⁶Gatsby Computat. Neurosci. Unit, Univ. Col. London, London, United Kingdom; ⁷HHMI & Stanford Univ., Stanford Univ. & HHMI, Stanford, CA

Abstract: The neural computations that enable flexible cognition and behavior are realized through the dynamical evolution of activity in recurrently connected circuits. Direct neural perturbations have emerged as an important tool for establishing causal links between neural activity and behavior, and are therefore critical for refining and updating current hypotheses about neural computation. We delivered optogenetic excitation and electrical intracortical microstimulation (ICMS) in M1 and PMd of macaques engaged in a reaching task. We reproduced longstanding behavioral findings that optogenetic excitation in macaque motor cortex only rarely produces behavioral effects, whereas ICMS can readily alter reach kinematics. During stimulation, we recorded local neural responses with multiple independent electrodes (optogenetic) and a Neuropixels probe (ICMS) and developed a novel computational approach to remove the stimulation artifact. Through simultaneous stimulation and recording of neural population activity, we sought to investigate the neural basis of these differences and to arrive at a mechanistic understanding of network properties that explain the observed population responses. Through statistical and theoretical modeling, we demonstrate that the empirical population responses exhibit structure that is expected in high-dimensional recurrent circuits whose dynamics obey Dale's law and implicitly contain low-dimensional structure. Additionally, we showed that optogenetic stimulation altered neural activity via a purely additive perturbation, preserving the underlying geometry of task activity. ICMS, in contrast, distorted the underlying task activity, and the degree of distortion correlated well with the evoked effects on reaching behavior. Our findings reveal general network mechanisms that facilitate robust computation, offer insight into the constraints under which computation is embedded within neural populations, and contribute to a more fundamental understanding of how modern tools for targeted manipulation engage with neural dynamics.



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Poster

490. Physiological and Neural Mechanisms of Working Memory

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 490.29

Topic: H.05. Working Memory

Support: NIMH Grant

Title: Medial prefrontal serotonin input regulates cognitive flexibility in mice

Authors: *N. D. ALVES¹, A. A. MORGAN¹, G. S. STEVENS¹, A. MACKAY¹, A. ZIOLKOWSKI-BLAKE¹, D. SARGIN², T. T. YEASMIN¹, C. HANNA¹, A. L. ADIB¹, J. RYBNICEK², E. K. LAMBE², M. S. ANSORGE¹;

¹Columbia Univ., Columbia Univ., New York, NY; ²Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: The medial prefrontal cortex (mPFC) regulates cognitive flexibility and emotional behavior. Furthermore, neurons that release serotonin (5-HT) project to the mPFC, and drugs targeting the 5-HT system influence emotional regulation and cognitive flexibility. Yet, the specific role of endogenous 5-HT release in the mPFC on neurophysiology and behavior is unknown. Here we selectively mapped, monitored, and manipulated 5-HT input into the mPFC to gain insight into the functional roles of this pathway. Using *in vitro* optogenetics paired with whole-cell slice electrophysiology we observed strong and dominant 5-HT_{1a} receptor-mediated

inhibition of mPFC pyramidal neurons. *In vivo* fiber photometry recordings revealed task-specific activity signatures in 5-HTergic neurons projecting from the dorsal raphe to the mPFC during a cognitive flexibility task but not in the open field test. Furthermore, *in vivo* optogenetic activation of the 5-HTergic dorsal raphe-to-mPFC pathway selectively improved extradimensional rule shift performance while inhibition impaired it, demonstrating sufficiency and necessity for mPFC 5-HT release in cognitive flexibility. Collectively, our data reveal a powerful and specific modulatory role of endogenous 5-HT release from dorsal raphe-to-mPFC projecting neurons in cognitive flexibility.

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Poster

491. Social Cognition: Circuits and Neural Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 491.01

Topic: H.06. Social Cognition

Support: DHHS-NATIONAL INSTITUTES OF HEALTH 1R56MH122810-01A1
UNIVERSITY OF PENNSYLVANIA MDBR-21-125-SETBP1

Title: Physiology of mPFC neurons projecting to MD in a mouse model of SETBP1 disorder

Authors: *P. LYUBOSLAVSKY¹, A. KIZIMENKO², A. C. BRUMBACK³;

¹Univ. of Texas, Austin, Austin, TX; ²Neurol., Univ. of Texas At Austin, Austin, TX;

³Neurology, Pediatrics, Neurosci. and The Ctr. for Learning and Memory, Ctr. For Learning & Memory, Univ. of Texas, Austin, Austin, TX

Abstract: The prefrontal cortex and its reciprocal connection with mediodorsal (MD) thalamus are involved in executive functioning, which is a core area of challenge for many people with neurodevelopmental disorders (NDDs) including *SETBP1*-related disorder. We hypothesized that disruption of the *SETBP1* gene would disrupt the intrinsic physiology of neurons in the mPFC → MD network. We compared adult (8 - 12-week-old) male and female heterozygous *C57BL/6J-Setbp1^{em8Lutz/Mnjax}* (*Setbp1^{indel}*; Jax #33281) with wildtype littermate controls. We used cholera toxin, subunit B (CTB) to retrogradely label Layer 5 mPFC → MD projection neurons. We used brain slice whole cell electrophysiology to measure subthreshold properties (resting membrane potential, input resistance, membrane time constant, voltage sag) and action potential spiking properties (rheobase, action potential threshold, frequency / current relationship, and accommodation). During recordings, neurons were filled with biocytin. We processed the fixed tissue and then traced the dendritic arbors using Neurolucida. We used Sholl analysis to quantify dendritic length and branching. During analysis, investigators are masked to the group identify of

the neurons. At the time of presentation, we will have unmasked data to determine if there are differences between *Setbp1*^{indel} mice and wildtype control littermates.

Disclosures: P. Lyuboslavsky: None. A. Kizimenko: None. A.C. Brumback: None.

Poster

491. Social Cognition: Circuits and Neural Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 491.02

Topic: H.06. Social Cognition

Support: DHHS-NIH Grant 1R56MH122810-01A1

Title: Reciprocal connections between the medial prefrontal cortex and the mediodorsal thalamus influence social exploration in mice.

Authors: *S. TOWERS¹, F. MENG², C. HABERL³, A. ALARIO³, A. KIZIMENKO², P. N. LYUBOSLAVSKY², A. C. BRUMBACK^{4,3,2,1};

¹Inst. for Neurosci., ²Neurol., ³Neurosci., ⁴The Ctr. for Learning and Memory, Univ. of Texas at Austin, Austin, TX

Abstract: In humans, abnormal connectivity between the medial prefrontal cortex (mPFC) and mediodorsal thalamus (MD) is associated with neuropsychological conditions characterized by affective and social dysfunction. In mice, excitation of neurons in mPFC is sufficient to decrease social exploration. mPFC and MD have reciprocal connections, and mPFC neurons project to MD and other subcortical structures. Here, we used *in vivo* optogenetics to investigate whether mPFC↔MD circuitry is required for social exploration in mice. We targeted neurons projecting from MD→mPFC or mPFC→MD to assess if directionality of this pathway is important. Furthermore, we targeted pan-excitatory (CamKII or Synapsin expressing) mPFC neurons, and Layer 5 dopamine receptor 2 expressing (Drd2+) and Layer 6 Neurotensin receptor 1-positive (Ntsr1+) subcortical projection neurons to assess if social exploration is regulated by specific subpopulations of mPFC neurons. Adult male and female mice were injected with viral vectors encoding channelrhodopsin into the MD or mPFC and implanted with bilateral fiber optic probes. Four weeks after surgery, mice were assessed for behavioral changes in response to photostimulation. Each mouse was evaluated twice on the following behavioral assays: open field; novel social investigation; novel object investigation; and olfaction habituation/dishabituation tests. Mice were randomly assigned to light on or light off during week 1 and received the other condition the following week. We found that pan-neuronal excitation of neurons projecting from either mPFC→MD or MD→mPFC decreases social sniffing of same sex conspecific mice. When examining specific populations of neurons, we found that excitation of L5 Drd2+ neurons projecting from mPFC→MD is sufficient to decrease duration of social sniffing, without affecting locomotion, time spent in the center of an open field, time spent sniffing a non-social stimulus, or olfactory function. Activation of Drd2+

MD→mPFC projections, however, did not influence social exploration. Our preliminary findings show that activating L6 Nstr+ mPFC→MD projections show an increase in exploration of social and nonsocial stimuli but do not significantly affect the other behaviors measured. Our findings directly demonstrate that reciprocal mPFC↔MD circuitry is important for social exploration and begins to dissect the specific circuitry and cell types involved. This work contributes to our understanding of the thalamocortical and corticothalamic network and its potential role in neuropsychiatric disease.

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Poster

491. Social Cognition: Circuits and Neural Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 491.03

Topic: H.06. Social Cognition

Support: Simons Foundation Autism Research Initiative Pilot Award 610850
NIMH R01MH118297
The Osaka Medical Research Foundation for Intractable Diseases 27-3-2

Title: Role of autism risk genes in frontal-thalamic projections underlying social processing in mice

Authors: *K. OKAMURA, A. LIDOSKI, B. STEVENS, K. YAMAMURO, M. LEVENTHAL, H. MORISHITA;
Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Challenges in social processing are associated with autism spectrum disorders (ASDs), yet little is known about the link between mutations in ASD risk genes and neural circuits underlying social processing. Recent genetic and transcriptomic studies have shown that many ASD risk genes are enriched in fetal and infant prefrontal cortical (PFC) layer 5/6 projection neurons. Based on our recent finding that the posterior paraventricular thalamus (pPVT) as one of prominent projection targets of PFC L5/6 neurons that is preferentially recruited by social interaction (Yamamuro et al., Nat. Neurosci., 2020), we aim to examine the impact of different ASD risk genes on PFC L5/6 projection neurons to the pPVT. We first assessed the electrophysiological functions of medial PFC (mPFC) neurons projecting to the pPVT (mPFC→pPVT neurons) of adult mice harboring mutations in multiple ASD risk genes (Fmr1-KO, Tsc2-Ht and Pten-Ht). Patch clamp recordings from retrobeads labeled mPFC→pPVT neurons revealed reduced excitability in both Fmr1-KO and Tsc2-Ht mice, and increased inhibitory drive in all three ASD mouse models we tested. To identify the source of excessive inhibitory drive onto mPFC→pPVT neurons, we next examined inhibitory inputs from specific sub-class of GABAergic interneurons. Optogenetic stimulation of ChR2 expressing

GAD2(+) cells, SST(+) cells or PV(+) cells in mPFC revealed increased evoked inhibitory inputs to mPFC-pPVT cells regardless of which sub-class of interneurons were stimulated in adult Fmr1-KO mice. Collectively, these findings support that the frontal-thalamic projection to pPVT is a converging circuit vulnerable to multiple ASD risk genes. Identification of specific PFC circuits that modulate social behavior and whose functions are affected by mutations in ASD risk genes will point toward potential targets that allow circuit-based amelioration of social processing challenges in ASD.

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Poster

491. Social Cognition: Circuits and Neural Mechanisms II

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 491.04

Topic: H.06. Social Cognition

Support: NIMH R01MH118297
NIMH F31MH127805

Title: Regrouping after juvenile social isolation precipitates deficits in social behavior and corticothalamic projection neurons in mice

Authors: **M. B. LEVENTHAL**, K. OKAMURA, A. LIDOSKI, M. JANIS, B. STEVENS, L. WALTRIP, H. MORISHITA;
Mount Sinai, New York, NY

Abstract: Background: Juvenile social isolation (JSI) is known to disrupt social behavior in adulthood, but little is known about the neural mechanisms of social experience-dependent brain maturation that are disrupted by JSI. Previous studies suggest that, in male mice, there is a critical period between postnatal day (p) 21 and p35 when isolation will reduce adult sociability and induce prefrontal cortex (PFC) abnormalities, such as dampened excitability in medial PFC neurons projecting to the posterior paraventricular thalamus (mPFC-pPVT neurons). Interestingly, these circuit abnormalities were not present at the end of the isolation period (p35), raising the question of when and how JSI-induced social deficits emerge over the course of development. **Methods:** To investigate the developmental progression of JSI-induced social dysfunction, we performed the three-chamber sociability test on a weekly basis between the end of isolation and adulthood. Additionally, during the post-isolation developmental period, we conducted tests of affiliative behavior and aggression among cage mates and used patch clamp electrophysiology to examine the excitability of mPFC-pPVT neurons. **Results:** We found that JSI-induced social dysfunction in the three-chamber test is delayed (not fully emerging until between p50-52) and that dysfunction in the three-chamber test, where subjects interact with novel mice, is preceded by negative social interactions between JSI cagemates during the first

week after the end of isolation. Further, excitability of mPFC-pPVT neurons is already decreased at p50-52. **Conclusions:** These results suggest that JSI may disrupt adult social behavior not only by impairing social development during the isolation period, but also by disrupting subsequent development during the post-isolation developmental period, highlighting a critical consideration for studies of social experience-dependent maturation. We propose that the prevailing “social deprivation model”, where adult social deficits are attributed to disruption of developmental processes occurring *during* the isolation period, should be supplemented by the “developmental mismatch model”, where social deficits are attributed to disruption of developmental processes occurring *after* the isolation period.

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Poster

491. Social Cognition: Circuits and Neural Mechanisms II

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 491.05

Topic: H.06. Social Cognition

Support: CFREF/BrainsCAN Accelerator Grant
SSHRC Insight Grant

Title: Dissociable effects of acute versus cumulative violent video game exposure on the action simulation circuit

Authors: *S. A. H. COMPTON¹, M. RITCHIE¹, L. D. OLIVER³, E. FINGER², D. G. V. MITCHELL¹;

¹Brain and Mind Inst., ²Robarts Inst., Univ. of Western Ontario, London, ON, Canada;

³Campbell Family Mental Hlth. Res. Inst., Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Whether violent video games negatively impact social functioning has been widely debated in popular culture and within the scientific literature. Discrepancies in the literature may be due to methodological concerns and a lack of research into the neurocognitive mechanisms behind the purported effects or into trait differences which may influence susceptibility.

Empathy and its neural correlates are one plausible mechanism by which violent video game exposure (VGE) could influence socially relevant outcomes. Action simulation (i.e., motor empathy), the imitation or internal simulation of motor responses, shares a partially overlapping neural network with emotional empathy processes. As such, action simulation tasks can be used as a covert measure of processes related to emotional empathy. Using a combined experimental and cross-sectional approach, we examined the impact of VGE on neural correlates associated with social cognition as a function of trait coldheartedness (i.e., low empathy). Healthy university students played either a violent or non-violent version of *Grand Theft Auto V* before

completing an fMRI measure of action simulation circuit (ASC) activity. No significant difference in simulation-related activity was found between groups; however, the violent group did display greater overall activation in the left inferior frontal gyrus (IFG). Unexpectedly, there was no evidence that trait coldheartedness interacts with violent gaming to affect activity within the ASC. However, a significant negative correlation between prior cumulative VGE and simulation-related activity was identified within a subsection of the IFG. Our results provide evidence that cumulative VGE is inversely related to simulation-related activity within the neurocognitive building blocks of empathy. Disturbances in the function of neural regions associated with such processes could indicate one mechanism by which VGE may influence risk for some of the antisocial associations observed in the literature. This study also identifies a potential dissociation between the effects of acute and cumulative violent gaming, and challenges the assumption that directionality of effects for cross-sectional associations always mirror those of acute exposure.

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Poster

491. Social Cognition: Circuits and Neural Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 491.06

Topic: H.06. Social Cognition

Support: NIH Grant R01MH115267
NIH Grant K99MH126164

Title: Sexual coordination in a whole-brain map of pair-bonding

Authors: *M. L. GUSTISON¹, R. MUÑOZ-CASTAÑEDA², P. OSTEN², S. M. PHELPS¹;
¹Univ. of Texas at Austin, Austin, TX; ²Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Social bonds are central to the human experience, and monogamous prairie voles enable investigation of the neurobiology of attachments. We developed a whole-brain imaging and computational pipeline to identify circuits involved in prairie-vole pair-bonding. Subjects were paired with either a same-sex sibling or a novel opposite-sex mating partner for up to 22h while we continuously tracked their movements and USVs. We extracted brain tissue at four time points (0h, 2.5h, 6h, 22h) and used iDisco immunolabeling to quantify the brain-wide distribution of the immediate-early gene c-Fos, a proxy for neuronal activity. The brain-wide distribution of the immediate-early gene c-Fos implicated 68 brain regions in pair-bonding, with little evidence for sexual dimorphism. Bonding pairs exhibited profound mating-induced male-female correlations across regions, a pattern predicted by ejaculation rates. The bed nucleus of the stria terminalis (BST) emerged as a central node in the pathway translating sexual experience into attachment; novel regions such as the optic area and medial amygdala, recently

implicated in social reward, responded strongly to bonding and were coordinated across pairs even after bonds formed. These data offer novel systems-level insights into sociosexual attachments.

Disclosures: **M.L. Gustison:** None. **R. Muñoz-Castañeda:** None. **P. Osten:** Other; Certego Therapeutics, Certerra, Inc.. **S.M. Phelps:** None.

Poster

491. Social Cognition: Circuits and Neural Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 491.07

Topic: H.06. Social Cognition

Title: Uncertainty in the mentalizing network: Dorsal medial prefrontal cortex activation tracks with the level of uncertainty across mental and nonmental inferences

Authors: ***D. BERKAY**, A. C. JENKINS;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Previous fMRI studies in the social cognition literature have consistently identified a set of brain regions that are more active during mentalizing tasks than nonmentalizing tasks. One common interpretation of these findings has been that these brain regions, which are collectively referred to as the mentalizing network, are specialized for reasoning about others' minds and have a domain-specific role. However, an alternative possibility is that activation in at least some of these brain regions reflects engagement of some domain-general cognitive processes which happen to be more heavily relied on in mentalizing than nonmentalizing tasks. In particular, given higher levels of uncertainty inherent to social contexts that require inferring others' mental states, one possibility is that at least some of these brain regions comprising mentalizing network contribute to processing or reducing uncertainty. Here, by experimentally manipulating uncertainty in both social and nonsocial contexts requiring mental and nonmental inferences, respectively, we investigate whether the level of activation observed in brain regions within the mentalizing network can be accounted for by a domain-general role associated with uncertainty rather than a domain-specific role in processing social content. In an fMRI study (N = 46), participants viewed initial information about people's personality traits (e.g., "shy"), people's physical characteristics (e.g., "muscular"), and objects' properties (e.g., "wooden") and made subsequent inferences about other attributes of those people or objects that varied systematically in the degree to which they were uncertain given the first piece of information (e.g., given that they are shy, how likely are they to be calm?). Across categories, we observed a parametric effect of uncertainty on activation in one region typically associated with social cognition: the dorsal medial prefrontal cortex (DMPFC). Specifically, DMPFC activation increased as a function of uncertainty, whereas this effect was not observed in other mentalizing regions such as the right temporoparietal junction. These results suggest that DMPFC activation during mentalizing tasks may reflect the engagement of a domain-general process related to uncertainty.

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Poster

491. Social Cognition: Circuits and Neural Mechanisms II

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Topic: H.06. Social Cognition

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German Research Foundation GR 3619/16-1

Title: Oxytocin activity in the Paraventricular and Supramammillary Nuclei of the Hypothalamus is Essential for Social Recognition Memory in Rats

Authors: *K. THIRTAMARA RAJAMANI¹, M. BARBIER¹, A. LEFEVRE³, K. NIBLO¹, N. CORDERO², S. NETSER⁴, S. WAGNER⁴, V. GRINEVICH⁵, H. HARONY-NICOLAS¹;
¹Psychiatry, Seaver Autism Ctr. for Res. and Treatment, ²Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY; ³UCSD, San Diego, CA; ⁴Univ. of Haifa, Haifa, Israel; ⁵Central Inst. of Mental Health, Univ. of Heidelberg, Heidelberg, Germany

Abstract: Social cognition in mammals is fundamental for several conserved behaviors including distinguishing prey from a conspecific, identification of mating partners and for kinship maintenance. Social recognition memory is a form of social cognition that requires the discrimination of a novel from a familiar conspecific. Deficits in social recognition have been reported in several psychiatric disorders including Autism spectrum disorder and Schizophrenia. Thus, identifying the neural and biological correlates of social recognition memory (SRM) can spur a greater underlying of these behaviors in disease. Oxytocin (OXT) is a neuropeptide that is synthesized and released by neurons in the paraventricular (PVH), supraoptic (SON) and accessory nuclei of the hypothalamus. It is implicated in social behaviors including maternal care, social bonding, and SRM. Despite a clear role for OXT in SRM, it is still unclear which of the three nuclei within the hypothalamus is necessary for the formation of this form of memory. Furthermore, little is known about the role of downstream neural substrates, targeted by OXT axonal projections, in SRM. We hypothesized that PVN-OXT neurons are necessary for both short- and long-term SRM. To address this, we used designer receptors activated by design drugs (DREADDs) to specifically silence OXT neurons (OXT-hM4DGi) in the PVH of Sprague Dawley rats (n=14/Males) and assessed their performance on both short and long term SRM. We found that silencing PVH-OXT neurons significantly impaired both long (**P=0.003) and short-term SRM (**P=0.005). In order to determine which of the downstream targets of PVH-OXT

axonal projection regions may contribute to SRM, we focused on the supramammillary nucleus (SuM), as it plays an important role in hippocampal-dependent learning and memory. We first demonstrated that the SuM contains OXT fibers using OXT specific antibodies. We then injected an OXT promoter driven specific anterograde (AAV-OXTp-Venus) or OXT promoter driven synaptophysin GFP (OXTp-Synaptophysin-GFP) in the PVH and found that OXT fibers in the SuM originate in the PVH but not SON. Using *in situ* fluorescent hybridization, we confirmed that OXT receptors are found predominantly on glutamatergic neurons in the SuM. Finally, we found that blocking OXT receptors in the SuM inhibits short (n=10, * $P=0.001$) and long-term SRM (n=12, *** $P=0.0008$), suggesting that the PVH-SuM may be a novel neural circuit necessary for this form of memory. Taken, together these findings attribute a novel role for PVH-OXT neurons and PVH-SuM neural circuitry in social recognition memory.

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Poster

491. Social Cognition: Circuits and Neural Mechanisms II

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Program #/Poster #: 491.09

Topic: H.06. Social Cognition

Support: IBS-ROO2-D1
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Title: Suppressed prefrontal neuronal firing variability and impaired social representation in IRSp53-mutant mice

Authors: ***W. KIM**¹, J. SHIN³, Y. JEONG⁴, K. KIM¹, J. BAE¹, Y. NOH¹, S. LEE³, W. CHOI², S.-B. PAIK², M. JUNG^{1,3}, E. LEE⁴, E. KIM^{3,1};

¹Dept. of Biol. Sci., ²Dept. of Bio and Brain Engin., KAIST, Daejeon, Korea, Republic of; ³Ctr. for Synaptic Brain Dysfunctions, Inst. for Basic Sci., Daejeon, Korea, Republic of; ⁴Dept. of Anat., Col. of Medicine, Yonsei Univ., Seoul, Korea, Republic of

Abstract: Social deficit is a major symptom of neuropsychiatric disorders, including autism spectrum disorders, schizophrenia, and attention-deficit/hyperactivity disorder, but its neural mechanisms remain unclear. IRSp53 is an excitatory postsynaptic scaffolding and adaptor protein that is implicated in neuropsychiatric disorders. Via tetrode single-unit recording, we examined neuronal discharge characteristics in the medial prefrontal cortex (mPFC) of IRSp53-mutant male mice, which show social deficits, during social approach. Our results show a decrease in the proportion of IRSp53-mutant excitatory mPFC neurons encoding social

information but not that of those encoding non-social information. In addition, the activity of IRSp53-mutant excitatory mPFC neurons was less differential between social and non-social targets. In order to search for a causal mechanism, we examined the basic firing properties of IRSp53-mutant excitatory mPFC neurons. We found that they displayed decreases in variability and dynamic range of firing rates during social and non-social target approaches. Moreover, they showed reduced burst firing compared to wild-type controls. Memantine treatment, which rescues the social deficits in IRSp53-mutant mice, ameliorated the decreased burst firing of mPFC excitatory neurons *in vitro*, suggesting an association between reduced burst and social deficit. Overall, these results suggest that insufficient neuronal activity dynamics and burst may underlie impaired cortical encoding of social information and social behaviors in IRSp53-mutant mice.

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Poster

491. Social Cognition: Circuits and Neural Mechanisms II

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Program #/Poster #: 491.10

Topic: H.06. Social Cognition

Support: NRF-2019M3C7A1032262
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S0254-22-1002

Title: Self-forgiveness is related to fusiform gyrus in healthy individuals

Authors: H.-J. KIM¹, J. SEO³, M. BANG², *S.-H. LEE¹;

²Psychiatry, ¹CHA Bundang Med. Ctr., Seongnam, Korea, Republic of; ³CHA Univ. Sch. of Med., Seongnam, Korea, Republic of

Abstract: Background: Self-forgiveness (SF) involves a process through which negative moral emotions directed at the self are replaced by benevolence and acceptance. Lower SF scores can be associated with less self-compassion, higher psychological distress, and lower life dissatisfaction. However, neural correlates of SF have not been investigated yet. **Methods:** We enrolled a total of 79 healthy individuals. The Self-Forgiveness Scale (SFS), Self-Compassion Scale (SCS), Connor-Davidson Resilience Scale (CD-RISC), Beck Depression Inventory-II (BDI-II), Beck Anxiety Inventory (BAI), and World Health Organization Quality of Life Instrument, Short Form (WHOQOL-BREF) were evaluated. Multiple regression models with age, sex, years of education, and total intracranial volume as covariates were performed. Spearman's correlation analyses also investigated the exploratory correlations between the adjusted GMVs of the dispositional SF-related regions and other psychological characteristics

among healthy individuals. **Results:** Voxel-wise correlational analyses showed a significant positive correlation between the total SFS scores and gray matter volumes (GMVs) in the fusiform gyrus (FG). In addition, the GMVs in the FG were significantly positively associated with the total SCS, CD-RISC, and WHOQOL-BREF scores and negatively correlated with the total BDI-II and BAI scores. **Conclusions:** Our findings suggest that healthy individuals with high SF showed increased GMVs in the FG, presumably mediated by guilt or shame. Their correlations were associated with other psychological characteristics (high resilience, high self-compassion, high life satisfaction, and low psychological distress). Further, they showed life satisfaction and a negative association with psychological distress such as depression and anxiety in healthy individuals.

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Poster

492. Cortical Networks and Behavior

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Topic: H.08. Learning and Memory

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Title: Reciprocal loop of excitatory projections from EC into CA1 and their back projections in contextual memory modulation

Authors: *M. HERNANDEZ-FRAUSTO, T. BUTOLA, J. BASU;
Neurosci. and Physiol., NYU Neurosci. Institute, NYU Langone Med. Ctr., New York, NY

Abstract: Interaction between entorhinal cortex (EC) and hippocampus CA1 area (HC-CA1) promotes sequential organization that lead to the formation of episodic memories of people, places, objects, and events. Within the EC, the medial part (MEC) acts as a spatial information detector that conveys position in space, and the lateral subdivision (LEC) functions as a contextual non-spatial sensor that conveys contextual features of the environment, related to objects, novelty, and odor. While HC-CA1, is crucial for the memory formation and recall. Interactions between EC-CA1 and their back projections are thought to comprise a main role in episodic memory. EC sends direct and indirect excitatory projections to CA1, with an implication of the indirect pathway in contextual memory and spatial navigation, however we know little about the functional role of direct projections. Furthermore, CA1 area sends a back

projections to EC that is thought to underlie sensory processing and memory recall, yet the detailed role in memory recall remains elusive. Here, we propose a reciprocal loop from EC and HC-CA1 with an important function in episodic memory. First, we suggest that LEC sends direct excitatory projections into HC-CA1 with a role in contextual non-spatial and spatial representations. Next, we propose that the back projections from HC-CA1 includes a novel feedback circuit into EC with a direct projections into layer 3 besides the canonical pathway that goes from HC-CA1 to layer 5 of EC, enabling novelty coding for cortical sensory and spatial information. To address the functionality of the reciprocal loop between EC to HC-CA1 and their back projections. First, with chemogenetic strategies and fiber photometry recordings in freely moving mice, I assessed the functionality of the excitatory projections from LEC into HC-CA1 in two contextual learning behaviors, Novel Object Recognition (NOR) and Novel Object Location (NOL) tasks and one spatial task, Barnes Maze. Next, to investigate the behavioral relevance in memory processing of HC-CA1 back projections into EC layer 3, I optogenetically silenced the circuit during the same contextual and spatial behavioral paradigms, NOR, NOL and Barnes maze. Our results propose that the reciprocal loop between EC and HC-CA1 have a differential role during encode phases of contextual and spatial learning. Furthermore, we propose that this newly discovered back projections modulate cortical sensory processing through novelty.

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Poster

492. Cortical Networks and Behavior

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Program #/Poster #: 492.02

Topic: H.08. Learning and Memory

Support: German Research Foundation CRC 779 (TP A07)

Title: Successful mnemonic discrimination is linked to functional connectivity between hubs in the frontoparietal and default mode network

Authors: *P. ILIOPOULOS^{1,2}, J. GÜSTEN¹, A. MAASS², E. DÜZEL^{1,2};

¹Inst. of Cognitive Neurol. & Dementia Res., Magdeburg, Germany; ²German Ctr. for Neurodegenerative Dis. (DZNE), Magdeburg, Germany

Abstract: Successful memory depends on the process of mnemonic discrimination to establish discrete memory representations of similar episodes. Although previous neuroimaging research has focused on the well-known role of the hippocampus, less is known about how brain areas belonging to the frontoparietal (FPN) and default-mode network (DMN) interact during this process. The present study investigated the functional connectivity between the hubs of these networks during a mnemonic discrimination task. Our sample consisted of 55 young adults (age: M = 23.67 years, SD = 3.38, 61.8% female), who had to discriminate similar objects and scenes

(‘lures’) from identically repeated items (‘repeats’). Stimuli were presented in sequences of 12 items. The first six stimuli were always new images, while each of the following six stimuli could be either a lure or a repeat trial. During the task, 3T functional magnetic resonance imaging data were collected (resolution 2 mm, TR = 2.2 s). The imaging data were preprocessed using the standard ‘fmripred’ pipeline (MNI152 normalization) and statistically modeled using generalized psychophysiological interaction (gPPI). We performed region of interest (ROI) to-ROI analyses. During successful mnemonic discrimination (lures correct versus repeats contrast), we found lower functional connectivity in a set of connections: between the 1) right lateral prefrontal cortex (rLPFC; FPN hub) and right lateral parietal cortex (rLP; DMN hub), the 2) rLPFC and posterior cingulate cortex (PCC; DMN hub), as well as between the 3) right posterior parietal cortex (rPPC; FPN hub) and the rLP. Notably, the lure discrimination behavioral performance was linked to the functional connectivity in the rLPFC-rLP and rPPC-rLP connections: the higher the lure behavioral performance, the higher the functional connectivity observed. Our results implicate a role of functional communication between DMN and FPN hubs for task mnemonic discrimination, extending previous findings in the literature. In future studies, we will examine its relationship to additional behavioral mnemonic discrimination measures, as well as how cognitive training may affect such functional communication between brain areas involved in memory.

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Poster

492. Cortical Networks and Behavior

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Program #/Poster #: 492.03

Topic: H.08. Learning and Memory

Support: NSF EAGER Grant 1753677
James S. McDonnell Foundation

Title: Individualized targeting and non-invasive stimulation of functional brain networks reveals stimulation-specific impacts on resting-state functional correlations

Authors: *P. F. AGRES¹, L. HAN¹, M. Y. CHAN¹, A. S. NAIR², C. A. CARRENO¹, G. S. WIG^{1,3};

¹Univ. of Texas at Dallas, Dallas, TX; ²Univ. of Delaware, Newark, DE; ³Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Transcranial magnetic stimulation (TMS) has been utilized as a tool to study brain function and cognition, and as a therapeutic intervention to modify brain function in an effort to treat disease. Towards these goals, recent efforts have attempted to target and modify large-scale functional brain networks using TMS. This is predicated on observations that the organization of the human functional brain network, measured at rest, is related to cognitive performance, aging-

related cognitive decline, and disease state. Large-scale brain networks consist of nodes (brain areas) which vary in their functional and topological characteristics. Nodes are parts of distinct subnetworks that represent functionally relevant brain systems. Functional brain network organization can differ in specific topography across individuals. As such, increased focus has been placed on identifying individual-specific TMS targets to reduce group-level bias. The present study used resting-state functional correlations (RSFC) to create individualized functional brain networks and identify distinct stimulation targets in a group of healthy young adults (N=17). RSFC was collected prior to, and 24 hours after a 5-day 20Hz repetitive TMS protocol. Two cortical targets with distinct functional and topological properties were identified: left angular gyrus (ANG) and left middle frontal gyrus (MFG), with each target serving as a control condition for the other. TMS to each target resulted in RSFC changes between the respective target node and its connections, whereas RSFC changes were not observed in off-target stimulation. RSFC changes were related to the baseline RSFC and Euclidean distance between each target and their respective network connections, and was also specific to on-target stimulation. However, baseline RSFC strength was found to explain stimulation-related changes in RSFC strength above and beyond Euclidean distance between nodes. On-target TMS to the ANG decreased RSFC within the default network and on-target TMS to the MFG decreased RSFC within the frontoparietal control network; off-target control stimulation had no impact on RSFC within these subnetworks. Topological measures of participation coefficient (the relative number of connections outside a node's subnetwork) and within-module degree (the relative number of connections within a node's subnetwork) were not related to observed RSFC changes, though this may be due to the limited number of subjects included in the study. These data imply that individualized on-target TMS modifies RSFC in a target-specific manner, and is related to functional properties of that node within the brain network.

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Poster

492. Cortical Networks and Behavior

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Program #/Poster #: 492.04

Topic: H.08. Learning and Memory

Support: JST SOUHATSU
JST CREST

Title: The neural basis of the crowding effect for reading comprehension

Authors: *C. HOSODA^{1,2}, K. HOSOKAWA²;

¹Tohoku Univ., Miyagi, Japan; ²NTT communication science laboratory, Tokyo, Japan

Abstract: What accounts for the enormous differences in how fast and accurately people can read? Reading skills, core to academic progress underpins content-area learning. The crowding effect is one of the factors that explain individual differences in reading skills. Crowding is a universal phenomenon that limits our ability to identify individual stimuli when multiple objects are displayed in their vicinity. In reading, individual letters and words must be identified accurately and fluently despite competition from neighboring letters and words. Therefore, in visual word processing systems, individual differences in the attentional mechanisms that lead to accurate processing of words in the vicinity of a fixated word have the potential to be extracted as crowding effects on individual differences. Furthermore, it is speculated that the individual differences in the crowding effect may be related to differences in reading skills. Moreover, it is possible that the neural basis of crowding and reading comprehension may be shared. In the present study, we aimed to elucidate the relationship between reading comprehension, crowding, and the neural substrate. Sixty-five healthy university students participated in the study. We measured the brain structure of all participants using magnetic resonance imaging scanning (3T-MRI, Siemens PRISMA). Reading comprehension was measured using the Reading skill test (Web-based RST) by Arai et al. (2017). The overall score on the RST was used as a measure of reading ability. In the crowding task, a target letter surrounded by flanker letters was displayed. The gap between the target and flanker varied among 0, 5, 10, 20, 40, and 80% of the letter size. Observers were asked to keep watching the fixation point and answer what letter was displayed as a target. A target letter was displayed about 10 degrees from the fixation point. A cumulative normal distribution curve was fitted to the contrast sensitivity curve for each participant, and the contrast value at the peak of the function after fitting was used as the individual score. Lastly, to clarify the neural basis of the crowding and the reading comprehension, we did a correlation analysis between the grey matter volume and fractional anisotropy (FA) and the RST score/crowding score. Reading comprehension requires, as a matter of course, the ability to read a text accurately as a bottom line. The results suggest that individual differences in crowding effects may affect the accuracy of reading comprehension. These findings may be helpful for effective teaching of reading comprehension.

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Poster

492. Cortical Networks and Behavior

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Program #/Poster #: 492.05

Topic: H.08. Learning and Memory

Support: NIMH R01MH108729
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Title: Perirhinal and postrhinal suppression leads to differential impairment in appetitive sensory conditioning

Authors: *X. PENG, D. L. POETA, R. D. BURWELL;
Dept. of Cognitive, Linguistic, and Psychological Sci., Brown Univ., Providence, RI

Abstract: The postrhinal cortex (rodent homologue to the parahippocampal cortex) and perirhinal cortex are important areas in the medial temporal lobe that communicate both directly and indirectly with the hippocampus. Multiple studies have shown that they contribute to various forms of associative learning. Here we used an appetitive sensory preconditioning procedure to investigate their involvement in higher-order stimulus learning. Chemogenetic suppression (Designer Receptors Exclusively Activated by Designer Drugs) in the postrhinal or perirhinal cortex was induced during preconditioned stimulus testing. Preliminary results showed that postrhinal suppression completely abolished the sensory preconditioning effect, whereas perirhinal suppression produced only moderate impairment. Response latencies were not impacted. Effectiveness of the suppression was verified using contextual fear conditioning, a task for which both areas are necessary. The results indicate significant yet different contribution of these two medial temporal lobe areas to reward learning.

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Poster

492. Cortical Networks and Behavior

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Topic: H.08. Learning and Memory

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NIA (F32AG069354)

Title: Neuronal ensembles reactivate during sleep

Authors: *J. LINES, R. YUSTE;
Neurotechnology Center, Dept. Biol. Sci., Columbia Univ., New York, NY

Abstract: Memory consolidation is believed to rely on the spontaneous neuronal activity during sleep, defined as periods of reduced animal behavior accompanied by low frequency increases in the local field potential. One potential substrate for memory is the synchronous firing of neuronal ensembles. Neuronal ensembles in the primary visual cortex have been shown to be evoked by sensory stimulation and can occur within spontaneous activity in the absence of visual presentation. Moreover, reactivation of ensembles can trigger perceptions and behavior. To investigate the structure of spontaneously active neuronal ensembles within the visual cortex across wake and sleep, we used two-photon calcium imaging to measure the activity of neuronal populations, using GCaMP6s under the VGLUT1 promoter in primary visual cortex of transgenic mice in vivo. Electrocorticogram (ECoG) and electromyography (EMG) was recorded simultaneously to rate the vigilance state of the animal. When comparing spontaneous cortical

activity between vigilance states, we found a reduction in neuronal and neuronal ensemble frequency during sleep. Quantification of ensemble frames showed a reduction in additional neuronal activity during sleep, suggesting an increase in signal to noise of ensembles during sleep, compared to wake. Visual presentations of drifting gratings evoked neuronal ensembles, and these ensembles were spontaneously reactivated in sleep. Our results underscore the importance of offline processing during sleep and its relationship with memory storage.

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Poster

492. Cortical Networks and Behavior

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Topic: H.08. Learning and Memory

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NSF GRFP

Title: Brain network dynamics differentiate autobiographical and working memory processes and explain variability in behavioral performance

Authors: *F. PECK¹, A. J. WESTPHAL², J. RISSMAN¹;

¹Dept. of Psychology, UCLA, Los Angeles, CA; ²Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Despite the immense complexity of the brain, a finite set of archetypal brain activity patterns will emerge and recede over the course of any cognitive act, with one such pattern dominating the cortical landscape at any given moment. A strategy to capture these moment-to-moment dynamics in fMRI data is to identify a set of coactivation patterns (CAPs) that each reflect a “brain state” and together capture maximal variance across a dataset. Here, we evaluate the temporal sequence of CAPs as participants alternate between two declarative memory processes that markedly differ with respect to attentional orientation. On each trial, participants were cued to recall a specific autobiographical memory (AM), requiring internally-oriented attention to the retrieved mnemonic contents, and then performed a 2-back working memory (WM) task, requiring externally-oriented attention to abstract visual stimuli. To identify how these respective memory processes are supported by different CAPs, we analyzed fMRI data from 26 participants (13 male) who each completed 48 trials; sex differences were not evaluated. Using a k-means clustering algorithm run on the fMRI timeseries data concatenated across all participants, we identified CAPs related to increased and decreased activity levels in the default mode network (DMN), dorsal attention network (DAN), frontoparietal control network (FPCN), and ventral attention network (VAN). We evaluated two core metrics, dwell time and fractional occupancy, that quantify the temporal stability and prevalence of CAPs across each task. These metrics were significantly different between the AM retrieval and WM tasks for all CAPs listed

above. To investigate brain-behavior relationships we used multilevel modeling on the single-trial CAP data, with participant as a random factor. We found that the maximum dwell time of the DMN-dominant and DAN-dominant CAPs significantly and differentially relate to the corrected recognition score (hit rate minus false alarm rate) for each trial of the WM task, with the former CAP associated with worse performance and the latter CAP associated with better performance. Additionally, we found that more CAP transitions (e.g., more neural instability) was significantly related to decreased WM task performance across participants. These findings provide insights into the different moment-by-moment brain dynamics that underlie internally- and externally-oriented memory processes. Most notably, we were able to relate brain state stability as indexed with CAP dwell time, fractional occupancy, and transition frequency to performance on the 2-back WM task.

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Poster

492. Cortical Networks and Behavior

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Nanyang Assistant Professorship, Nanyang Technological University

Title: Emergence of cortex-wide circuit motifs for sensorimotor transformation during learning

Authors: *X. CHIA, J. TAN, H. MAKINO;
Nanyang Technological Univ., Singapore, Singapore

Abstract: Learning adaptive behaviors requires refinement of coordinated activity across individual neurons residing in multiple brain areas. However, because past studies mostly focused on a single brain region, how such coordination of activity emerges during learning is poorly known. Using longitudinal calcium imaging with a two-photon random access mesoscope, we simultaneously probed activity of layer 2/3 pyramidal neurons across eight regions of the mouse cortex during learning of a delayed-response task. Among the eight regions, the anterior lateral motor (ALM) cortex exhibited learning-related strengthening of the choice-related activity and the trial-type non-selective ramp-up activity during the delay epoch. We confirmed that the increased amplitude in the ramp-up activity reflected enhanced robustness in the attractor dynamics based on a simulation with a recurrent neural network (RNN). Importantly, such learning-related activity modulations in the ALM were accompanied by trial-by-trial coordination of choice-related activity with the posterior parietal cortex (PPC). Furthermore, extracting coupling among functionally characterized neurons, we found that

choice-encoding neurons from the PPC to ALM became more functionally connected across learning, while cortex-wide connectivity was globally weakened. Selective ablation of the PPC-ALM functional coupling resulted in disruption of choice-related activity in the ALM, suggesting that choice information was routed from the PPC to ALM. Thus, learning creates cortex-wide circuit motifs with specific inter-areal communication channels to support efficient and robust sensorimotor transformation.

Disclosures: X. chia: None. J. Tan: None. H. Makino: None.

Poster

492. Cortical Networks and Behavior

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Title: Network modularity predicts executive function performance throughout multimodal training

Authors: *A. ANGEBRANDT¹, P. ROBLES GRANDA¹, C. E. ZWILLING¹, E. D. ANDERSON¹, C. H. HILLMAN², A. F. KRAMER², N. J. COHEN¹, S. CULPEPPER¹, A. K. BARBEY¹;

¹Univ. of Illinois Urbana-Champaign, Urbana, IL; ²Northeastern Univ., Boston, MA

Abstract: Prior research has found that cognitive abilities can be enhanced through experimental intervention; however, little is known about the factors that influence individual variability in intervention success. One growing area of interest is the relationship between intervention success and brain network modularity. The community structure of a network is modular to the extent that it forms non-overlapping groups or communities, such that higher levels of modularity indicate dense connectivity within a module and sparse connectivity between modules. Across a variety of training modalities and populations, recent studies have found that brain network modularity measured prior to an intervention predicts training-related cognitive improvements. The present study aims to expand on these findings in a novel cohort of young adults (N = 83; mean age 23.12 years, sd 4.67; 49 females; 48% Caucasian). While extant literature primarily measured the effects of training on cognition through the analysis of pre-post change scores, the present study instead investigates continuous measures of performance on multiple executive function tasks over an extended training program. Subjects in this study were examined in the context of a larger intervention trial and underwent 16 weeks of multimodal

training (fitness and cognitive, N = 41; fitness, cognitive, and mindfulness, N = 42). We conducted a linear mixed effects model to explore the effect of baseline network modularity on the performance on seven executive function tasks over the course of all training sessions. The model included fixed effects for baseline modularity, experimental group, and session. We found that task performance improved with training (Session: $\beta = 0.023$, $df = 6.026$, $t=8.038$, $p < 0.001$) and was significantly associated with baseline modularity ($\beta= 0.1782$, $df = 8391$, $t = 5.179$, $p < 0.001$). Intervention group did not relate significantly to task performance ($p = 0.076$). Our findings demonstrate that modularity reliably predicts performance on multiple executive function tasks over the course of the 16 week interventions, extending prior research that has observed similar effects for unimodal cognitive and exercise training interventions over shorter timescales. Future work will further investigate the relationship between modularity and learning in these data through the classification and analysis of learning curves (to assess learning profiles) and by examining whether the strength of this relationship is moderated by individual or group differences in a host of mental health and lifestyle measures collected in this sample.

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Poster

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Topic: H.08. Learning and Memory

Support: NIH 1R01NS116589-01

Title: Emergence of population dynamics causally driving delayed paired associations working memory

Authors: ***A. BELLAFARD**, G. NAMVAR, J. KAO, P. GOLSHANI;
UCLA, Los Angeles, CA

Abstract: While several brain regions vital for working memory have been identified, and persistent activity in these regions is recorded during working memory tasks, how these activity patterns emerge and the necessity of these activity patterns for behavioral performance is poorly understood. To gain more insight into these problems, we have trained head-fixed mice to perform an olfactory paired association working-memory task, during which head-fixed mice made decisions depending on the sequential identity of two odors presented separated by 5 seconds. We utilized a highly efficient soma-targeted *Guillardia theta* anion-conducting channelrhodopsin (stGtACR2) to perform optogenetic silencing at different time intervals while the animal was performing the task. Silencing of M2 neurons during delay and reward periods dramatically worsened behavioral performance, while stimulation at other intervals had no

effect. Therefore, M2 is both important for maintaining the working memory during the delay period and comparing the memory of the first stimulus with the second stimulus to drive decision making. To examine the emergence and stability of the M2 neuronal population representations, we have used mesoscopic two-photon calcium imaging to capture the activity of hundreds of L2/3 neurons while mice learned and performed the task. We show that most neurons exhibit mixed selectivity for different task parameters, including odor identity and choice. We show that most task-related parameters could be decoded from the population activity of the M2 cortex in well-trained but not novice mice. Future work will determine the mechanisms that drive the development of delayed activity with learning.

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Poster

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Topic: H.08. Learning and Memory

Support: 26-D-70200-73387-110258

Title: Hippocampo-cortical coupling varies with depth of NREM sleep

Authors: *R. SWANSON¹, J. BASU², G. BUZSAKI³;

¹New York Univ., New York, NY; ²NYU, New York, NY; ³Neurosci., New York University, Langone Med. Ctr., New York, NY

Abstract: During NREM sleep, the brain is in a self-organized excitable regime in which alternations between spiking and near cessation of spiking propagate along the forebrain, termed slow oscillations (SOs) or UP and DOWN states in the neocortex, and sharpwave-ripples (SPW-Rs) in the hippocampus (Levenstein 2019). Both gain and loss of function studies have demonstrated the importance of tight temporal coordination between SOs and SPW-Rs systems consolidation. However, where, when, and how this tight coupling is spontaneously achieved across regions is unknown, despite being essential for understanding whole-brain mechanisms of systems consolidation. Towards this goal, we developed a chronic preparation in mice that combines widefield imaging of dorsal neocortex and ipsilateral extracellular silicon probe recordings of hippocampus (HPC) and retrosplenial cortex (RSC), allowing us to monitor multi-scale interaction between regions during sleep and further develop an existing theory of NREM sleep. We find that interaction between HPC and RSC is well matched by a proposed model whereby both RSC and HPC are in reciprocally perturbable excitable regimes, and the degree to which they can perturb one another depends on both the strength of input received and state of the receiving region. 1. SPW-Rs can cause DOWN states in RSC that may propagate to lower order visual areas conditional on the magnitude of the SPW-R and the state of cortex, and 2. transitions to UP states are sufficiently synchronous to drive SPW-Rs in hippocampus. Taken

together, we propose depth of NREM mediates a tradeoff between integration and segregation of reactivated assemblies, serving as a biophysical substrate for compositional reactivation.

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Poster

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Topic: H.08. Learning and Memory

Support: NIH Grant 5R01DC007703-17
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Title: A novel approach to studying cue-guided food seeking

Authors: *E. BARASH¹, D. SVEDBERG¹, H. GERMAINE², D. B. KATZ¹;
¹Brandeis Univ., Brandeis Univ., Waltham, MA; ²Brandeis Univ. Grad. Neurosci. Program, Brandeis Univ. Grad. Neurosci. Program, Boston, MA

Abstract: Survival is inextricably tied to consumption decisions; toxic foods can lead to illness/death, while nutrient-rich foods promote good health. Thus, it is useful to associate cues (ex. the color of a fruit) with a post-consumption outcome (ex. eating a red fruit made me sick) to guide approach-avoidance decisions. While cue-driven-association research is common, little research focuses on the role of cue-food associations in driving foraging/consumption. We have developed a novel experimental framework dedicated to this question. Results from cue-“food reward” association tasks in literature show that rats learn associations in ~11 - 15 days, but the tasks used are often minimally structured. We aimed to not only improve upon the learning rate but do so in an interpretation-rich multi-step response task. We designed a paradigm with a cue-trigger/retrieval-reward sequencing, with visual-auditory cues paired with unique chemosensory food properties - citric acid, water, and sodium chloride - ranging from least to most palatable, respectively. Our findings indicate that the optimal procedure to increase speed of learning involves first training rats the “retrieval-reward” component with a neutral palatability water reward, then expanding to “cue-trigger-retrieval-reward” with varying palatability rewards. The preliminary results show a fast-learning rate as well as expected behavior with rats displaying a decreased latency to response for cues indicating higher palatability rewards, and the inverse for cues indicating lower palatability rewards. In the future this paradigm will be expanded to include electrophysiological interrogation of neural representations of anticipation, decision, and response in the gustatory cortex to understand the neural underpinnings of the differential behavior for different palatability cues.

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Poster

492. Cortical Networks and Behavior

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Neuroscience and Biomedical Sciences

Title: Cortical sensory processing changes with experience across days and even initial trials

Authors: ***D. SVEDBERG**¹, T. GRAY³, D. B. KATZ²;
²Neurosci., ¹Brandeis Univ., Waltham, MA; ³Biol., Brandeis Univ. Grad. Neurosci. Program,
Waltham, MA

Abstract: Laboratory rodents are typically raised in highly impoverished environments, and much of the corpus of post-developmental sensory research has operated under the assumption that perception in adults is innate—that the first exposure to a stimulus is processed “normally” by a system that is fully prepared to handle that stimulus. However, behavioral phenomena such as neophobia, and recent electrophysiological studies, suggest that taste perception changes as animals grow familiar to sensing, at least on the time scale of days. Here, we investigate whether taste responses in rats' primary gustatory cortical (GC) ensembles change with familiarization to novel stimuli within a single session. We observe that the properties of “normal” taste responses rapidly emerge across the initial ~5 exposures to taste stimuli. Specifically, our analyses show that taste-quality coding, and typical temporal structure in taste responses, emerge with broad changes in spontaneous ensemble firing across the 1st 5-10 min of the first exposure session. This rapid evolution of ensemble activity in GC indicates that brief taste exposure may be necessary to establish normal perception and that this exposure may alter the “dynamical landscape” of neural circuits in GC. These experiments add to a growing literature indicating that exposure to even simple sensory stimuli to be a learning experience, and demonstrate that this learning process occurs across multiple time scales. As such, they reveal the danger of starting an experiment with a truly ‘naïve’ subject.

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Poster

492. Cortical Networks and Behavior

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Topic: H.08. Learning and Memory

Support: NIH Grant R01NS109965
DP2NS111134

Title: Perirhinal cortex acquires a predictive map of the task environment through error learning and associative learning

Authors: *D. G. LEE^{1,2}, C. MCLACHLAN^{3,2}, A. E. CAREY^{3,2}, G. HOUSE³, G. LAGANI³, D. LAMAY³, R. NOGUEIRA^{5,6}, S. FUSI^{5,6}, J. L. CHEN^{1,2,3,4};
¹Biomed. Engin., ²Ctr. for Neurophotonics, ³Biol., ⁴Ctr. for Systems Neurosci., Boston Univ., Boston, MA; ⁵Ctr. for Theoretical Neurosci., ⁶Neurosci., Columbia Univ., New York, NY

Abstract: Through learning, the brain creates relationships between stimuli, events, and outcomes. Internal models built on these relationships must be flexible to accommodate unreliable stimuli and novel associations. Perirhinal cortex (Prh) is a region interconnected with sensory cortex and hippocampus which encodes both complex sensory features and their associations. Anatomical tracing shows reciprocal connections between Prh and somatosensory cortices. We sought to investigate how Prh participates in goal-directed learning of abstract tactile representations. Mice were trained across multiple learning stages to classify sequential whisker stimuli during a tactile working memory task. Chemogenetic inactivation of Prh in mice ($n = 9$ inactivated, $n = 13$ control) trained to the task using automated home-cage training systems demonstrated that Prh is necessary for task learning. To understand how these representations evolve in Prh, we performed chronic two-photon imaging of layer 2/3 neurons (200-500 neurons per animal, $n = 7$ animals) over each training session (26-68 sessions per animal). Single-cell activity was analyzed using generalized linear models and population activity was decoded using support vector machines. Population decoder performance to task-relevant directional stimuli decreased with behavioral learning. In contrast, decoder performance to task-irrelevant speed information increased. This suggests that Prh learns a model of task-relevant stimuli and signals the difference between the expected and experienced stimuli. Network activity encoding reward prediction also accompanies sensory prediction activity. Stable reward associations also appeared during early learning, expanding temporally from representations of reward outcome to reward prediction. These generalized to incorporate novel stimulus-reward associations. These results suggest that Prh combines error learning and associative learning to form an internal model of learned task behavior.

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Poster

492. Cortical Networks and Behavior

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Topic: H.08. Learning and Memory

Support: DGAPA-PAPIIT IA201420
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Title: Establishment of conditioned taste preference induced by glucose requires glutamatergic and noradrenergic signaling within the insular cortex

Authors: *K. G. MEDINA-MEDINA, F. BERMÚDEZ-RATTONI, D. OSORIO-GÓMEZ;
Neurociencia cognitiva, Inst. De Fisiología Celular - UNAM, Mexico city, Mexico

Abstract: Conditioned taste preference (CTP) is a form of associative learning by which animals prefer tastants that have been previously associated to the postingestive effects of nutrients. Long-term CTP establishment requires the neural integration of orosensory and viscerosensory information. Currently it is known that the insular cortex (IC) has a functional role in the integration of taste and visceral signals. However, there is scarce information about the neurochemical signaling within the IC involved in CTP establishment. Therefore, we evaluated neurotransmitters release within the IC during exposure to a novel taste and a positive post-ingestive stimulus necessary for CTP acquisition. First, we developed a CTP protocol in male Wistar rats through the presentation of a novel taste paired with an administration of i.p. glucose. Accordingly, we performed a dose-response curve in which two bottles containing 30 mL of saccharin (0.3%) were given to animals and 15 min later different doses of glucose were injected intraperitoneally. Our results show that the i.p. administration of glucose 350 mg/kg induces a long-term CTP. Afterwards, we monitored, through microdialysis in free-moving animals, the changes in norepinephrine and glutamate release within the IC during saccharin intake and i.p. glucose administration. Results show that saccharin consumption induces an elevation of norepinephrine. Interestingly, i.p. glucose administration promotes an increase in both norepinephrine and glutamate release. To assess the functional role of cortical glutamate and norepinephrine in CTP establishment, we administered NMDA (APV) or β -adrenergic (PROP) receptor antagonists into the IC immediately before i. p. glucose and evaluated long-term memory 72 hours later. Our results demonstrate that NMDA and β -adrenergic receptors blockade within the IC hinders CTP establishment. Additionally, we evaluated whether the blockade of NMDA and β -adrenergic receptors within the IC impairs memory acquisition or consolidation processes; to achieve this, we administered APV or PROP 30 minutes after the CTP acquisition session. Short-(4 hrs) and long-(72 hrs) term memories were tested. Results show that while blockade of β -adrenergic receptors spares short and long-term CTP, NMDA receptors blockade within the IC only impairs long-term CTP. These results suggest that glutamatergic and adrenergic signaling within the IC plays an important role in the establishment of CTP. Additionally, our results demonstrate that the activation of NMDA receptors is related to the consolidation of taste preference memories.

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Poster

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Topic: H.08. Learning and Memory

Support: NSERC

Title: Assessing the role of parvalbumin containing GABAergic inhibitory interneurons in the perirhinal cortex in object category learning and recognition in mice

Authors: *H. A. COLLETT¹, K. H. JARDINE¹, J. L. FOURNIER², C. E. WIDEMAN¹, A. E. HUFF¹, B. D. WINTERS¹;

¹Psychology, ²Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada

Abstract: Accurate object category recognition is necessary for producing object-appropriate behavioural responses. Objects from a familiar category may be recognized with the use of generalized category representations which may be refined through exposure to various category exemplars. Presumably, to refine category representations, extraneous object features must be inhibited, which may be achieved through inhibitory GABAergic signaling. Previously we demonstrated that disrupting GABAergic transmission impairs category learning on a mouse object category recognition (OCR) task. Mice that were administered bicuculine, a GABA_A receptor antagonist, prior to exposure to category exemplars were later unable to discriminate between a novel and familiar category object on the OCR task with a 1-h retention delay. Mice require prior category exemplar exposure to perform the OCR task at longer retention delays such as 1-h but can perform the task with novel categories with shorter delays up to 30-min. Parvalbumin-containing GABAergic interneurons (PVINs) specifically have been implicated in visual stimulus learning, including the enhancement and inhibition of relevant and irrelevant visual stimuli, respectively. Furthermore, PVINs provide feedforward inhibitory control in the perirhinal cortex (PRh), an area necessary for object recognition and object feature-binding. To determine the role of GABAergic PVINs in the PRh in the formation of generalized category representations, we infused male PV^{cre} mice intracranially in PRh with adeno-associated virus containing either excitatory or inhibitory designer receptors exclusively activated by designer drugs (DREADDs). The DREADD agonist compound 21 (C21) was administered systemically (i.p., 1mg/kg) prior to exposure to object category exemplars. Both inhibition and stimulation of PRh PVINs prior to pre-exposure sessions impaired subsequent OCR task performance with a 1-h, but not a 30-min, retention delay. With a 30-min retention delay and novel category objects (i.e., no pre-exposure), task performance was also disrupted by pre-sample PVIN inhibition or stimulation; previous object category exemplar exposure ameliorated this deficit with both a 30-min and 1-h retention delay. Interestingly, neither pre-sample PVIN PRh inhibition nor stimulation produced a deficit on the spontaneous object recognition task with a 30-min retention delay. These findings suggest that PVIN activity in the PRh plays an essential role in the refinement of object category representations for long-term memory, as well as allowing for novel object category exemplars to be generalized over a relatively short delay.

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Poster

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Title: Neural correlates of multimodal object recognition in the perirhinal cortex

Authors: *H.-Y. LIM, I. LEE;
Dept. of Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Object recognition is normally accomplished in a multimodal and cross-modal fashion in natural settings. Known as the critical region for object recognition, the perirhinal cortex (PER) receives direct inputs from the cortical perceptual areas specialized in processing specific sensory modalities. However, the neural mechanisms are largely unknown regarding how those individual sensory-perceptual modalities are bound to represent an object and, once learned, the degree to which an object representation is activated by cross-modal cues in the PER. To examine these issues, we recorded spiking activities of single neurons in the PER while rats performed a multisensory object recognition task. Specifically, we trained eight male Long-Evans rats to recognize two different objects, each associated with unique visual and auditory features (i.e., boy-shaped figure with 5kHz frequency sine-wave tone versus egg-shaped figure with 10kHz frequency sine-wave tone). The rat initiated the onset of the multimodal object stimulus by poking the center nose-poke hole and, once the object stimulus appeared, poked its nose into either the left or right reward port on the basis of the identity of the multimodal object. Once trained, a 24-tetrode-carrying hyperdrive was implanted to target the PER. Once the neural recording commenced, only either visual or auditory modality was used during the object sampling phase in some trials, intermixed with the multimodal conditions. Rats performed successfully in both multimodal ($86 \pm 5.4\%$, $p < 0.0001$ compared to 50% chance level in a one-sample t-test) and unimodal conditions (visual: $67.2 \pm 4.6\%$, $p < 0.0001$; auditory: $68.3 \pm 13.7\%$, $p = 0.007$), although their performance was better in the multimodal trials than in the unimodal trials ($p = 0.0006$, one-way repeated-measures ANOVA). Our preliminary analysis suggests that the neural firing patterns are correlated with the diverse combinations of modality conditions (i.e., multimodal, visual-only, or auditory-only) in object-representing neurons of the PER. We are currently characterizing further the neural firing patterns of the different subclasses of

neurons in association with different modality conditions during object recognition and the relationships between the neural firing patterns and behavioral performance.

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Poster

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Title: Histone deacetylase 4-dependent regulation of cortical synaptic plasticity during adulthood.

Authors: *S. CHUNG^{1,2}, J.-H. JEONG³, H. JIE¹, Y.-H. KIM³;

¹Brain Korea 21 project, Dept. of Physiology, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; ²BnH Res. Co., LTD., Goyang-si, Korea, Republic of; ³BnH Res. Co., LTD., Goyang-si, Gyeonggi-do, Korea, Republic of

Abstract: It is known that the primary sensory cortex contributes to the perceptual decision-making process. Still, a detailed synaptic mechanism explaining the relationship between sensory input and perceptual decision-making remains elucidated. Previously, we demonstrated sensory deprivation restores critical-period-like synaptic plasticity to the barrel cortex in the unilateral infraorbital injury (IO) mice. Therefore, we explored the relationship between adult primary synaptic input and perceptual decision-making in adult IO mice using the head-fixed texture discrimination test and brain slice patch-clamp technique. We also explored a molecular mechanism underlying IO-induced reactivation of TC synaptic plasticity in the barrel cortex. IO mice learned the spared TC input-mediated perceptual decision-making task more effectively than sham-controls with enhanced perceptual discrimination and prolonged memory retention. This improvement in perceptual learning was intimately correlated with spared TC synaptic efficacy. In terms of a possible molecular mechanism for the IO-induced reactivation of TC synaptic plasticity, HDAC4 expression was significantly reduced in layers 4 and 5 of the barrel cortex in IO mice compared with Sham-controls. In addition, the application of HDAC4 inhibitor to the S1 barrel cortex via an osmotic pump effectively potentiated TC synaptic efficacy without alteration of excitation/inhibition balance similar to IO mice. These results suggest that the

reactivation of synaptic plasticity of primary TC input may be involved in perceptual decision-making during adulthood.

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Poster

492. Cortical Networks and Behavior

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Topic: H.08. Learning and Memory

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DC007703

Title: Orexin neurons contribute to cortical taste responses

Authors: ***K. C. MAIGLER**¹, D. B. KATZ²;
¹Brandeis, ²Brandeis Univ., Brandeis Univ., Waltham, MA

Abstract: Primary taste cortex (gustatory cortex, GC) is important for taste decisions and related learning, but functions as part of a network, not alone. The taste system is a distributed and versatile network that hinges on GC, but cooperates within itself to generate proper behavioral output. My preliminary optogenetic data suggests that GC, in fact, cooperates with lateral hypothalamus (LH) in the processing of the hedonic value of the taste (palatability). The current research aims to unravel the nature of this across-region interaction by determining what type of projections are being sent from LH to GC. Unlike GC, LH contains a diverse population of peptide-expressing neuronal subtypes. One of these groups of neurons, orexin+ cells, appear to be designed to influence feeding via axons linking them to reward areas and cortex. To test whether LH orexin+ neurons are involved in the GC taste response, I selectively perturbed this orexin+ pathway (achieved by using transgenic rats and optogenetics) and examine whether this manipulation alters GC activity and taste-evoked orofacial response. We confirmed that orexin neurons are a specific subset of taste responsive neurons in LH. When disrupting orexin signaling, we found a significant impact on GC taste responses, indicating that the orexin projection between LH and GC is important contributor in maintaining the functionality of the taste system.

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Poster

492. Cortical Networks and Behavior

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Title: Accelerated learning in auditory, motor, and cognitive tasks using vagus nerve stimulation

Authors: ***T. DANAPHONGSE**¹, A. CARROLL¹, D. PRUITT¹, J. RILEY¹, C. T. ENGINEER², S. A. HAYS³, M. P. KILGARD⁴;

²TxBDC, ¹Univ. of Texas at Dallas, Richardson, TX; ³Bioengineering, Univ. of Texas at Dallas, Dallas, TX; ⁴Behavioral and Brain Sci., Univ. of Texas at Dallas, Richardson, TX

Abstract: Vagus nerve stimulation (VNS) promotes recovery of both motor and sensory function in animal models of neurological injury. While the benefits of VNS therapy have been repeatedly demonstrated in injured models, little is known about what beneficial effects VNS may confer in uninjured animals. Therefore, in this study, we tested whether VNS results in accelerated learning during auditory, motor, and cognitive tasks. We first trained rats on 2 separate speech discrimination tasks. In the first task, rats were trained to discriminate between 8 different consonant pairs. After learning to discriminate between 2 consonants, they moved on to the next pair until they learned to discriminate all 8 pairs. In the second task, rats were trained to respond to a single target speech syllable and withhold responding to four non-target speech syllables. In each experiment, rats were divided into VNS and Sham groups. Rats in the VNS group received stimulation during behavior. The Sham group did not receive any stimulation. We tested how quickly uninjured rats learned to discriminate target from non-target sounds in each task, and whether VNS accelerated the rate of learning compared to Sham subjects. In the motor task, another cohort of rats was trained to use their right forelimb to reach, grasp, and rotate a knob. The difficulty of the task was progressively increased by increasing the distance for the rats to reach the knob and also increasing the required rotation angle. Here, we investigated how quickly naïve rats can learn the supination task while receiving VNS. Finally, for the cognitive task, rats were trained on a visual paired-associate task. Rats learned to associate each of 3 stimulus patterns (horizontal, vertical, and diagonal lines) with a corresponding section of a touchscreen display (left/middle/right). Each trial, two patterns would show: one in the correct placement on the screen and the other placed incorrectly. Rats were given a pellet reward if they touched the correct pattern and position. After learning the task, rats were divided into VNS and Sham groups and required to re-learn the task with new pattern-place associations. We tested if VNS delivered during behavior could enhance extinguishing of previously learned associations and accelerate learning of new ones. In each experiment, we hypothesized that by giving VNS while learning novel tasks to uninjured rats, they would learn them significantly faster. However, across all auditory, motor, and cognitive tasks, the VNS rats failed to show any accelerated learning. Future studies should investigate whether more specific paradigms of tasks would result in accelerated learning compared to what we studied here.

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Poster

492. Cortical Networks and Behavior

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

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Topic: H.08. Learning and Memory

Support: Whitehall Foundation Research Grant 2020-05-71

Title: Dendritic coding of outcome in premotor cortex during motor learning

Authors: *J. SCHEIB¹, J. GABLE¹, Z. NEWMAN¹, M. HEAD^{2,1}, S. BLIESE¹, B. DOUGEN¹, A. KERLIN¹;

¹Univ. of Minnesota, Minneapolis, MN; ²Univ. of Waikato, Hamilton, New Zealand

Abstract: During motor learning, signals regarding the achievement of goals must remodel the brain networks controlling the desired action. How these networks generate action, while simultaneously integrating information regarding the outcome of past action, remains largely unknown. Computational models of cortical learning suggest outcome-related information may target the apical dendrites of pyramidal neurons, compartmentalizing the integration of outcome information from feedforward sensorimotor input onto the basal dendrites. We studied the representation of outcome information in the activity of dendrites of neurons in the anterior lateral motor (ALM) cortex, a premotor area critical for planning orofacial movements. Head-fixed mice were trained to expert performance of a delayed-response directional licking task. We then changed the locations of the licking targets and monitored behavioral variables as mice updated their responses to reflect the new target locations. Bilateral optogenetic suppression of ALM activity during the outcome epoch of this task did not impact baseline performance when licking targets were at the trained location, but did prevent mice from updating tongue trajectories when targets shifted to a new location, indicating that activity in ALM cortex during the outcome epoch is necessary for learning. Two-photon calcium imaging of the apical dendrites of layer 5 neurons indicated robust encoding of movement success or failure during motor learning. Selective optogenetic suppression of dendrite activity bilaterally in the superficial layers of ALM cortex impaired motor learning, similar to the effect of full ALM suppression. Further work will compare recordings and selective manipulations of layer 5 neuron spiking activity with these results. Our results suggest that a dendritic representation of movement outcome in the premotor cortex may play a critical role in motor learning.

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Poster

492. Cortical Networks and Behavior

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Title: Neural ensemble dynamics of auditory thalamus reflect sensory learning and task features

Authors: *M. HASEGAWA^{1,2}, Z. HUANG¹, J. GRÜNDEMANN^{1,2};
¹DZNE Bonn, Deutsches Zentrum für Neurodegenerative Erkrankungen, Bonn, Germany; ²Univ. of Basel, Basel, Switzerland

Abstract: Sensory cortices receive and process uni- and multisensory information to guide behavior. However, so far, the encoding of uni- and multisensory inputs during complex behaviors in sensory thalamic nuclei preceding cortex is poorly understood at the single cell and population level. Here, we established a gradient refractive index lens-based two-photon calcium imaging approach to record the activity of large populations of individual auditory thalamus (medial geniculate body, MGB) neurons in mice during a multisensory go/no-go task. We monitored MGB neurons across learning and found distinct neuronal ensembles encoding auditory, visual and multisensory stimuli as well as task-features. MGB neurons clustered into different functional groups with stable, enhanced or inhibited responses to sensory stimuli upon learning. Additionally, MGB neurons developed a ramping activity during the delay period between the predictive sensory stimulus and reward consumption window, indicating that MGB neurons can retain task information to guide action in expert animals. On the population level, trial-by-trial population vector correlations were stable during the stimulus period, yet increased with learning during the delay period in expert animals, lending further support to the notion that MGB populations encode task features. Finally, graph theory analysis revealed that the ensemble structures of co-activated MGB neurons changed after learning, suggesting that MGB ensembles can be flexibly recruited during learning. Our data suggests that MGB neurons dynamically encode task-related multisensory information across learning at both, the single cell as well as the population level, supporting the view that sensory thalamic nuclei contribute to cognitive processing beyond classic relay functions.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Topic: H.08. Learning and Memory

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Title: An I For an I, Competitive Inhibition and Information Asymmetry in STDP Neural Network Model

Authors: *J. R. HURTADO¹, J. A. BROEK³, B. MISHRA⁴, A. A. FENTON²;

¹Neurobio. of Cognition Lab., Ctr. for Neural Science, New York Univ., New York City, NY;

²Neurobio. of Cognition Lab., Ctr. for Neural Science, New York Univ., New York, NY;

³Departments of Computer Sci. and Mathematics, Courant Inst. of Mathematical Sciences, New York Univ., New York, NY; ⁴Departments of Computer Sci. and Mathematics, Courant Inst. of Mathematical Sci., New York City, NY

Abstract: Inhibitory (I) neurons coordinate synchronous activity in networks of excitatory (E) neurons, but how does network synchrony affect coactivity amongst inhibitory neurons? To better understand these cross-scale interactions, we examined a standard recurrent neural network model of integrate-and-fire E and I neurons, with the EE, EI, IE, and II connections adjusting according to E and I Spike Timing Dependent Plasticity (STDP) rules in response to exploration of a ring environment. To generate sparse and spatially distinct “place” activity in the E neurons (Firing Rate (FR) < 5 AP/s), II connections must be pre arranged into mutually inhibitory competitive groups. Spatial distinctiveness (SpD) > 0.9 is defined as 1 minus the mean correlation between all pairs of spatial activity vectors. To investigate the effect of network synchrony, we added a sinusoidal 7-Hz “theta” input to all I neurons. Low theta amplitudes (10-40% AP threshold) are destabilizing, causing runaway excitation (mean eFR > 10 AP/s) and decreasing SpD (< 0.8). Theta amplitudes beyond the destabilizing range ($\geq 50\%$ AP threshold) lead to network phase transitions that segregate the I neurons into active and inactive groups (Grouping Index (GI) > 0.40, defined as the proportion of I neurons with iFR > mean iFR). We investigated interactions between global network synchrony and the temporally sensitive local learning rules, analyzing subthreshold dynamics in E and I neurons. The Voltage-Phase Coupling of E neurons to the theta input is defined as eVPC = average phase of highest depolarization in each neuron, from its 25% most depolarized phases. When GI is high, eVPC is homogeneous, but becomes inhomogeneous under low theta amplitudes. This weakens EI connections to zero and decreases GI by 50% compared to no theta input. To make the network generally robust to theta, we used principles from signaling game theory to modify the local learning rules as agent-based utility functions with costly signaling. We quantified the conflict between simultaneous E and I inputs onto postsynaptic targets, termed here as “postsynaptic frustration” (PFR), and included it into the STDP learning rule functions. Adjusting connections in proportion to PFR deviations causes the network to become robust to amplitude-dependent instabilities (GI > 0.40, SD > 0.9), while randomly adjusting the weights does not. These findings demonstrate that poorly-timed and conflicting E and I inputs cause suboptimal interactions between i) temporally sensitive local learning rules, ii) information asymmetry encoded in spike-time differences, and iii) low levels of network synchrony.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: Disruption of place cell remapping by scopolamine during aversive learning

Authors: *G. J. BLAIR^{1,2}, C. GUO^{3,4,5}, M. S. FANSELOW^{2,5}, P. GOLSHANI^{3,4,5}, D. AHARONI^{3,4,5}, H. T. BLAIR^{2,5};

¹Ctr. for Neural Sci., NYU, New York, NY; ²Psychology, ³Neurol., ⁴David Geffen Sch. of Med., ⁵Integrative Ctr. for Learning and Memory, UCLA, Los Angeles, CA

Abstract: Hippocampal (HPC) place cells can “remap” their spatial tuning properties with the passage of time or in response to behaviorally significant events, which may enable them to encode information not just about the animal’s present location in space, but also about memories of past experiences or predictions of future events at that location. Here we performed in-vivo calcium imaging of HPC place cells in freely behaving rats (Long Evans, 3mo, n=14) to investigate whether scopolamine, which impairs aversive learning, also disrupts place cell remapping that occurs during such learning. 1.2uL AAV9-Syn-GCamp7s was injected in the HPC CA1 layer and 2 weeks later, a 1.8 mm diameter GRIN lens stack (~0.5 pitch) was implanted to image CA1. After recovery, rats began running one 15 m session every 48 h on a 2.5x1.25 m rectangular maze where they earned 20 mg chocolate pellets by alternating between two rewarded corners. On each trial, rats were free to choose a direct short path (2.5 cm) or an indirect long path (5.0 m) to reach the next reward. During early sessions, rats rapidly acquired a preference for choosing the short path; they were then trained to avoid the short path by electrifying its middle segment with a 1.0 mA shock current during the final 5 min of a 15 min training session, followed on subsequent days by shock-free extinction sessions. Drug-free avoidance training and extinction caused place cells to remap near the shocked location, in accordance with prior studies. Systemic pre-training injections of scopolamine (1 mg/kg) did not impair immediate shock avoidance within the session, and also did not disrupt spatial tuning properties or shock-evoked responses of place cells during training. However, scopolamine impaired 48 h retention of avoidance and also prevented place cells from remapping near the shocked location. In addition, when rats navigated the maze on scopolamine, CA1 population vectors showed degraded between-session similarity with drug-free maze visits on prior days, suggesting that mAChR antagonism acutely degraded the fidelity with which stored HPC representations were retrieved. Based upon these results, we propose that scopolamine causes amnesia by degrading the dynamics of HPC memory retrieval at the population level, thereby

shielding stored memory representations from being modified by experience to encode new memories of events that occur under the influence of the drug. Our findings provide novel evidence that place cell remapping plays a role in memory encoding during aversive learning, and suggests that mAChR antagonists produce amnesia by interfering with experience-dependent plasticity of HPC population codes.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Topic: H.08. Learning and Memory

Support: R01MH115304

Title: Towards identifying which hippocampal synapses are crucial for long-term storage of active place avoidance memory

Authors: *A. B. GRAU-PERALES¹, L. KWOK¹, C. GARCIA JOU², T. C. SACKTOR³, A. A. FENTON¹;

¹Ctr. for Neural Sci., New York Univ., New York, NY; ²City Univ. of New York, New York, NY; ³Dept Physiol. & Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract: Memory is stored by persistent modifications of synaptic strength amongst the neurons that were active during learning, but which hippocampal synaptic populations change to store memory is unknown. Because PKM ζ is necessary and sufficient for maintaining hippocampal LTP, and hippocampal PKM ζ is crucial for persistence of active place avoidance memory, we used PKM ζ immunohistochemistry (IHC) to identify synaptic populations that persistently store the memory. *Arc-CreER^{T2}STOP-floxed-ChR2-EYFP* mice (n=4) received active place avoidance memory training (2x 30-min daily trials) and 4-OH Tamoxifen (TAM) given 30 min before trial 3, to tag memory-activated neurons with membrane-associated ChR2-EYFP. Control mice (n=4) got identical experience but were never shocked. Prior to sacrifice, 1-mo memory is evaluated without shock. IHC and confocal microscopy identified memory-activated (EYFP) and LTP-maintaining (PKM ζ) neurons in dorsal hippocampus. In principal cell layers, EYFP+PKM ζ + co-label increases ~2x in trained vs. control mice at the outer and middle molecular layers of dentate gyrus, and *stratum radiatum* (*str. rad.*) of CA3 and CA1, tracing the trisynaptic circuit standardly related to memory storage and recollection. Co-label is strong but indistinguishable between trained and control *strata lacunosum moleculare* (*str. lac. mol.*) of CA3 and CA1 where entorhinal inputs terminate. We deleted *Prkcz* from excitatory and inhibitory neurons using *Prkcz*-floxed mice crossed with mice that express *CreER^{T2}* in either *CaMKII α* - or *GAD2*-expressing cells. Relative to control littermates, TAM administration

decreases PKM ζ to 35% of controls in both *CaMKII α -Cre*-expressing principal cells and *Gad2-Cre*-expressing inhibitory cells. *Prkcz* deletion and compensation by PKC ι/λ , the other aPKC, was assessed by IHC and RNA-scope. The *Prkcz* deletion upregulates PKC ι/λ by 220% at CA1 *strata rad.* and *lac. mol.* in *CaMKII α -Cre* but not in *Gad2-Cre* mice. After cell-type specific *Prkcz* deletion, place avoidance learning and 1-week memory are strong in the PKC ι/λ -compensated mice but *Prkcz* deletion in *Gad2-Cre*-expressing mice prevents learning across 3 training days, despite mice improving within each day, consistent with a selective role for PKM ζ in long-term memory maintenance. The findings elucidate hippocampal synaptic circuits for long-term storage of a memory, demonstrate molecular compensation for PKM ζ loss by PKC ι/λ , and point to a crucial role in long-term memory storage for PKM ζ at post-synaptic inhibitory neurons.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: Photoactivated proteolysis targeting chimeras (PHOTACs) for optical control of synaptic proteins

Authors: *A. FENTON¹, C. JOU³, T. KO², A. B. GRAU-PERALES¹, M. REYNDERS², D. TRAUNER⁴;

¹Ctr. for Neural Sci., New York Univ., New York, NY; ²Chem., New York Univ., New York City, NY; ³Psychology, The CUNY Grad. Center/Hunter Col., New York City, NY; ⁴Chem., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Hundreds of proteins determine the function of synapses, and synapses define the neuronal circuits that subserve myriad brain and behavioral functions. It is thus crucial to precisely manipulate specific proteins at specific locations and times to elucidate the roles of particular proteins and synapses in brain function. We developed PHOtochemically TArgeting Chimeras (PHOTACs), a strategy to optically degrade a target protein with high spatial and temporal precision. PHOTACs are based on small molecules that, upon wavelength-selective illumination, target particular proteins for ubiquitination by an E3 ligase complex and subsequent degradation by the endogenous proteasome. Here we validate the PHOTAC strategy, by targeting the Ca²⁺/calmodulin-dependent protein kinase II alpha (CaMKII α ;) isoform because it is crucial for baseline synaptic function of excitatory neurons. We describe the design and chemical properties of CaMK2-PHOTAC that targets CaMKII α ;, and show that the PHOTAC is

effective in mouse brain tissue. Light activation (385 nm) of CaMK2-PHOTAC (n = 10) decreased CaMKII α immunolabeling to 30% of controls, specifically, the non-activated CaMK2-PHOTAC (dark condition, n = 8), tissue incubated with ACSF with light (n = 7) or without illumination (n = 4). No differences were observed in the number of DAPI-stained nuclei, MAPK (n's=4), or parvalbumin immunolabeling (n's=4), demonstrating specificity of the CaMK2-PHOTAC. Using optical dissection we confirmed significant loss of CaMKII α was limited to within 25 μ m of the illuminated surface. Because CaMKII α maintains synaptic structure, we next tested whether CaMK2-PHOTAC depresses synaptic transmission in the medial perforant path (MPP). While 100 ms 385 nm light pulses every 10 s did not change responses in the control slices, the illumination depressed baseline synaptic responses in CaMK2-PHOTAC-incubated slices (n = 7), as predicted for loss of CaMKII α in maintaining baseline synaptic function. The effect required light activation because the synaptic responses persisted in the CaMK2-PHOTAC slices that were not illuminated (n = 5). These biochemical and electrophysiological findings demonstrate that CaMK2-PHOTAC is effective in brain tissue, is cell permeable, and targets CaMKII α with no evidence of toxicity. The PHOTACs methodology should be broadly applicable to other key proteins implicated in synaptic function, especially for evaluating their precise roles in the maintenance of long-term potentiation and long-term memory within subcellular dendritic domains.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: How to interpret the results of causal optogenetic manipulations?

Authors: *C. JOU¹, E. PARK², D. DVORAK², A. A. FENTON²;

¹City Univ. of New York, New York, NY; ²New York Univ., New York, NY

Abstract: Optogenetics have provided an unprecedented ability to causally and selectively manipulate specific cell populations to test their hypothesized function. For example, the so-called “engram cell” literature reports that hippocampus neurons that are active during training of a memory task can be optogenetically tagged such that they can be later stimulated to discharge in novel conditions. Amazingly, optogenetic stimulation causes behavioral expression of the memory. Are the tagged neurons the cells that store memory? What is the correct interpretation of such results? Consider two hippocampus place cells, one with a firing field in

the north, the other with a field in the south of an arena. Both are active during training trials, but not at the same time. Once tagged, both cells will be stimulated to cofire. Why would one interpret this novel cofiring to be familiar and related to prior experience? Optogenetic manipulations may be precise manipulations, but they manipulate a complex neuronal population with adaptive, non-linear, and inertial interactions. Accordingly, we evaluate the effects of these manipulations by recording ensembles of CA1 cells during optogenetic stimulation of CA1 in Arc-CreERT2-ChR2-EYFP mutant mice. We find that optogenetic stimulation of active place avoidance memory-tagged hippocampus CA1 neurons activates ~14% of cells with sub-second latency. The CA1 network rapidly adapts with cells increasing, decreasing, or maintaining their baseline activity, despite continued stimulation with 15-ms light pulses in 4-Hz or 10-Hz trains for 10min. Even the opto-tagged neurons adapt by progressively decreasing their response to light pulses from 94% to 70%. The discharge pattern of the CA1 population is invariant to the stimulation, preserving their intrinsic ensemble discharge relationships, as well as the low-dimensional manifold organization of their population dynamics. This network resistance to faithfully follow optogenetic stimulation correctly predicted that the optogenetic stimulation is sufficient to elicit the conditioned active place avoidance memory in a neutral environment where the avoidance is not otherwise expressed. We conclude that causal manipulations such as optogenetic engram-cell stimulation should be interpreted as the expression of the population dynamics of a complex system, rather than the precise causal demonstration of a functioning circuit.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: Inside out: CA1 remapping changes the registration between internally-organized population discharge and the environment without reorganizing most neural discharge relationships

Authors: *S. CARRILLO SEGURA¹, E. R. LEVY¹, E. PARK¹, W. REDMAN², J. HURTADO¹, S. CHUNG¹, A. A. FENTON¹;

¹Ctr. For Neural Sci., New York Univ., New York, NY; ²Interdepartmental Grad. Program in Dynamical Neurosci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: How hippocampus represents spatial knowledge is unclear. The active subset of principal cells discharge in cell-specific locations called “place fields.” Standard “*external*” place

field-based hypotheses assume place cells respond to places, only consider the active subset of place cells, and assume place fields are stable within environments. We performed a classic remapping experiment, using GCaMP6f and miniature microscopes to record CA1 ensemble activity while mice explored a box and a cylinder in 36 5-min sessions spread across 3 weeks. We consider the data in light of the standard hypothesis and an alternative “*internal*” hypothesis that assumes neural activity is internally-organized, founded on the cofiring relationships amongst all CA1 principal cells, and compatible with non-stable spatial tuning. This hypothesis asserts that neural computations are informed by the properties of low-dimensional trajectories of conjoint neural population activity through the neural state space. We find that CA1 place fields are unreliable across weeks, identify a <25% minority of the CA1 population, and remap between environments, permitting decoding of the current environment based on firing fields. Decoding from the cofiring patterns of the entire population is at least as accurate because of multi-stable dynamics that resemble remapping expectations, despite modest cofiring changes across environments. The cells that contribute the most to discriminating the environments are the ~30% “anti-cofiring cells,” whose 1-s timescale discharge is negatively correlated to the rest of the cells. The anti-cofiring subset is environment specific (9% in both environments), and place cells are no better than chance to be anti-cofiring. Other than the anti-cofiring subset, CA1 cofiring is stable across the environments, is organized on a low-dimensional manifold that is indifferent to the environments and stable across weeks. The environments are discriminated by the anti-cofiring cells that anchor the neural manifold within the neural state space. Firing field remapping across environments is not a reorganization of neural coactivity, contradicting the definition of remapping; instead the internally-organized activity manifold changes where it anchors in neural state space and its registration to the environment. These findings demand we reject standard external hypotheses and design experiments to investigate the internal hypothesis that hippocampus represents information about external variables by registering the internally-organized neural activity to external features, controlled by anti-cofiring discharge.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: The hippocampus as a sorter and reverberatory integrator of sensory inputs

Authors: *M. NOMOTO^{1,2,3}, E. MURAYAMA^{1,2,3}, S. OHNO^{1,2,3}, R. OKUBO-SUZUKI^{1,2,3}, S.-I. MURAMATSU^{4,5}, K. INOKUCHI^{1,2,3};

¹Univ. of Toyama, Toyama, Japan; ²Res. Ctr. for Idling Brain Sci., Toyama, Japan; ³CREST, JST, Toyama, Japan; ⁴Jichi Med. Univ., Shimotsuke / Tochigi, Japan; ⁵The Univ. of Tokyo, Tokyo, Japan

Abstract: In entorhinal-hippocampal networks, the trisynaptic pathway, including the CA3 recurrent circuit, processes episodes of context and space. Recurrent connectivity can generate reverberatory activity, an intrinsic activity pattern of neurons that occurs after sensory inputs have ceased. However, the role of reverberatory activity in memory encoding remains incompletely understood. Here we demonstrate that in mice, synchrony between conditioned stimulus (CS) and unconditioned stimulus (US)-responsive cells occurs during the reverberatory phase, lasting for approximately 15 s, but not during CS and US inputs, in the CA1 and the reverberation is crucial for the linking of CS and US in the encoding of delay-type cued-fear memory. Retrieval-responsive cells developed primarily during the reverberatory phase. Mutant mice lacking N-methyl-D-aspartate receptors (NRs) in CA3 showed a cued-fear memory impairment and a decrease in synchronized reverberatory activities between CS- and US-responsive CA1 cells. Optogenetic CA3 silencing at the reverberatory phase during learning impaired cued-fear memory. Our findings suggest that reverberation recruits future retrieval-responsive cells via synchrony between CS- and US-responsive cells. The hippocampus uses reverberatory activity to link CS and US inputs, and avoid crosstalk during sensory inputs.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: Forgetting unveils a temporal transition of engram function

Authors: *A. CHOUCRY^{1,2,3,4}, K. ABDOU^{1,2,3,4}, R. OKUBO-SUZUKI^{1,2,3,4}, E. MURAYAMA^{1,2,3,4}, K. INOKUCHI^{1,2,3,4};

¹Biochem., Univ. of Toyama, 2630 Sugitani, Japan; ²Core Res. for Evolutional Sci. and Technol. (CREST), University of Toyama, Japan; ³Japan Sci. and Technol. Agency (JST), University of Toyama, Japan; ⁴Res. Ctr. for Idling Brain Sci. (RCIBS), University of Toyama, Japan

Abstract: Memory engrams have been shown to hold silent memory traces that could ``vocalize`` their content by artificial reactivation. The persistence of these silent traces after memory consolidation and the generation of cortical traces begets the question of what cognitive roles these silent engrams may still serve within the hippocampus. We used the novel object place recognition task in mice to generate a long-term memory that naturally faded within three days. We added a short-term memory-only protocol either one or four days after the original event, to target both states when the engram was naturally vocal or silent, respectively. Interestingly, only the silent state, henceforth referred to as a mute state, induced the consolidation of the second event, and recovered the original memory in the process. Bidirectional engram manipulation confirmed this observation, as vocalization of the mute engram or muting of the vocal one abolished and induced their ability to consolidate the new event`s memory, respectively. Inhibition of synaptic GluA2-containing AMPA receptors endocytosis prevented both the vocal to mute state transition and the subsequent consolidation of the weak memory. Our data suggest that despite adding the same event unto the same initial training, the cognitive outcome may differ depending on the state of the original hippocampal engram. Silent hippocampal engrams may thus continue to affect the processing of future eligible episodes long after the consolidation process has concluded.

Disclosures: **A. Choucry:** None. **K. Abdou:** None. **R. Okubo-Suzuki:** None. **E. Murayama:** None. **K. Inokuchi:** None.

Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 493.09

Topic: H.08. Learning and Memory

Support: JSPS KAKENHI JP18H05213
JST CREST JPMJCR13W1
MEXT JP25115002
Takeda Science Foundation

Title: Orbitofrontal Cortex Governs Wise Decisions Throughout Idling States

Authors: ***M. FAYED**^{1,2,3}, **K. GHANDOUR**^{1,2,3}, **E. MURAYAMA**^{1,2,3}, **R. OKUBO-SUZUKI**^{1,2,3}, **K. ABDOU**^{1,2,3}, **M. NOMOTO**^{1,2,3}, **K. INOKUCHI**^{1,2,3};

¹Grad. Sch. of Med. and Pharmaceut. Sciences, Univ. of Toyama, Toyama, Japan; ²Res. Ctr. for Idling Brain Science, Univ. of Toyama, Toyama, Japan; ³CREST, JST, Toyama, Japan

Abstract: Wise decisions are supposed to be guided by the accumulated experiences and life skills that are acquired throughout life. It stands to reason that this complex cognitive function is negatively affected by improper sleep or rest, however, direct experimental evidence is still deficient. We hypothesize that sleep or resting periods, which we refer to as idling states, affect the neural underpinnings of the wise decision-making process. We have established a behavioral protocol where after appropriate training on separate days, mice can attain the knowledge to have a free choice between a mentally challenging high gain choice and another that is associated with a safe low reward. We have confirmed that mice can attain their same free choices on separate days, while when sleep deprived (SD) before the test, their choices were significantly biased away from wise decisions compared to the other testing day. The orbitofrontal cortex (OFC) has been shown to affect value-guided decision-making. Therefore, OFC represented a perfect candidate area that upon its silencing just before the test, the behavioral output was correlated with the effect of SD. On the contrary, pre-test activation of the OFC compensated for the deteriorating effects of SD. Interestingly, we found that not only the pre-test idling state is decisive for wise choices, but also the post-training idling state if perturbed could trigger a distant significant negative bias away from wise decisions. Inactivation of the OFC for this specific post-training period gave rise to the same latter behavioral output. These findings indicate that idling states are indispensable for wise behavior and knowledge preservation.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 493.10

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JST CREST JPMJCR13W1
MEXT JP25115002
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Takeda Science Foundation

Title: Weaving cognitive inference in prefrontal network during NREM and REM sleep

Authors: *A. IBRAHIM ZAHER IBRAHIM^{1,2,3}, K. ABDOU^{1,2,3}, K. CHOKO^{1,2,3}, M. H. ALY⁴, M. NOMOTO^{1,2,3}, K. GHANDOUR^{1,2,3}, R. OKUBO-SUZUKI^{1,2,3}, S.-I. MURAMATSU⁵, K. INOKUCHI^{1,2,3};

¹Grad. Sch. of Med. and Pharmaceut. Science, Univ. of Toyama, Toyama, Japan; ²Res. center for Idling Brain Science, Univ. of Toyama, Toyama, Japan; ³CREST, JST, Toyama, Japan; ⁴British Univ. in Egypt, Cairo, Egypt; ⁵Jichi Med. Univ., Jichi Med. Univ., Shimotsuke / Tochigi, Japan

Abstract: Inferential reasoning is a prominent property of higher-order cognition which relies on systematic organization of existence knowledge. Creative inference without conscious awareness facilitates the flexibility of future decisions in daily novel situations. Rapid eye movement (REM) and non-rapid eye movement (NREM) sleep have been correlated with a potential role for inference, gaining insight and innovative problem solving. However, it is still unclear how and when the subconscious mind during sleep assists in eliciting novel ideas and how sleep stages could have distinct roles in this process. Here, we show that cortical offline, but not online, activity is essential for inference evolution and that REM activity is sufficient to boost creativity even from inadequate knowledge. We have designed a transitive inference paradigm in which mice could learn a pair-wise relationship between five different contexts to be used in building an organized hierarchy to help in inferring newly unlearned information. Mice could gain inference after one day, but not shortly, after complete training. Inhibiting the neuronal populations in anterior cingulate cortex (ACC) during post-learning sleep, but not wakefulness, disrupted emergence of inference without affecting the existing knowledge. After insufficient training, artificial activation of medial entorhinal cortex-ACC cross-talk during REM sleep only enhanced inference evolution. In vivo calcium imaging showed that inferential behavior was represented by two distinct neuronal populations in ACC. The first population developed gradually during randomized training and both sleep stages, while the other population started to emerge during REM sleep. Post-training NREM sleep showed high reactivation of original memory to build up the hierarchy. While, REM sleep showed an important role for using this hierarchy to weave inference-related ensembles. These findings establish causal evidence for a complementary role of different sleep stages in reorganizing the available knowledge to reach novel inference, thereby highlighting the power of idling brain in gaining insight and creativity. These findings could aid the development of novel approaches to improve the cognitive performance of normal and diseased subjects.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Topic: H.08. Learning and Memory

Support: JSPS KAKENHI JP18H05213
CREST JPMJCR13W1
MEXT JP25115002
PREST JPMJPR1684

Title: Dual roles of idling moments for past and future memories

Authors: *K. GHANDOUR^{1,2}, T. HAGA³, N. OHKAWA⁴, C. FUNG³, M. NOMOTO^{1,2}, M. SATO⁵, T. FUKAI³, K. INOKUCHI^{1,2};

¹Univ. of Toyama, Toyama, Japan; ²Res. Ctr. for Idling Brain Sci., Toyama, Japan; ³OIST, Okinawa, Japan; ⁴Dokkyo Med. Univ., Tochigi, Japan; ⁵Hokkaido Univ. Grad Sch. Med., Hokkaido Univ., Sapporo, Hokkaido, Japan

Abstract: We experience daily episodes and store new memories every day. Although memories are stored in corresponding engram cells, how different sets of engram cells are selected for current and next episodes, and conduct their memories remains unclear. We report that hippocampal CA1 neurons show an organized synchronous activity in the home cage sleep before learning that is correlated with the learning ensembles, only in engram cells, termed pre-configured ensembles. Moreover, after learning, a subset of non-engram cells develops a population activity, constructed during post-learning offline periods through synaptic depression and scaling, and emerges to represent the next new learning. Together, there are two parallel processes occurring during offline periods: conserving past memories through reactivation and preparing for upcoming ones through offline synaptic plasticity mechanisms.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: A novel dimensionality reduction method "iSeq" reveals that higher-order functions of medial prefrontal cortex are represented as a combination of simpler functions

Authors: *S. OHNO^{1,2,3}, M. NOMOTO^{1,2,3}, K. INOKUCHI^{1,2,3};

¹Univ. of Toyama, Toyama, Japan; ²Res. Ctr. for Idling Brain Sci., Toyama, Japan; ³CREST, JST, Toyama, Japan

Abstract: Although the medial prefrontal cortex (mPFC) plays an important role in rule learning and decision making, it is still unclear how these functions are represented in the mPFC. Related to this, many literatures have shown that the dynamics of neurons associated with behavior or

cognition tend to have a sequential structure. However, since the functions of mPFC are of a higher-order, it is unlikely that these are realized by a single sequence.

Therefore, we hypothesized that the functions of mPFC is represented by dividing it into multiple elements. In other words, we thought that the complex neuronal activity in the mPFC can be viewed as a combination of neuronal sequential activities that encode simple behavior and cognition, respectively. To detect them, we have developed a novel dimensionality reduction method called iSeq. iSeq is an improvement of convNMF[1], and it automatically extracts a statistically valid number of sequential activities from a time series of hundreds of neuronal activities.

To investigate the neuronal activity of the mPFC in animals learning rules on their own, we trained mice to perform a Y-maze task over 6 days, during which their mPFC was recorded by calcium imaging. Analysis of this data with iSeq revealed that the mPFC during the task contains neuronal sequences which express strategic behavior and response to or prediction of reward. These neuronal sequences had necessary and sufficient information to represent the rule of the task. In other words, characteristic combinations of neuronal sequences were observed to occur during successful and unsuccessful task, respectively, and conversely, task success and failure could be decoded from the chronological changes in the neuronal sequences. In addition, neuronal sequences and decoders from one day's data can also be applied to data from other days to confirm temporal variation and invariance of neuronal representations.

We have used this method to show that in the mPFC, the higher-order function of rule learning is decomposed into units such as behavior and reward, each of which is represented in the form of neuronal sequences, but it can also be applied to other brain regions. In this conference, we propose a comprehensive analysis pipeline to automatically detect neuronal sequences and analyze their correspondences with behavioral data and their temporal changes.

[1] Smaragdis. IEEE/ACM Trans Audio Speech Lang Process., 15:1-12, 2007

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: Experience Expands Hyperbolic Representations in the Hippocampus

Authors: H. ZHANG¹, P. D. RICH², A. K. LEE³, *T. SHARPEE⁴;

¹Neurosciences, Univ. Of California San Diego Neurosciences Grad. Program, La Jolla, CA;

²Janelia Farm, Ashburn, VA; ³HHMI Janelia Res. Campus, Arlington, VA; ⁴Salk Inst., San Diego, CA

Abstract: Daily experience suggests that we perceive distances near us linearly. However, the actual geometry of spatial representation in the brain is unknown. Here we report that neurons in the CA1 region of the hippocampus that mediate spatial perception represent space according to a nonlinear hyperbolic geometry. This geometry uses an exponential scale and yields greater positional information compared to a linear scale. We find that the size of the representation matches the optimal predictions for the number of CA1 neurons. The representations also dynamically expanded proportional to the logarithm of time that the animal spent exploring the environment, in correspondence with the maximal mutual information that can be received. The dynamic changes tracked even small variations due to changes in the running speed of the animal. These results demonstrate how neural circuits achieve efficient representations using dynamic hyperbolic geometry.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Topic: H.08. Learning and Memory

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One Mind Otsuka Rising Star Award
McKnight Memory and Cognitive Disorders Award
Klingenstein-Simons Fellowship Award in Neuroscience
Mount Sinai Distinguished Scholar Award
Brain Research Foundation Award

Title: Ensemble remodeling supports memory-updating

Authors: W. MAU, D. MORALES-RODRIGUEZ, Z. DONG, Z. T. PENNINGTON, T. FRANCISCO, A. M. BAGGETTA, M. G. BAXTER, T. SHUMAN, **D. J. CAI**;
Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Memory-updating is critical in dynamic environments because updating memories with new information promotes versatility. However, little is known about how memories are updated with new information. To study how neuronal ensembles might support memory-updating, we used a hippocampus-dependent spatial reversal task to measure hippocampal ensemble dynamics when mice switched navigational goals. Using Miniscope calcium imaging,

we identified neuronal ensembles (co-active neurons) in dorsal CA1 that were spatially tuned and stable across training sessions. When reward locations were moved during a reversal session, a subset of these ensembles decreased their activation strength, correlating with memory-updating. These 'remodeling' ensembles were a result of weakly-connected neurons becoming less co-active with their peers. Middle-aged mice were impaired in reversal learning, and the prevalence of their remodeling ensembles correlated with their memory-updating performance. Therefore, we have identified a mechanism where the hippocampus breaks down ensembles to support memory-updating.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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McKnight Memory and Cognitive Disorders Award
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Title: Ensemble reactivation during an offline period links recent experience with past memories

Authors: *Y. ZAKI¹, D. M. MORALES-RODRIGUEZ², T. FRANCISCO³, Z. T. PENNINGTON⁴, A. LABANCA³, Z. DONG¹, T. SHUMAN⁶, K. RAJAN⁵, D. J. CAI⁷;
¹Icahn Sch. of Med. at Mount Sinai, New York, NY; ²Neurobio., Univ. of Chicago, New York, NY; ⁴Dept. of Neurosci., ⁵Neurosci., ³Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; ⁶Icahn Sch. of Med. At Mount Sinai, New York, NY; ⁷Mount Sinai, New York, NY

Abstract: The compilation of memories, aggregated across a lifetime, defines our human experience. But memories are not static representations of our experiences; rather, we are constantly updating and linking memories across time, especially when a past event can help us predict future outcomes. How are memories dynamically updated to make causal inferences about the world? Using in vivo calcium imaging (with open-source Miniscopes in freely behaving mice), chemogenetics, and novel behavioral designs, we tested how hippocampal networks link an aversive experience to a neutral memory formed days prior. We found that fear

from an aversive experience can transfer to a neutral event experienced days prior. We termed this phenomenon retrospective memory-linking. Memory-linking was asymmetric, as fear from an aversive experience did not transfer prospectively to a neutral event experienced days later. We found retrospective memory-linking was modulated by negative valence; the more negative the aversive experience, the more likely fear would transfer to a past memory. We imaged hippocampal ensembles while mice learned a neutral context, received a strong or weak shock in a separate aversive context two days later, and while mice rested in their homecage after aversive learning. We found that mice that received a weak shock—and did not display memory-linking—displayed reactivation of the aversive ensemble, but not of the neutral ensemble from days prior. In contrast, mice that received a strong shock displayed reactivation of both the aversive and neutral ensembles. Finally, inhibiting ensemble reactivation during the offline period abolished memory-linking. These results suggest that fear from an aversive event can transfer to neutral events experienced days prior, and is driven by offline reactivation of hippocampal ensembles representing multiple experiences. We are currently investigating the specific brain states (i.e., sleep states) that drive offline reactivation of memory ensembles. In nature, the causal link between predictors and outcomes is often uncertain; thus, it may be useful to link memories across days to predict future outcomes. Our work points to a neural mechanism that supports causal inference and explains how episodic memories are linked across days.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Topic: H.08. Learning and Memory

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McKnight Memory and Cognitive Disorder Award
One Mind-Otsuka Rising Star Research Award
Mount Sinai Distinguished Scholar Award
DP2 MH122399-01

Title: Ensemble-specific deficit in neuronal intrinsic excitability in aged mice.

Authors: ***T. FRANCISCO**¹, **A. BAGGETTA**², **L. CHEN**³, **S. RAMIREZ**⁴, **R. L. CLEM**⁵, **T. SHUMAN**⁶, **D. J. CAI**⁷;

²Neurosci., ¹Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; ³Mt Sinai Sch. of Med., NEW YORK, NY; ⁴Boston Univ., Boston, MA; ⁵Neurosci.,

Mount Sinai Sch. of Med., New York, NY; ⁶Icahn Sch. of Med. At Mount Sinai, New York, NY;
⁷Mount Sinai, New York, NY

Abstract: With the prevalence of age-related cognitive deficits on the rise, it is essential to identify cellular and circuit alterations that contribute to age-related memory impairment. Increased intrinsic neuronal excitability after learning is important for memory consolidation, and changes to this process could underlie memory impairment in old age. Some studies find age-related deficits in hippocampal neuronal excitability that correlate with memory impairment but others do not, possibly due to selective changes only in activated neural ensembles. Thus, we tagged CA1 neurons activated during learning and recorded their intrinsic excitability 5 hours or 7 days post-training. Adult mice exhibited increased neuronal excitability 5 hours after learning, specifically in ensemble (learning-activated) CA1 neurons. As expected, ensemble excitability returned to baseline 7 days post-training. In aged mice, there was no ensemble-specific excitability increase after learning, which was associated with impaired hippocampal memory performance. These results suggest that CA1 may be susceptible to age-related impairments in post-learning ensemble excitability and underscore the need to selectively measure ensemble-specific changes in the brain.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Program #/Poster #: 493.17

Topic: H.09. Spatial Navigation

Support: WT Wellcome Trust - 207481/Z/17/Z

Title: Neuronal signature of spatial choice in freely moving rats using miniature endoscopic calcium imaging in the hippocampus

Authors: *R. MITCHELL-HEGGS¹, F. GOBBO¹, D. TSE¹, S. R. SCHULTZ², R. G. M. MORRIS¹;

¹Ctr. for Discovery Brain Sci., The Univ. of Edinburgh, Edinburgh, United Kingdom; ²Dept. of Biosengineering, Imperial Col. London, London, United Kingdom

Abstract: *Aim:* Our aim is to elucidate how the spatial information of a goal location represented in the hippocampus can be used to guide the animal's navigation. Out-of-field prospective replay of place cell activity has been suggested to provide animals with the necessary information (Pfeiffer & Foster *Nature* 2013), but it is unclear if the replayed information reflects remembered past paths, and future paths to be taken, or the evaluation of possible routes. Here we used endoscopic calcium imaging to record neural activity during a goal-oriented task to explore how hippocampal activity can support decision making.

Experiment: 5 WT male Lister-Hooded rats performed the everyday memory task (Bast et al J.Neurosci. 2005). Miniature endoscopes with GRIN lenses targeted to the rat CA1 were used to record from several sessions across consecutive days. We expressed GCaMP6f via AAV injection and recorded neuronal activity using the Inscopix system (mean 191 cells per animal, SEM 43) during the exploration of the arena and performance in the task. Rats learned to retrieve food from one of 3 possible sandwells during sample trials whose position changed every session and were tested for memory recall 45 min after learning. Rats entered the arena from a startbox, making it possible to distinguish the start point (decision) from the trajectory through the arena to an anticipated goal. After defining place cells in daily exploration, we found that the goal sandwell could be correctly predicted from the neuronal activity during the 2-10 s preceding the leaving of the startbox. Importantly, while very high for correct trials, the startbox neural prediction declined when animals made the wrong choice (visiting a non-rewarded Sandwell first). 93% of the active cells in this time window were also active during exploration, and 71 % of the activity matched one of the three possible trajectories. We observed that, although the correct location was over-represented in the startbox neural activity, alternative paths to the incorrect, non-rewarded sandwells were also replayed. Our results support the view that prospective replay activity likely represents possible alternatives that support decision-making. *Implications:* To make a decision about their destination, animals need to have access to information about where to go. Our data show that the activity of neurons encoding the goal is prospectively replayed before the animals make their decision. However, during decision-making, other alternative goal locations are replayed, which may be used by the animal to take them into consideration in planning possible routes. Our data therefore reveal a role for replay during navigation planning.

Disclosures: **R. Mitchell-Heggs:** None. **F. Gobbo:** None. **D. Tse:** None. **S.R. Schultz:** None. **R.G.M. Morris:** None.

Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Program #/Poster #: 493.18

Topic: H.09. Spatial Navigation

Support: Wellcome Trust Grant 207481/Z/17/Z

Title: Comparison of Egocentric and Allocentric Spatial Strategies in Rats performing an Episodic Memory task with Miniscope Ca²⁺ imaging

Authors: ***F. GOBBO**¹, **R. MITCHELL-HEGGS**^{1,2}, **D. TSE**^{1,3}, **N. GARCIA-FONT**¹, **S. SCHULTZ**², **R. G. M. MORRIS**^{1,4};

¹CDBS, Univ. of Edinburgh, Edinburgh, United Kingdom; ²Ctr. for Neurotechnology, Imperial Col. London, London, United Kingdom; ³Dept. of Psychology, Edge Hill Univ., Ormskirk,

United Kingdom; ⁴Simons Initiative for the Developing Brain and Patrick Wild Centre⁴Simons Initiative for the Developing Brain and Patrick Wild Ctr., Edinburgh, United Kingdom

Abstract: *Aim:* The study of spatial navigation in goal-oriented tasks gives an opportunity to understand how space is represented (egocentric vs allocentric), and how spatial information related to the goal is stored and accessed during memory retrieval and planning. The behavioral strategy may change with time and familiarity with the task, as paradigmatically shown by Packard & McGaugh (*Neurobiol.Learn.Mem.* 1996). This study used endoscopic calcium imaging in the rat hippocampus to determine the neuronal representation during planning and later execution of goal trajectories when rats use these two different strategies.

Experiment: We employed 10 WT male Lister-Hooded Rats performing in variants of the everyday memory task designed to require the use of egocentric or allocentric strategies, respectively (Broadbent et al *Eur.J.Neurosc* 2019). GCaMP6f was expressed virally in CA1 and neuronal activity is recorded with miniature microscopes using the Inscopix system (average number of cells 195 per animal, SEM 50.7) during the task performance. Rats learned to retrieve food during sample trials from one of 6 possible sandwells whose position changed every session, and are tested for recall 60 min after learning. The animals entered the arena from 4 possible start locations, generating different possible origin-goal trajectory combinations; 27 training sessions were performed to ensure coherent, asymptotic performance, and tests conducted to confirm that animals were using an egocentric or allocentric strategy. For instance the removal of visual cues caused performance to drop to 56% in allocentric testing, but not egocentric - 96% correct. The two groups were then switched to the opposite strategy, reaching 94% and 79% performance in the now egocentric and allocentric groups, respectively. 7 consecutive sessions were recorded for the two groups for each strategy training (sessions 28-34). Matched spatial trajectories are being compared across groups with respect to neuronal representation. The variance between identical reference trajectories is compared to test trajectories which are identical in egocentric frames but distinct in allocentric terms. The analysis also compares the representation of same prospective goal location from multiple starting points. *Implications:* What goals and paths are can assume a different meaning depending on the spatial framework used. Our data provide new information on how the hippocampus computes representations at the neuronal level in egocentric and allocentric terms. Our hypothesis being tested is that the neural representation at the start box should differ for allocentric vs egocentric coding.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Program #/Poster #: 493.19

Topic: H.09. Spatial Navigation

Support: UCLA IDDRC 441587-PG-31669

Title: Hippocampal neural dynamics of spatial navigation in the Morris water maze

Authors: *R. RESHEF¹, M. SHAHI², D. AHARONI¹, P. GOLSHANI¹;

¹Dept. of Neurol., ²Dept. of Bioengineering, UCLA, Los Angeles, CA

Abstract: Learning and memory enables the organism to acquire, store, retrieve, and update knowledge of the world through experience as well as to use this knowledge to navigate through a myriad of stimuli to facilitate survival. Lesions or inactivation of the hippocampus impair the encoding and retrieval of spatial memories. Hippocampal place and head direction (HD) cells activity has long been proposed to be related to the representation of one's location in space and the heading direction in that space. Yet, how place and HD neuronal population dynamics change with spatial learning and how this activity drives navigation to a learned goal is poorly understood. To address these questions, using wire-free miniaturized microscopy of genetically encoded calcium indicator GCAMP7f, we imaged the activity of CA1 neurons during spatial learning of a target oriented two-dimensional navigational task, the Morris Water Maze - a gold standard task for spatial navigation. Using generalized linear models (GLM) we were able to disentangle the activity of place cells and HD cells in CA1 as animals navigate in the maze. Our results demonstrate that the tuning of space and HD cells becomes significantly more selective as animals learn to navigate to the goal (n=5 animals). As we were able to track the activity of cells across days, we also found that tuning selectivity of individual neurons increases. Preliminary data shows these changes in selectivity are not evident in animals unable to learn the goal location (n=2 animals). Thus, cells become more tuned to location and heading direction as learning continues. Future work will determine whether this increased selectivity in tuning is essential for learning.

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Poster

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Topic: H.09. Spatial Navigation

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ELSC graduate students' scholarship

Title: Hippocampal Astrocytes Encode Reward Location

Authors: *A. DORON¹, A. RUBIN², A. BENMELECH-CHOVAV³, N. BENAİM³, T. CARMI³, R. REFAELI⁵, N. NOVICK³, T. KREISEL⁴, Y. ZIV⁷, I. GOSHEN⁶;
¹Hebrew Univ., Hebrew Univ., Jerusalem, Israel; ²Weizmann Inst. of Sci., Weizmann Inst. of Sci., Rehovot, Israel; ⁴The Hebrew Univ., ³The Hebrew Univ., Jerusalem, Israel; ⁵Hebrew Univ. of Jerusalem, ⁶The Hebrew University, Edmond and Lily Safra Ctr. For Brain Sci., Hebrew Univ. of Jerusalem, Jerusalem, Israel; ⁷The Weizmann Inst. of Sci., The Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Calcium dynamics in astrocytes have been implicated in the encoding of sensory information, and modulating them was shown to impact behavior. However, real-time astrocytic calcium activity in the hippocampus of awake mice has never been investigated longitudinally. We chronically imaged CA1 astrocytes using 2-photon microscopy as mice ran in familiar or novel virtual environments to obtain water rewards. We found that astrocytes exhibit persistent ramping activity towards the reward location in a familiar environment, but not in a novel one. Shifting the reward location within a familiar environment also resulted in diminished ramping. Following additional training, as the mice became familiar with the new context or the new reward location, ramping was reestablished. Using linear decoders, we could predict the location of the mouse in a familiar environment from astrocytic activity alone. We could not do the same in the novel environment, suggesting that the spatial modulation of astrocytic activity is experience-dependent. This is the first indication that astrocytes can encode the expected reward location within a specific spatial context, thereby extending their known computational capabilities, and their role in cognitive functions. Next, we set out to investigate the functional significance of astrocytes to neuronal computation during the performance of similar spatial tasks. Specifically, we ask whether astrocytes, which cover discrete physical domains with minimal overlap between their fine processes, also have functional implications for neurons residing in their territories.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 493.21

Topic: H.09. Spatial Navigation

Support: NIH-NINDS Grant R01 NS104829-01

Title: Inputs to CA1 cells override distributed local LFP theta

Authors: *M. WANG¹, B. E. PFEIFFER²;

¹UCSD Dept. of Neurosciences, La Jolla, CA; ²Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: To perform complex cognitive functions, the brain must coordinate the activity of large populations of neurons. Hippocampal local field potential (LFP) theta rhythm (4-12 Hz) is believed to play a key role in coordinating place cell activity during memory encoding. However, the phase of theta is not synchronous along the longitudinal axis of the hippocampus, raising questions regarding how activity from spatially distant hippocampal populations can be coordinated. Here, we implanted tetrodes distributed across a large area of the rat hippocampus to simultaneously record distributed LFPs and hundreds of bi-hemispheric CA1 neurons in freely moving rats on open-field and linear-track tasks. We observe stable and widely distributed LFP theta phase offsets among recording sites. However, despite distributed LFP phase offsets at each recording site, population firing patterns of recorded neurons appeared to be synchronized to a single global reference, indicating that populations are largely insensitive to the phase of their 'local' theta oscillation. Analysis of cells with bimodal firing with respect to the theta oscillation indicated two sources of global synchrony, likely driven by two upstream inputs to CA1 during theta state. In support of this hypothesis, we found two globally synchronous groups of cells during REM sleep: a dominant group likely to reside in the deep sublayer, and a secondary group more likely to reside in the superficial sublayer, suggesting that EC3 and CA3 inputs respectively underlie theta phase-locked firing for these two populations. The above findings suggest that asynchronous LFP theta across the hippocampus has minimal impact on information representation by distributed CA1 cell populations, and that theta-locked firing in area CA1 is predominately driven by upstream inputs rather than local circuit operation.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: Co-allocation to overlapping dendritic branches in the retrosplenial cortex integrates memories across time

Authors: *M. SEHGAL¹, D. ALMEIDA-FILHO², G. I. KASTELLAKIS⁴, S. KIM⁵, J. LEE⁵, S. MARTIN¹, I. D. MEJIA¹, A. PEKCAN¹, S. HUANG¹, A. LAVI¹, W. HEO⁶, P. POIRAZI⁷, J. TRACHTENBERG¹, A. SILVA³;

¹Univ. of California Los Angeles, Univ. of California Los Angeles, Los Angeles, CA;

²Neurobio., ³Dept Neurobiol, UCLA, Los Angeles, CA; ⁴IMBB/FORTH, Heraklion, Greece;

⁵KAIST, Daejeon, Korea, Democratic People's Republic of; ⁶IBS/KAIST, Daejeon, Korea, Democratic People's Republic of; ⁷IMBB-FORTH, Heraklion, Crete, Greece

Abstract: Events occurring close in time are often linked in memory, providing an episodic timeline and a framework for those memories. Recent studies suggest that memories acquired close in time are encoded by overlapping neuronal ensembles, and that this overlap is necessary for memory linking. Transient increases in neuronal excitability drive this ensemble overlap, but whether dendritic plasticity plays a role in linking memories is unknown. Here, we show that contextual memory linking is not only dependent on ensemble overlap in the retrosplenial cortex (RSC), but also on RSC branch-specific dendritic allocation mechanisms. We used miniature microscopes and activity-dependent labelling to demonstrate that neuronal ensemble overlap within the RSC underlies linking of two contextual memories. Using longitudinal two-photon imaging of RSC dendrites, we show that the same dendritic segments are preferentially activated by two linked (but not independent) contextual memories, and that spine clusters added after each of two linked (but not independent) contextual memories are allocated to the same dendritic segments. Importantly, with a novel optogenetic tool selectively targeted to activated dendritic segments following learning, we show that reactivation of dendrites tagged during the first context exploration is sufficient to link two contextual memories. These results demonstrate a causal role for dendritic mechanisms in memory linking and reveal a novel set of rules that govern how linked, and independent memories are allocated to dendritic compartments.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: Compartment-specific tuning of dendritic feature selectivity by intracellular Ca²⁺ release

Authors: *J. K. O'HARE¹, K. C. GONZALEZ¹, S. A. HERRLINGER¹, Y. HIRABAYASHI², V. L. HEWITT¹, H. BLOCKUS¹, M. SZOBOSZLAY¹, S. V. ROLOTTI¹, T. GEILLER¹, A. NEGREAN¹, V. CHELUR¹, F. POLLEUX¹, A. LOSONCZY¹;

¹Zuckerman Inst., Columbia Univ., New York, NY; ²Sch. of Engin., The Univ. of Tokyo, Tokyo, Japan

Abstract: Synaptic plasticity, the process by which neurons adjust the strengths of their thousands of inputs, allows animals to adapt to the environment. Decades of research have established calcium as a central mediator of synaptic plasticity. Historically, most investigations have focused on calcium influx through voltage-gated channels. However, a large body of *in vitro* research suggests that an alternate source of calcium may also play a potent role in shaping plasticity: the endoplasmic reticulum (ER). The ER stores calcium in vast quantities within a cell which it can release in response to strong synaptic input through intracellular calcium release (ICR). The ER is therefore poised to shape the magnitude and spatial distribution of calcium during plasticity induction. Despite its potential role in synaptic plasticity, ICR has never been investigated in mammalian neurons *in vivo*. To test whether ICR participates in experience-dependent plasticity, we focused on pyramidal neurons of hippocampal area CA1 (CA1PNs). CA1PNs receive excitatory inputs from multiple afferent circuits, carrying complementary streams of information about an animal's environment, that target distinct compartments of the CA1PN dendritic arbor. During exploration, CA1PNs integrate these inputs to form spatially-tuned receptive fields, known as place fields (PFs), that manifest as a neuron firing when an animal occupies a particular location. Recent work has characterized an *in vivo* plasticity mechanism, behavioral timescale synaptic plasticity (BTSP), that drives rapid PF formation in CA1PNs. Here we used CA1PNs and BTSP as a model system to test whether ICR participates in the experience-dependent emergence of feature selectivity. We implemented a series of tools based on single-cell electroporation allowing us to (1) augment the cytosolic impact of ICR at single-cell resolution using conditional gene deletion, (2) optogenetically induce PFs and (3) image somatic and dendritic activity dynamics simultaneously during spatial navigation. We found that augmenting ICR in single adult CA1PNs dramatically increased the level of spatial co-tuning observed in their apical dendrites relative to the soma of CA1 place cells; a phenomenon not observed in basal dendrites which were already highly co-tuned with the soma in control CA1PNs. Maximizing stabilized PFs over days and altered the integrative properties of apical dendrites to shape output-level receptive fields. Therefore, ICR cooperates with circuit-level architecture *in vivo* to promote the emergence of behaviorally-relevant forms of plasticity in a compartment-specific manner.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Title: Neuronal gain control by an activity-regulated gene, Arc, in the hippocampal CA1 area

Authors: L. YUAN¹, *A. TASHIRO²;

²Nanyang Technological Univ., ¹Nanyang Technological Univ., Singapore, Singapore

Abstract: Activity-regulated cytoskeleton-associated protein, Arc, is known to mediate synaptic plasticity and contribute to memory. However, it is unknown how Arc regulates information coding of individual neurons and neuronal population. Here we examined this issue by knocking down Arc gene using virus-mediated RNA interference and monitored the activity of principal cells in the hippocampal CA1 area, in which many principal cells show place cell activity. First, we found that Arc knockdown significantly reduces firing rate of CA1 principal neurons including place cells. This reduction occurred specifically in single isolated spikes but not burst firing and more extensively outside of place fields than inside. These results indicate that Arc knockdown reduces input-to-output gain so that principal neurons less frequently fire at situations where inputs are weaker. Second, due to larger reduction of firing outside of place fields, spatial firing pattern was more specific. This effect resulted in increased spatial information content per spike. We also found similar increased firing specificity when we analyzed firing patterns along theta oscillations. Although spatial information content per spike was increased, overall information content represented by individual neurons was reduced because of reduction in the number spikes per unit time. In population analyses, we found that Arc knockdown principal cells reduced cofiring frequency and population information content while increasing errors in position estimation. These results indicate that Arc knockdown compromised spatial coding of neuronal population. Finally, we examined relationship between the parameters affected by Arc knockdown in datasets from normal neurons that did not undergo Arc knockdown. We found these parameters are significantly correlated, suggesting that they are normally co-regulated in individual neurons, potentially through a common mechanism. Overall, our findings indicate that Arc increases neuronal gain to make principal neurons to fire more sensitively to weak inputs. Although this increased gain sacrifices firing specificity, more frequent firings improve information coding of single neurons and, in turn, neuronal population. Considering activity-induced nature of Arc gene expression, we propose that activated neurons (e.g. those that participated in memory formation) undergo these Arc-dependent changes.

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Poster

493. Learning and Memory: Hippocampal and Prefrontal Circuits

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Topic: H.09. Spatial Navigation

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Title: Hippocampal involvement in robot avoidance paradigm in rats - immediate early gene imaging and chemogenetic approach

Authors: *J. SVOBODA, B. KRAJCOVIC, P. ZITTA, D. CERNOTOVA, S. KUBIK, A. STUHLIK;

Inst. of Physiol. of the Czech Acad. of Sci., Praha, Czech Republic

Abstract: Animals can efficiently organize their spatial behavior with respect to other moving animals or objects, but the underlying neural substrate is poorly understood. To model spatial interactions between an animal and a moving object, we developed an aversive task in which a rat placed on a circular arena must avoid a 25cm distance from a small programmable robot to keep itself from a mild footshock. Our previous work showed that the dorsal hippocampus plays a vital role in this process. Bilateral muscimol inactivations of the dorsal hippocampus impaired avoidance of a moving but not stable robot. In addition, unit recordings from CA3 found neurons responding to the mutual position of a rat and the robot. In the present experiment, we aimed to investigate the neural substrate in more detail using immediate-early gene imaging. Male Long Evans rats either underwent training to avoid a stable (S) or moving (M) robot in four 20-min daily sessions. On the last day, they either continued in this condition (SS group, MM group), or the condition changed (SM group, MS group). Control group remained in the cage on the last day (CG). After the last session, rats were sacrificed and perfused. Brains were either stained for the presence of c-Fos protein or the expression of other immediate-early genes, Homer1a and Arc, which were determined using cellular compartment analysis of temporal activity by fluorescence in situ hybridization (catFISH). In dorsal hippocampus, in all but the control group, we found increased c-Fos activity in the dentate gyrus, followed by a less active CA1. Interestingly in CA3 and CA1, groups initially trained to avoid a mobile robot (MM, MS) displayed higher activity than groups trained with the stable robot (SS, SM), irrespective of the condition on the last day. A similar pattern of c-Fos staining was observed in the ventral hippocampus, except for the dentate gyrus, in which the activity remained low across all groups. These data suggest that not only the dorsal but also the ventral hippocampus participates in robot avoidance, and CA3 and CA1 plasticity may be related to the experience of the robot's mobility. Detailed analysis of catFISH data is in progress. In a subsequent study, we transduced PV-cre rats with pAAV-hSyn-DIO-hM3D(Gq)-mCherry in CA1 in the dorsal hippocampus to investigate the role of CA1 PV interneurons in robot avoidance. Rats were first well-trained to avoid under both M and S conditions and then intrahippocampally injected with C21 to increase PV activity. We observed no significant deterioration of avoidance in either condition, suggesting PV activity in CA1 does not directly control avoidance behavior.

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Poster

494. Schizophrenia: Translational Studies

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Program #/Poster #: 494.01

Topic: H.13. Schizophrenia

Support: NIH Grant R61-MH121560

Title: Outpatients with schizophrenia show differential activation of cortical regions during emotional working memory task

Authors: *A. R. KNIPPENBERG¹, M. J. SPILKA¹, L. LUTHER¹, L. SWEET¹, D. SABATINELLI¹, S. SCHWEIZER², G. P. STRAUSS¹;

¹Univ. of Georgia, Athens, GA; ²Univ. of New South Wales, Sydney, Australia

Abstract: Working memory (WM) has long been a focus of cognitive neuroscience research since WM is implicated in many different cognitive processes. Individuals with schizophrenia (SZ), a serious and debilitating mental illness, display specific WM deficits for emotional stimuli; however, it is unclear which neural processes drive these abnormalities and whether they are associated with clinical outcomes. The current study examined the neural basis of emotional working memory performance (EWM) in SZ, and associations with clinical outcomes. Thirty-two outpatients with SZ completed clinical ratings and an EWM n-back task during functional magnetic resonance imaging (fMRI). A region of interest (ROI) analysis was employed to evaluate differential contributions of cortical ROIs to the processing of emotional versus neutral stimuli. Results indicated that task accuracy was poorer on more demanding n-back trials, yet similar on emotional versus neutral trials. Overall accuracy was positively correlated with vIPFC and mPFC activation under greater emotional load, and negatively correlated with dIPFC activation under lower load. Greater negative symptom severity was associated with poorer accuracy for emotional trials, and this association was driven by increased vIPFC activation. Findings suggest that negative symptoms in schizophrenia are associated with EWM abnormalities that result from altered activation of prefrontal circuits. Our group is following up these results by evaluating whether cognitive training interventions designed to enhance EWM can improve negative symptoms via a direct mechanistic effect on prefrontal activation.

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Poster

494. Schizophrenia: Translational Studies

Location: SDCC Halls B-H

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Program #/Poster #: 494.02

Topic: H.13. Schizophrenia

Support: NIMH Grant R01MH117323
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Title: A model of reward and perceptual computations underlying hallucinations

Authors: *J. BUCK^{1,2}, K. IIGAYA^{1,2}, G. HORGA^{1,2};

¹Columbia Univ., New York, NY; ²New York State Psychiatric Inst., New York, NY

Abstract: Hallucinations are false percepts typically experienced with high confidence. Neuroimaging studies have consistently shown a specific positive correlation between hallucination severity and striatal dopamine release. However, the process by which striatal dopamine drives hallucinations remains unclear. Both reward and perceptual learning, which have been linked to mesolimbic and nigrostriatal dopamine function respectively, are disrupted in patients with psychotic disorders. These alterations have fostered both reward-based and perception-based theories of hallucinations, but these theories have never been operationalized and tested within a common quantitative framework. Here, we mathematically ground these theories with a computational model. Given considerable evidence that hallucination propensity strongly correlates with false alarms in signal-detection tasks we designed our paradigm building from this literature. Our extended signal-detection model updates both perceptual and reward expectations based on experience and integrates these expectations with current sensory evidence to determine if a signal was present or absent on each trial. We simulated model observers with a variety of learning alterations on a standard signal-detection task and found that both reward and perceptual alterations could result in patterns of false alarms displayed by hallucination-prone participants in previous studies. Further, a model recovery analysis suggested that reward and perceptual alterations were indistinguishable under standard task conditions. To dissect independent contributions of reward and perceptual computations to false alarms, we thus designed a novel auditory signal-detection task. In our task, observers are incentivized to develop perceptual and reward expectations and incorporate these expectations into their decision process. Importantly, simulated task observers with reward alterations show behavioral signatures distinguishable from those with perceptual alterations, as supported by model-recovery analyses. Overall, we propose a new framework for understanding reward and perceptual computations underlying hallucinations—which could inform psychopathological

models and perhaps ultimately personalized treatments—and describe a novel perceptual decision-making task designed to interrogate this framework.

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Poster

494. Schizophrenia: Translational Studies

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Program #/Poster #: 494.03

Topic: H.13. Schizophrenia

Title: Combining Data-Driven and Theory-Driven Approaches in Computational Psychiatry: Cross-Task predictors in schizophrenia

Authors: *A. GEANA¹, J. WALTZ², J. M. GOLD³, M. J. FRANK⁴;

¹Brown Univ., Providence, RI; ²Univ. of Maryland MPRC, Baltimore, MD; ³Psychiatry, Univ. of Maryland, Baltimore, MD; ⁴Brown Univ., Carney Inst. for Brain Sci., Providence, RI

Abstract: A central goal of computational psychiatry is to develop mechanistic models based on theoretical and functional principles to explore how aberrations in such mechanisms lead to mental illness. However, most studies in this domain employ computational modeling to clinical samples who have been tested on a single task. A more comprehensive approach is needed to expose clusters of symptom-producing mechanisms that could then be targeted for treatment development. Here we applied computational multidimensional functional profiling to assess neurocognitive alterations in 112 patients with schizophrenia and 32 controls performing 5 "mechanistic" tasks amenable to computational modeling, as well as a battery of standard neuropsychological assessments. We used cross-validation to test whether mechanistic tasks and computational modeling aid patient classification compared to neuropsychological tests. We found that task-based classifiers matched or outperformed those based on neuropsychological assessments, but only when computational model parameters were used. Moreover, classifiers based on model parameters exhibited greater specificity but worse sensitivity compared to neuropsychological tests. Finally, canonical correlation analysis revealed that model parameters related to uncertainty and exploration across tasks were predictive of negative symptoms. Taken together, these findings indicate that mechanistic tasks and computational modeling can complement neuropsychological tests to aid patient classification and mechanism discovery.

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Poster

494. Schizophrenia: Translational Studies

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Title: Behavioral measures of cortical hyper-excitability in subclinical Autism (ASD) and Schizotypy

Authors: *W. A. TORRENS¹, J. N. PABLO², M. E. BERRYHILL⁴, S. M. HAIGH³;
¹Integrative Neurosci., ²Cognitive Brain Sci., ³Dept. of Psychology and Inst. for Neurosci., Univ. of Nevada, Reno, Reno, NV; ⁴Psychology, Cognitive and Brain Sci. and Integrative Neurosci., Univ. of Nevada, Reno, NV

Abstract: Individuals with Autism Spectrum Disorder (ASD) and individuals with schizophrenia (SZ) both report subjective experiences of sensory flooding despite ASD being linked with cortical hyperexcitability and SZ often exhibiting cortical hypo-excitability. Experiences of sensory flooding are consistent with symptoms of cortical hyperexcitability. However, the use of medications in both clinical populations can obscure the mechanisms underlying each condition. Subclinical traits of ASD and SZ (schizotypy) can be measured in the general population within a spectrum of severity, avoiding medication related confounds. Therefore, we examined the relationship between a behavioral measure of cortical hyper-excitability - the Pattern Glare test (PGT) - and subclinical traits of autism and schizotypy in the general populations. The rationale is that more excitable brains experience a greater number of illusions. 479 undergraduates participated via online survey (Qualtrics). Schizotypal and Autism traits were measured using the Schizotypal Personality Questionnaire - Brief Revised (SPQ-BR) and Autism-Spectrum Quotient (AQ). Participants viewed a single horizontal grating for 5 seconds and reported any perceived illusions. SPQ and AQ scores significantly predicted the number of illusions reported ($F(2,476)=15.50, p<.001$), though only SPQ significantly predicted total illusions reported ($\beta;=.02, p<.001$). A second multiple regression tested if individual SPQ factors (cognitive perceptual, interpersonal, and disorganized) and AQ factors (attention switching, attention to detail, imagination, communication, and social skills) significantly predicted the number of illusions experienced. The model was statistically significant ($F(8,470)=4.90, p<.001$); the significant factors within the model were the SPQ disorganized ($\beta;=.04, p=.02$) and the AQ attention switching factor ($\beta;=.11, p=.01$). Individuals with more schizophrenia-like traits experience more illusions, consistent with cortical hyperexcitability. This relationship was weaker in individuals with ASD traits. The disorganized schizotypy and the attention switching factor of the AQ were predictive of the number of illusions, suggesting that the mechanisms underlying their experiences of sensory flooding may be influenced by attentional deficits.

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Poster

494. Schizophrenia: Translational Studies

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Title: Cerebro-cerebellar gray matter abnormalities underlying executive dysfunction in patients with schizophrenia

Authors: *N. KANG¹, S. CHUNG², H.-Y. JUNG¹, Y.-G. HWANG¹, Y. HEO¹, S.-H. LEE¹, M. BANG¹;

¹Dept. of Psychiatry, CHA Bundang Med. Center, CHA Univ. Sch. of Med., Seongnam, Korea, Republic of; ²CHA Univ. Sch. of Med., Pocheon, Korea, Republic of

Abstract: Background: Schizophrenia is a brain disorder involving the cerebral and cerebellar structures. The cerebellum is reciprocally connected with the cerebrum and constitutes cerebro-cerebellar networks responsible for the cognitive and affective function, as well as the traditional sensorimotor network. We aimed to investigate the volumetric alterations in cerebro-cerebellar gray matter (GM) in patients with recent-onset and chronic schizophrenia compared to healthy controls (HCs) and explore their relationships with executive function. **Methods:** Seventy-two patients with recent-onset schizophrenia (50 women), 43 patients with chronic schizophrenia (26 women), and 127 HCs (66 women) underwent T1-weighted magnetic resonance imaging (MRI) scan. The regional volume difference in the cerebellum among the three groups was examined using voxel-based morphometry (VBM) and its associations with cerebral cortical volumes were assessed using FreeSurfer. Executive function was measured using the Wisconsin Card Sorting Test. **Results:** Compared to HCs, both groups of participants with schizophrenia had significantly smaller GM volumes in the left lobule V, left lobule X, left Crus I, left lobule VIIa, right lobule VIIb, and right lobule I-IV; no significant differences were observed between participants with recent-onset schizophrenia and those with chronic schizophrenia. The GM volumes in these cerebellar regions significantly correlated with the GM volumes in the fronto-temporal cortices associated with the higher-order cognitive and affective function. The smaller GM volume in the left Crus I was significantly correlated with poorer executive performance in participants with schizophrenia (total errors: $r = -0.298$, $p = 0.006$); non-perseverative errors: $r = -0.308$, $p = 0.004$; conceptual level responses: $r = 0.315$, $p = 0.006$). **Discussion:** Our findings suggest that patients with schizophrenia show cerebellar GM abnormalities from the early stages of illness. The volumetric changes in the cerebellum were associated with the GM volume in the functionally corresponding cerebral regions. Furthermore, in line with the theory of cognitive dysmetria, cerebellar GM abnormalities were correlated with executive performance, including attention and working memory that requires a fine adjustment of mental processing. We expect that these findings may expand our understanding of the neurobiology of schizophrenia based on the cerebro-cerebellar interconnectivity of the brain.

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Poster

494. Schizophrenia: Translational Studies

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MH078113

Title: Short-term and long-term temporal stability of antisaccade task performance in psychosis

Authors: *L.-Y. HUANG¹, B. S. JACKSON², S. S. KEEDY⁴, C. A. TAMMINGA⁵, M. S. KESHAVAN⁶, G. D. PEARLSON⁷, J. A. SWEENEY⁸, B. A. CLEMENTZ³, J. E. MCDOWELL²;

²Psychology, ³Departments of Psychology and Neurosci., ¹Univ. of Georgia, Athens, GA; ⁴Univ. of Chicago, Chicago, IL; ⁵UT Southwestern Med. Ctr., Dallas, TX; ⁶Wayne State Univ., Detroit, MI; ⁷Yale Univ., Hartford, CT; ⁸Univ. of Texas Southwestern, Dallas, TX

Abstract: Antisaccades require cognitive control, and psychosis cases have consistently shown elevated antisaccade error rates. Multiple large research consortia included antisaccade errors as a marker for schizophrenia and other psychosis cases. However, daily antisaccade practice for a week or more has shown improvements in subjects diagnosed with schizophrenia in both performance and brain activation. These practice effects had been observed in healthy subjects as well. Thus, to continue studying psychosis-related brain alterations using antisaccades as an endophenotype, it is of import to measure the level of state-independence of antisaccade tasks in the short-term as well as long-term. 82 stable psychosis subjects (with diagnoses of schizophrenia, schizoaffective, or bipolar 1 with psychosis) and 36 healthy subjects from the Bipolar-Schizophrenia Network for Intermediate Phenotypes 2 (B-SNIP2) consortium completed two identical antisaccade sessions, 6 months apart. Within each session, 80 trials were administered, divided into 4 sequential quarters of 20 trials with equal difficulty conditions. 3-way ANOVA of percent correct with diagnosis group (healthy vs. psychosis), visit (baseline or 6-month) and quarter (1st to 4th) as factors did not show any interactions, and the only main effect was between groups ($F(1,965) = 55.65, p < 0.0001$), with psychosis individuals committing more errors, replicating numerous previous studies. There were no significant within-session nor between-session changes. The long-term temporal stability shown here in psychosis cases replicated previous findings with a larger sample size. Together, results from this study indicate that for both healthy subject and psychosis cases, antisaccade performance does not change over time without regular and intentional practice, so it is appropriate for longitudinal tracking of cognitive control performance over reasonable testing intervals.

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Poster

494. Schizophrenia: Translational Studies

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 494.07

Topic: H.13. Schizophrenia

Title: Testing the Neurovisceral Integration Model in Schizophrenia and Obsessive-Compulsive Disorder: the connection between parasympathetic activity, subjective stress and cognitive inhibition

Authors: ***M. LAZARIDI**^{1,2}, **P. COVANIS**¹, **G. PANAGIOTAROPOULOU**^{3,1}, **T. KARANTINOS**¹, **E. KOUMANTAROU MALISIOVA**², **I. MOURIKIS**², **C. KLEIN**^{4,5,6}, **N. SMYRNIS**^{6,1};

¹Lab. of Cognitive Neurosci. and Sensorimotor Control, Univ. Mental Health, Neurosciences and Precision Med. Res. Inst. "COSTAS STEFANIS", Athens, Greece; ²1st Psychiatry Dept., Natl. and Kapodistrian Univ. of Athens, Med. School, Eginition Hosp., Athens, Greece; ³Dept. of Psychiatry, Charité- Universitätsmedizin Berlin, Berlin, Germany; ⁴Dept. of Child and Adolescent Psychiatry, Med. Faculty, Univ. of Freiburg, Freiburg, Germany; ⁵Dept. of Child and Adolescent Psychiatry, Med. Faculty, Univ. of Cologne, Cologne, Germany; ⁶2nd Psychiatry Dept., Natl. and Kapodistrian Univ. of Athens, Med. School, Univ. Gen. Hosp. "ATTIKON", Athens, Greece

Abstract: The neurovisceral integration model proposes a brain-heart linkage where prefrontal cortical brain areas related to executive functions are connected to midbrain areas that are responsible for the vagal control of the heart which in turn is related to mechanisms of stress reactivity and regulation in organism. There is evidence documenting autonomic dysregulation, specifically in parasympathetic system, among patients with schizophrenia (SZ) while much less is known in patients with OCD. SZ patients present deficits in the cognitive inhibition of saccadic eye movements related to prefrontal cortical areas. Studies examining the inhibitory control of OCD patients in saccadic eye movements show inconsistent results. The current study was to examine the relation between parasympathetic activity, subjective stress and inhibitory oculomotor control in SZ and OCD patients using indexes of heart rate variability (HRV) measured during the performance of saccade and antisaccade tasks. Besides, participants of all groups completed a Visual Analogue Scale for their momentary stress level three times: upon their arrival in the lab, in the middle of the antisaccade task and right after the completion of the task. Results from 30 healthy controls (HC), 30 SZ and 28 OCD confirmed group difference in antisaccade error rate (ER), indicating a deficit in cognitive inhibition mainly in schizophrenia less so in OCD patients ($F_{(2,86)} = 4.5, p = 0.01$). Electrocardiographic (ECG) recordings were used to determine autonomic activity during saccade and antisaccade task performance. RR intervals were detected in the ECG signal and used to measure the high frequency component of HRV (hfHRV), an indicator of parasympathetic activity. Results showed a group difference ($F_{(2,86)} = 7.8, p = 0.0008$). Planned comparisons confirmed that both schizophrenia and OCD patients had lower hfHRV during antisaccade task compared to controls. We found a strong relationship between the deficit in cognitive inhibition as measured with increased ER and parasympathetic activity only in schizophrenia (Pearson $r = -0.518, p = .003$). We did not find this relation between cognitive inhibition and parasympathetic activity in OCD patients. Nevertheless, we found a relation between subjective stress as reported in VAS in the middle of antisaccade task and antisaccade ER only in OCD group (Pearson $r = 0.383, p = .044$). Our results provide evidence for a physiological connection between cognitive inhibition and parasympathetic activity in schizophrenia. Whereas in OCD, our results highlight the connection between subjective stress and cognitive performance.

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Poster

494. Schizophrenia: Translational Studies

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Topic: H.13. Schizophrenia

Support: MH123603

Title: Amphetamine (AMPH)-enhanced targeted cognitive training (TCT) in antipsychotic (AP)-medicated schizophrenia (SZ) patients: Proof-of-concept demonstration and preliminary results

Authors: *N. R. SWERDLOW^{1,2,3}, J. TALLEDO², D. IWANAGA², J. DIN², J. MINHAS², Y. JOSHI^{2,3}, J. MOLINA^{2,3}, S. BHAKTA², G. A. LIGHT^{2,3};

¹UCSD Sch. of Med., Univ. Of California San Diego Neurosciences Grad. Program, La Jolla, CA; ²Dept. of Psychiatry, UCSD, La Jolla, CA; ³VA San Diego Healthcare Syst., VISN-22 Mental Illness Research, Educ. and Clin. Ctr. (MIRECC), La Jolla, CA

Abstract: Disabling effects of cognitive deficits persist in AP-medicated SZ patients. In a subset of SZ patients, these deficits respond to 30-40 hours (h) of sensory-based TCT, but a significant non-response rate, even after 40 h of training, has prompted a search for ways to predict, accelerate and enhance TCT response. We reported that in AP-medicated SZ patients the pro-attentional drug, AMPH (5 mg po) significantly increased learning of an auditory frequency discrimination task (Sound Sweeps) that is a key therapeutic module of TCT. Here we provide proof-of-concept data for the safety, efficacy and predictability of the therapeutic use of AMPH to enhance TCT response in AP-medicated SZ patients. Patients (n=19) were screened; to-date, 8 have completed 2 test days (T1, T2) to assess AMPH target engagement and identify potential neurocognitive and EEG-based biomarkers, followed by 30 1-h TCT sessions (2-3/week). On T1, all subjects received placebo (PBO) 1-h prior to testing; after T1, they were randomized (double blind) to PBO vs. AMPH (5 mg) administered 1-h prior to T2 and 1-h prior to each of the 30 TCT sessions. Neurocognitive, clinical and functional measures were assessed (pill-free) at baseline (screening) and 1 day after 10, 20 and 30 h of TCT. Preliminary results provide evidence for target engagement (Sound Sweeps learning in AMPH+TCT > PBO+TCT group), safety and tolerability (no adverse events; reduction or no change in specific scales that assess potential adverse AMPH effects, e.g., steady decline in Young Mania Rating Scale in AMPH+TCT group), potential cognitive gains (e.g., gains in attention/vigilance T-scores on MATRICS Consensus Cognitive Battery in AMPH+TCT > PBO+TCT group) and potential clinical gains (e.g., decline in PANSS Positive Symptom scores from baseline to session #30 in AMPH+TCT > PBO+TCT group). Ongoing enrollment and testing will be used to assess AMPH+TCT response in a fully-powered sample, as well as response durability (12 weeks after TCT session #30) and response predictability (based on putative T1 and T2 biomarkers) in AP-medicated SZ patients. Preliminary results to-date suggest that it is feasible to evaluate this novel approach to pharmacologic augmentation of targeted cognitive training in SZ. Supported by MH123603.

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Poster

494. Schizophrenia: Translational Studies

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Program #/Poster #: 494.09

Topic: H.13. Schizophrenia

Support: FRN114887
FRN364273

Title: The association of sleep disturbances with mesolimbic reactivity in the context of a monetary incentive delay task

Authors: ***J. OUELLET**, R. ASSAF, J. WALLACE, S. POTVIN, P. CONROD;
Univ. de Montréal, MONTREAL, QC, Canada

Abstract: Sleep deprivation is known to produce paradoxical effects on the dopaminergic system. On one hand, research shows an increase in presynaptic dopaminergic tones and an internalization of post-synaptic dopaminergic receptors. These mechanisms may shift the equilibrium of the mesolimbic system, engendering certain biases in the appraisal of reward and punishment. In this study, we assessed whether changes in blood-oxygen-level dependent responses could be detected in the context of a monetary incentive task. A sample of 127 teenagers (52% female) were recruited from Montreal high-schools. A past-month sleep score was derived from a three items sleep questionnaire (subjective sleep quality, wake after sleep onset and sleep latency). The monetary incentive delay task was used in the context of a functional magnetic neuroimaging sequence using the nucleus accumbens and ventral tegmental areas as regions of interest. This task uses feedbacks of target hit and miss in reward and punishment conditions, which makes it possible to derive four different contrasts (i.e. positive reinforcement, negative reinforcement, positive punishment and negative punishment). Data was analyzed using a multi-level model with a Bayesian estimator and controlling for sex and time. In the positive punishment condition, results showed a significant between-group activation of the ventral tegmental area (Est=0.977, SD=0.628, p=0.031) and a trending between-group activation of the nucleus accumbens (Est=0.789, SD=0.361, p=0.075). No differences were found in the other three contrasts. While preliminary, these results suggests that the mesolimbic system may be more reactive to positive punishment in individuals having experienced periods of sleep disruptions. This could have implications in the context of mood and anxiety disorders.

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Poster

494. Schizophrenia: Translational Studies

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Program #/Poster #: 494.10

Topic: H.13. Schizophrenia

Title: The neural impacts and treatment strength of CBTp and cognitive remediation for adults living with thought disorders

Authors: Z. PIERCE¹, *J. BLACK²;
¹Social Work, ²Boston Col., Chestnut Hill, MA

Abstract: Background: Individuals living with thought disorders experience positive, negative, and cognitive symptoms. Cognitive behavioral therapy for psychosis (CBTp) and cognitive remediation (CR) are two frontline treatments indicated for helping individuals mediate these symptoms. Neuroscientific research has been expanding the knowledge base around how each intervention impacts psychotic neurophysiology independently, yet there is no study to date that compares both interventions concurrently. Accordingly, our systematic review and meta-analysis compared the neurological impacts of CBTp and CR on positive, negative, and cognitive symptom severity and frequency. We hypothesized that CBTp would have a broader impact of region of interest (ROI) activation and deactivation when compared with CR. Methods: Using three databases, we initially collected $n = 110$ full-text articles. We subjected these articles to several selection criteria outlined in our PRISMA flow chart. This process yielded $n = 13$ full-text articles for consideration in our study. We collected post-test CGI-S scores and simple counts of activated and deactivated ROIs between both interventions. We subjected data for CBTp (Test 1) and CR (Test 2) to two independent samples t-tests. Results: Our review highlighted discrete clusters of ROIs that were activated and deactivated during each intervention. Test 1 revealed a statistically significant relationship between CGI-S scores and ROI activation during CBTp ($t = 3.16, p = .025$) when compared with ROI deactivation ($t = 1.65, p = .158$). Test 2 showed that there was no statistical significance with activation ($t = -.18, p = .869$) and deactivation ($t = 1.63, p = .178$) during CR treatment. Conclusions: This is the first study to compare neurophysiological activity between CBTp and CR with respect to positive, negative, and cognitive symptom remission for individuals living with thought disorders. T-test results suggest that CBTp had the greatest impact on ROI activity during treatment. Findings from this study will inform future comparative and individual intervention research into the neurophysiological impacts of CBTp and CR for treatment of thought disorders, considering they exhibit differences in treatment strength for symptom remission. Findings will also impact the domain of clinical intervention by bolstering the knowledge base of practitioners who use CBTp within serious mental illness (SMI) clinics or in practices with specialty areas germane to psychosis treatment, contributing helpfully toward neuroscientifically informing current mental health clinicians.

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Poster

494. Schizophrenia: Translational Studies

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Topic: H.13. Schizophrenia

Support: DANA
K23MH108711

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MH121790

Title: Engagement of the temporoparietal junction and posterior superior temporal sulcus by visual scanning of naturalistic social scenes in individuals at clinical high risk for developing schizophrenia: a pilot study

Authors: ***J. K. LEE**, N. CHOWDHURY, D. R. RUIZ-BETANCOURT, F. I. PLAZA, A. MURATI, N. M. MACILVANE, J. P. SANCHEZ-PEÑA, G. H. PATEL;
Columbia Univ. / NYSPI, New York, NY

Abstract: Schizophrenia is often marked by a lifetime of social functioning deficits. Although early intervention may mitigate these poor outcomes, it is unclear when these social functioning symptoms develop in relation to the onset of schizophrenia in young adulthood. Recently in schizophrenia participants (SzP) we found insufficient visual scanning of social cues for the inference of sarcasm, associated with reduced activation in the temporoparietal junction and posterior superior temporal sulcus (TPJ-pSTS) areas during free-viewing of a naturalistic stimulus. The present study evaluates the functionality of the TPJ-pSTS associated with naturalistic social scene processing in individuals at clinical high-risk for developing schizophrenia (CHR). We collected eye-tracking data in 41 healthy controls (HC), 26 CHR and 43 SzP while they performed The Awareness of Social Inference Test (TASIT). For each TASIT video frame, eye-positions were scored as a z-transformed distance from the mean eye-position of the HCs. In a subset of 22 HC, 17 CHR and 23 SzP, BOLD-fMRI data were also collected while the participants watched a movie clip of “The Good, the Bad and the Ugly” with no sound. Using HC as the reference, we measured the inter-subject correlation (ISC) as an index of functional integrity of the TPJ subdivisions and compared the ISC scores to the TASIT performance in CHRs. Overall, the three groups significantly differed in sarcasm inference accuracy ($F_{(2,59)}=16.67, p<.001$) with the accuracy in CHR and HC both higher than SzP. In CHRs, the eye-positions during the TASIT sarcasm trials were more deviant in periods where SzP eye-positions were divergent from that of the HC (z-transformed distance >2) compared to intervals where SzP gaze were not divergent ($t_{(35.09)}=3.75, p=.001$). Interestingly, only in CHRs, greater functionality of the posterior TPJ (TPJp) correlated with better sarcasm inference in TASIT ($r_{(15)}=.66, p=.004$). Despite the deviant visual scanning pattern in CHRs mimicking that of SzPs, their ability to infer sarcasm in naturalistic social scenes was not disrupted. Together, the juxtaposition of results from CHR and SzP suggest that increased activity within the TPJp node of the mentalization network may compensate for an otherwise compromised system. The current pilot study demonstrates the use of eye-tracking, a sensitive tool for detecting attentive processes of visual scanning, in refining naturalistic paradigms to investigate the neural mechanisms underlying the emergence of social cognitive deficits in schizophrenia.

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Poster

494. Schizophrenia: Translational Studies

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Program #/Poster #: 494.12

Topic: H.13. Schizophrenia

Title: The effect of Mental disorders on COVID-19 severity

Authors: G. JAVZANDULAM¹, E. BATKHUYAG², E.-U. PERENLEISAMBUU¹, K. ZUUNNAST³, *G. TUMUR-OCHIR⁴;

¹Natl. Ctr. for Mental Hlth. of Mongolia, Ulaanbaatar, Mongolia; ²Brain and Mind Res. Institute, Mongolian Acad. of Sci., Ulaanbaatar, Mongolia; ³Mental Hlth., Mongolian Natl. Univ. of Med. Sci., Ulaanbaatar, Mongolia; ⁴Brain and Mind Res. Inst., Ulaanbaatar, Mongolia

Abstract: Introduction: Coronaviruses disease 2019 (COVID-19) has evolved into a world-wide pandemic with more than 200 million individuals infected and 4 million deaths as of August 2021, resulting in a burden of over a decade in terms of potential years of life lost. Researchers have informed that people with mental disorders are at increased risk of infection with Covid-19. Recent studies have shown that psychiatric patients with Covid-19 have a higher need for ventilators and hospitalization in the intensive care unit, as well as a significant increase in the risk of death. But there is no research has been conducted in our country on people with mental illness who have Covid-19 disease. In our country, there is no large-scale study of psychiatric patients with Covid-19. **Materials and methodology:** The study was conducted using a hospital-based cross-sectional method and data were obtained from psychiatric patients with Covid-19 who were admitted to the Covid-19 treatment wards of the National center for mental health from April 22 to July 1, 2022 using 126 items questionnaire that classified in 4 sections. Moreover, we used electronic medical history database. The survey data were statistically processed using SSPS 21.0. **Results:** The study covered 39 (48.8%) men, 41 (51.3%) women aged 22-71 years, and a total of 80 psychiatric patients with Covid-19, and the average age were 44.5 ± 12.5 . According to the severity of Covid-19 disease, 26.3% (n = 21) had no clinical sign, 43.8% (n = 35) had mild, 28.8% (n = 23) had moderate and 1.3% (n = 1) had more severe clinical signs. In the study of adherence to the treatment regimen, patients with intellectual disabilities (F70) and schizophrenia (F20) did not have the ability to adhere to the treatment regimen with the support of medical staff. Although participants with alcoholism had severe form of Covid-19, 40.0% of patients with schizophrenia, 33.3% of patients with schizophrenic spectrum disorders, 36.4% of people with organic dementia, and 15.4% of people with intellectual disabilities had a mild form of Covid-19. **Conclusions:** Patients with mental retardation (F70) and schizophrenia (F20) were unable to follow the treatment regimen of Covid-19 on their own and people with alcoholism, schizophrenia, schizophrenic spectrum disorders, and organic dementia were likely to have severe forms of Covid-19 disease. **Key words:** depression, mental retardation, schizophrenia, treatment

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Poster

494. Schizophrenia: Translational Studies

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Program #/Poster #: 494.13

Topic: H.04. Executive Functions

Support: R21 MH122886
R01 MH108654

Title: Optimizing Cognitive Control Activity in First Episode Psychosis Patients and Controls

Authors: *A. BARBER¹, J. GALLEGGO², P. DEROSSE², M. BIRNBAUM², T. LENCZ², A. MALHOTRA²;

¹Feinstein Inst. for Med. Res., Manhasset, NY; ²Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Background: Cognitive impairment is a core feature of First Episode Psychosis (FEP). While cognitive control paradigms commonly identify dysfunction in several brain circuits, they fail to identify known sources of trial-to-trial variability. **Trial Specific Costs (TSCs)** include Block Restart, Previous Trial Interference, Response Switching, and Stimulus Items behavioral costs, each of which elicit robust effects. The current study examined whether modeling TSCs improves detection of brain dysfunction in FEP.

Methods: 51 Patients with FEP (59% male, age=23.8(3.9) years) and 31 Controls (46% male, age=28.0(5.0) years) were scanned on a Siemens 3T Prisma scanner with 64-channel head coil. This included a T1 scan (TR=2400ms, TE=2.22ms) and four SMS-EPI scans (multiband factor=8, TR=720ms, TE=33ms), in which the Multi-Source Interference Task (MSIT) was performed. Subject models included congruent, interference, and error regressors, in addition to TSC and nuisance regressors. Group models examined congruent, interference, and TSC activity, while controlling for group, sex, age, and scan motion. Model fit (Mean Squared Error/Degrees of Freedom) was compared between subject **TSC and standard (no TSC) models**. Effect sizes (T-values thresholded at voxel and cluster $p < 0.001$ for congruent and interference contrasts) were compared between group TSC and standard models. To determine whether TSC models could detect cognitive control dysfunction in FEP, Connectome-Based Predictive Modeling (CPM) with Leave One Out Cross Validation detected regional task activity that distinguished patients from controls. Follow up CPM analyses examined whether task activity was related to individual differences in cognitive efficiency (mean RT*error rate).

Results: Paired T-tests comparing the model fit for the subject models found significantly better TSC than standard model fit across the whole brain and for all networks except the visual network. Group effect sizes also tended to be greater in the TSC model, especially for the congruent condition in both FEP patients and controls. CPM analyses found that TSC contrast activity strongly contributed to FEP dysfunction, with high cognitive demand TSCs showing some of the greatest group differences. TSC activity also contributed to individual differences in cognitive efficiency. Different sets of TSC activity related to cognitive efficiency in patients and controls.

Conclusions: Models of cognitive control are improved by including TSC regressors for known

behavioral costs reflecting within condition trial variability. Cognitive control dysfunction in FEP can be better characterized by examining TSCs.

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Poster

494. Schizophrenia: Translational Studies

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Program #/Poster #: 494.14

Topic: H.04. Executive Functions

Support: Gruber Science Fellowship
R01 MH121095

Title: Altered brain dynamics in bipolar disorder and schizophrenia during rest

Authors: *J. YE¹, H. SUN², S. GAO³, D. SCHEINOST¹;
¹Interdepartmental Neurosci. Program, ²Biomed. Engin., ³Engin. and Applied Sci., Yale Univ., New Haven, CT

Abstract: Nonlinear manifold learning can be used to create a low-dimensional space shared by multiple tasks from which representative brain states can be found. Putatively, neuropsychiatric disorders—e.g., bipolar disorder (BD) and schizophrenia—are characterized by altered brain dynamics. Here, we explored how brain dynamics in this space vary in BD and schizophrenia during rest. Timeseries fMRI data from the Human Connectome Project (N=390; 6 tasks) were embedded into a low-dimensional space using 2-step Diffusion Maps. Four reoccurring brain states were identified with K-means clustering and characterized as high-cognition, low-cognition, cue, and fixation based on what task conditions people were performing during timepoints associated with each cluster. The centroid of each cluster was identified as that state's representative timeframe. We then extracted rest timeseries data using the Shen-268 atlas from healthy controls (N=104; 49 females; mean age: 30.3; age SD: 8.0), individuals with BD (N=35; 15 females; mean age: 34.1; age SD: 9.0) or schizophrenia (N=38; 9 females; mean age: 35.4; age SD: 9.0) in the UCLA Consortium for Neuropsychiatric Phenomics dataset. All four representative timeframes and rest volumes were standardized by dividing each time frame with its standard deviation. The four representative timeframes were next regressed from each rest volume using nonnegative least squares. This novel approach provides a continuous measure of state engagement across time and allows for the possibility that participants might recruit multiple states simultaneously at each timepoint during rest. Two summary measures, state contributions (each state's coefficients summed across volumes divided by the sum of all coefficients) and state contribution variability (the standard deviation of each state's coefficients), were computed for each individual and compared between groups using MANOVA. We found significantly different group differences in state contribution variability

($p=0.009$) but not in state contribution ($p=0.10$) for all four states across BD, schizophrenia and healthy control. These findings suggest altered brain dynamics in BD and schizophrenia during rest. Our results additionally seem to indicate that specific brain dynamic measures might be more sensitive to aberrant brain dynamics in certain scanning conditions. While our limited insight into people's thought content during rest might have made detecting group differences through state engagement challenging, another measure providing information on state transition sheds light on how brain dynamics might be altered in BD and schizophrenia during rest.

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Poster

495. Advances in Circuit Tracing

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Program #/Poster #: 495.01

Topic: I.03. Anatomical Methods

Title: A scalable approach for mapping microconnectivity in transcriptomically defined neuron types

Authors: *M. V. MOYA^{1,2}, S. SUBRAMANIAN¹, P. S. DAVE^{1,3}, T. D. WEBER¹, J. MERTZ^{1,4,2,5}, M. N. ECONOMO^{1,4,2,5};

¹Dept. of Biomed. Engin., ²Ctr. for Systems Neurosci., ³Grad. Program in Bioinformatics, ⁴Ctr. for Neurophotonics, ⁵Photonics Ctr., Boston Univ., Boston, MA

Abstract: Identifying the cell types that make up each region of the brain and the patterns of synaptic connections through which they are linked is key to understanding how neural circuits give rise to all perception, cognition, and behavior. Rapid improvements in optical, molecular, and computational technologies are enabling large-scale projects aiming to comprehensively map the cell types that comprise the mammalian brain. Nevertheless, defining the microconnectivity of the thousands of cell types in the brain remains challenging due to a lack of scalable methods. Here we demonstrate a new technology for addressing this methodological gap. Using a novel combination of high-sensitivity fluorescence voltage imaging and single-neuron optogenetic photostimulation, we map synaptic connectivity between brain regions with throughput two to three orders of magnitude higher than existing techniques. Importantly, leveraging an all-optical approach to mapping connectivity allows us integrate synaptic connectivity measurements with emerging techniques for highly multiplexed fluorescence *in situ* hybridization. In this way, we can identify the molecular identities of large neuronal populations and their connectivity. Here we demonstrate a proof of concept of this approach in the motor cortex, a region where knowledge of gene expression patterns has far outpaced our ability to identify connectivity motifs. Revealing the precise molecular identity of cells that receive long-range input from areas that drive motor cortical activity will provide new insights into the circuit mechanisms supporting voluntary movements.

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Poster

495. Advances in Circuit Tracing

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Program #/Poster #: 495.02

Topic: I.03. Anatomical Methods

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Brain Research Foundation BRF-SIA-2014-03
IARPA MICrONS D16PC0008

Title: Axonal Barseq, Novel Technique For High-Throughput Mapping Single-Cell Projections In Situ

Authors: ***L. YUAN**¹, **X. CHEN**², **H. ZHAN**¹, **H. L. GILBERT**¹, **A. M. ZADOR**¹;
¹Cold Spring Harbor Lab., Cold Spring Harbor, NY; ²Allen Inst., Seattle, WA

Abstract: Neurons are heterogenous and assembled into complicated circuits in the brain. However, neural projections are thin and intertwined, which presents a technical challenge for mapping long-range projection with single-cell resolution for large numbers of neurons. Here, we developed axonal BARseq, a high-throughput technique for mapping neural projections with single-cell resolution. As a proof of principle, we mapped the projections of > 8000 neurons in 2 mm area of primary auditory cortex from one single mouse. We identified major cell types using projection targets and axonal trajectory. Furthermore, the large cell number enable us to systematically quantify the projection diversity of intratelencephalic (IT) neurons. We found it is not uncommon for a cell to have different laminar terminations in different cortical areas and it is area-dependent. Also, simple grouping IT cells into three groups using soma cortical depth, reveal different laminar terminations, projection targets and axonal morphology across groups. Thus, axonal BARseq is a powerful technique for studying the heterogeneity of neural projections.

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Poster

495. Advances in Circuit Tracing

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 495.03

Topic: I.03. Anatomical Methods

Title: A machine learning, 3D atlas pipeline for classification of synaptic density of collateral axon projections to spinal cord after stroke

Authors: ***M. KENWOOD**¹, K. POINSATTE², A. AJAY², W. XU³, X. KONG², D. BETZ¹, E. J. PLAUTZ², D. M. RAMIREZ², M. GOLDBERG¹;

¹Neurol., UT Hlth. Sci. Ctr. San Antonio, San Antonio, TX; ²Dept. of Neurol., ³Dept. of Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Stroke is the leading cause of disability in the United States, leaving many of patients with significant motor impairment. The sprouting of the contralesional corticospinal tract (CST), which contains roughly 98% of the CST projecting neurons of the contralesional hemisphere, into the injured hemicord is associated with improved motor recovery after unilateral primary motor cortex stroke. Numerous studies have quantified either synaptic or axonal density in only the injured hemicord using histological serial sectioning with regional distributions determined manually, but these studies did not assess the distribution of synapses and axons relative to the contralesional hemicord. To accomplish this, we induced a photothrombotic motor cortex stroke or sham surgery in 8-11 week old male C57/B6 mice. These mice also received a contralesional motor cortex injection of an anterograde adeno-associated virus expressing both distinct membrane-targeted tdTomato and synaptically-targeted eGFP and were sacrificed at multiple post-stroke time points. Cervical spinal cords were subjected volumetric imaging via serial two-photon tomography (TissueCyte 1000) to characterize synaptic and axonal density of sprouting CST collaterals in the whole cervical spinal cord in both stroke and sham mice. Unbiased, global quantification of axonal and synaptic density across the entire cervical cord was accomplished by the development of a custom automated image analysis pipeline, incorporating a novel 3D spinal cord reference volume, published spinal cord annotations comprising 47 distinct anatomical regions (SpinalJ), and a machine learning based pixel classification workflow. In sham mice, synapse density is highest in Laminae 4,5,6,7, and 9 in the uninjured hemicord and highest in Laminae 1,10,7,4, and 9 in the injured hemicord. Stroke induced significantly increased synapse density from contralesionally sprouting CST neurons in Laminae 4 and 5 at 4 weeks post stroke compared to sham. Thus, laminae that have lost input from the degenerating CST are the same laminae reinnervated by new sprouting contralesional CST collaterals. Understanding the pattern of typical laminae innervation by the CST is essential to understand patterns of contralesional CST sprouting mediated reinnervation and subsequent mechanisms that govern sprouting and pathfinding after motor cortex stroke.

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Poster

495. Advances in Circuit Tracing

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Topic: I.03. Anatomical Methods

Support: Gatsby charitable foundation GAT3755
Wellcome 2196

Title: Anterograde transneuronal transfer of rabies via novel pseudotyping with HSV-1 glycoproteins gE, gI and US9

Authors: *S. L. THOMPSON¹, A. J. MURRAY²;
²Sainsbury Wellcome Ctr., ¹Univ. Col. London, London, United Kingdom

Abstract: Transsynaptic tracing methods have proved incredibly useful in furthering our understanding of brain-wide connectivity. In particular, rabies virus (RABV) has proven to be a highly useful retrograde tracer due to the ability to restrict transsynaptic transfer to one synapse. However, an equivalent monosynaptic anterograde tracer is not yet available. Here we attempt to produce an anterograde transsynaptic RABV by pseudotyping the virus with herpes simplex virus-1 (HSV-1) glycoproteins gE, gI and US9, which are known to facilitate anterograde spread. We first validated whether pseudotyping of RABV with HSV-1 glycoproteins would produce functional virions in vitro. Next, we developed a range of adeno-associated virus (AAV) complementation strategies to facilitate in vivo testing of multiple neural circuits in mice. Firstly, in wildtype mice RABV positive cells in the lateral vestibular nucleus were complemented with three AAVs, each expressing an individual glycoprotein, to test for anterograde spread to motor neurons within the spinal cord. Secondly, RABV positive Purkinje cells in TU/L7-Cre mice were complemented with two high expressing AAVs, one containing the receptor TVA and gE, another containing gI and US9, to test for anterograde transsynaptic spread to cells within the deep cerebellar nuclei (DCN). In both cases a small number of RABV labelled cells were found in each downstream area with no corresponding retrogradely labelled cells visible. This outcome demonstrates the potential viability of this novel pseudotyping approach though presently the efficiency remains low and further optimisation is ongoing.

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Poster

495. Advances in Circuit Tracing

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Program #/Poster #: 495.05

Topic: I.03. Anatomical Methods

Support: MH116989

Title: ATLAS - A method for anterograde transsynaptic tracing from genetically determined neurons

Authors: *H. HUANG, J. RIVERA, W. WENG, H. SOHN, D. B. ARNOLD;
Dept. of Biol. and Neurosci. Grad. Program, USC, Los Angeles, CA

Abstract: The study of neural circuits has been revolutionized by the advent of monosynaptic tracing from genetically determined cells in the retrograde direction using rabies virus. However, mapping circuits in the anterograde direction, which is equally important, has proven to be challenging. Although anterograde tracing methods have been developed, each tool has weaknesses, including the lack of ability to trace from genetically determined neurons, retrograde labeling, multi-synaptic labeling, and toxicity. Here we introduce ATLAS (Anterograde Transsynaptic Labeling using Antibody-like probes), a new method for anterograde tracing. ATLAS uses a rationally designed anterograde tracer that efficiently and specifically maps connections from genetically determined starter cells, monosynaptically and without retrograde labeling or toxicity. It is based on the AMPA FingR, a recombinant antibody-like protein that binds specifically to the N-terminal domain of the AMPA receptor GluA1. The AMPA FingR is released from presynaptic termini, diffuses across the synapse, and then binds to a subset of AMPA receptors undergoing endocytosis. ATLAS works by a well-defined mechanism and is composed of independent components that can be optimized or exchanged to give specific functionality. In these ways, it offers advantages over traditional viral tracers.

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Poster

495. Advances in Circuit Tracing

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Topic: I.03. Anatomical Methods

Support: NIH Grant R34NS116713
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Title: Axonal barcode analysis of pyramidal tract projections from mouse forelimb M1 and M2

Authors: *J. M. BARRETT¹, F. S. HAUSMANN¹, M. E. MARTIN¹, H. ZHAN², G. M. G. SHEPHERD¹;

¹Dept. of Neurosci., Northwestern Univ., Chicago, IL; ²Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Forelimb-related areas of the motor cortex communicate directly to downstream areas in the brainstem and spinal cord via axons that project to and through the pyramidal tract. To better understand the diversity of the brainstem branching patterns of these pyramidal tract projections, we used MAPseq, a molecular barcode technique for population-scale sampling with single-axon resolution. In experiments using mice of both sexes, we first confirmed prior results demonstrating the basic efficacy of axonal barcode identification of M1 pyramidal-tract type (PT) axons, including corticobulbar (CBULB) and corticospinal (CSPI) subclasses. We then used multiplexed MAPseq to analyze projections from M1 and M2 (caudal and rostral forelimb areas). The four basic axon subclasses comprising these projections (M1-CSPI, M1-CBULB, M2-CSPI, M2-CBULB) showed a complex mix of differences and similarities in their brainstem projection profiles. This included relatively abundant branching by all classes in the dorsal midbrain, by M2 subclasses in the pons, and by CSPI subclasses in the dorsal medulla. Cluster analysis showed graded distributions of the basic subclasses within the PT class. Clusters were of diversely mixed subclass composition, and showed distinct rostro-caudal and/or dorso-medial projection biases. Exemplifying these patterns was a subcluster likely enriched in cortico-cuneate branches. Overall, the results indicate high yet systematic PT axon diversity at the level of brainstem branching patterns; projections of M1 and M2 appear qualitatively similar, yet with quantitative differences in subclasses and clusters.

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Poster

495. Advances in Circuit Tracing

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Program #/Poster #: 495.07

Title: WITHDRAWN

Poster

495. Advances in Circuit Tracing

Location: SDCC Halls B-H

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Topic: I.03. Anatomical Methods

Support: NIMH R01MH110822
BRAIN Initiative R34NS122050

Title: Single neurons in macaque basolateral amygdala exhibit distinct patterns of collateral projections to frontal cortex and subcortical structures

Authors: *Z. R. ZEISLER, J. M. FREDERICKS, W. G. JANSSEN, F. M. STOLL, R. L. CLEM, P. H. RUDEBECK;
Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The macaque basolateral amygdala sends projections widely across frontal cortex. Yet, it remains unknown whether single neurons target multiple cortical areas: an understudied phenomenon known as collateralization. Collaterals have been speculated to play a critical role in regulating brain-wide states, but to date, obtaining enough data to support that claim has been nearly impossible. Whether amygdala neurons collateralize has been challenging to discern to date, as gold-standard single-neuron tracing approaches are limited in throughput. Intersectional viral approaches, too, can only assess projections to a small number of areas. Here, we optimized MAPseq, an RNA-barcoding technique originally developed in mice, to assess the connection patterns of individual amygdala neurons to frontal cortex and subcortical structures in rhesus macaques.

Of over 3000 single amygdala neurons analyzed from four hemispheres, approximately one-third of neurons did not project outside of amygdala. One-third projected to only one target area in frontal cortex or subcortical structures, and the remaining third sent projections to more than one area. The projection motifs identified by MAPseq closely recapitulate anatomical features identified by traditional tract-tracing techniques. Notably, those neurons that projected to only one area preferentially targeted non-frontal areas, including the hippocampus, entorhinal cortex, and striatum - particularly nucleus accumbens. Neurons that sent collaterals to multiple targets tended to connect to multiple areas in previously-defined anatomical or functional networks. We are currently validating these novel connectional motifs with standard tract-tracing approaches. In summary, by optimizing MAPseq in macaques we were able to reveal the projection patterns of thousands of amygdala neurons at single-neuron resolution. Our analyses reveal distinct patterns of collateralization in a significant proportion of these neurons, connection motifs that may play key roles in regulating brain-wide states. With this new approach optimized in macaques, future studies can seek to assess other areas in healthy animals, or to interrogate the nature of anatomical changes over the course of development or neurodegeneration.

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Poster

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Topic: I.03. Anatomical Methods

Support: “MOST 110-2326-B-007-001-MY3” (Taiwan) to CHC

Title: Anatomical analysis of collateral orbitofrontal cortex projections to the amygdala and the nucleus accumbens

Authors: *C.-W. SHIH, C.-H. CHANG;

Institutie of Systems Neuroscience, Natl. Tsing Hua Univ., Hsinchu, Taiwan

Abstract: The orbitofrontal cortex (OFC) is an important brain region that mediates the reward learning, decision making, reversal learning, self-control, and emotional regulation. It is composed of distinct subregions, including the lateral OFC (LO), medial OFC (MO), and ventral OFC (VO), which have different connections and functions. It has been well documented that the OFC projects to the basolateral amygdala (BLA) and to the nucleus accumbens (NAc), and these two downstream targets share some similar behavioral functions. In this study, we analyzed the distribution and collateralization of anatomical projections from different subregions in the OFC, as well as the prelimbic (PL) and the infralimbic (IL) divisions of the medial prefrontal cortex (mPFC), to the BLA and/or the NAc. Double neuronal retrograde tracing approach was used, in which Cholera toxin subunit B conjugated with the Alexa Fluor 488 (CTB-AF488) or Alexa Fluor 594 (CTB-AF594) were unilaterally injected into the BLA and the NAc, respectively, in male Long-Evans rats (n = 3). Among the sampled regions, there were more neurons labeled at the ipsilateral side compared to the contralateral side. Along the anteroposterior axis, more NAc- and/or BLA-projecting neurons were labeled toward the posterior end. The averaged percentage of the collateral projecting neurons remained a similar ratio across different subregions at about 10-20%. Further statistical analyses will be conducted with increased sample size.

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Poster

495. Advances in Circuit Tracing

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Topic: I.03. Anatomical Methods

Support: Brain initiative 1R01NS113104-01

Title: Fore- and mid- brain structural connectivity of male and female prairie voles

Authors: *K. R. GOSSMAN¹, E. ANDREWS¹, B. DYKSTRA³, K. TA³, A. S. SMITH²;
²Dept. of Pharmacol. & Toxicology, ¹Univ. of Kansas, Lawrence, KS; ³Univ. of Kansas, Lawrence, KS

Abstract: The socially monogamous prairie vole (*Microtus ochrogaster*) has been used for three decades to study the neurobiology of pair bonding or selective bonds between breeding pairs. This has led to well-defined behavioral characterization of selective affiliation toward a partner and stranger selective aggression and the influence by neurochemicals in certain brain regions. However, unlike other rodent models, limited knowledge is available regarding the overall neurocircuitry and interregional connections of the vole brain. Neuroanatomical tracing methods remain fundamental for elucidating the complexity of brain circuits. Here, we used both male and female prairie voles and cholera toxin subunit-B retrograde tracers (conjugated to one of four

Alex Fluors: 488nm, 555nm, 594nm, and 647nm) to map out the ipsilateral and contralateral fore- and mid- brain physical connections of the regions associated with the Social Decision-Making Network (SDMN) with the addition of the paraventricular nucleus of the hypothalamus (PVN) and the anterior cingulate cortex (ACC). These results will significantly expand our knowledge of the neural circuitry of the vole fore- and mid- brain and act as a guide for interregional connections for future research.

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Poster

495. Advances in Circuit Tracing

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Support: Mission Connect, A program of TIRR Foundation
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Title: Morphological Characterization of Sparsely-Labeled V2a Interneurons in the Murine Spinal Cord

Authors: ***F. JALUFKA**, J. DULIN, D. MCCREEDY;
Texas A&M Univ., Texas A&M Univ., College Station, TX

Abstract: Quantification and classification of neuronal morphology is an integral component of neural circuit mapping throughout the central nervous system. Current online neural atlases consist mostly of brain circuitries and morphologies and similar atlases of spinal cord circuitry remain underdeveloped. The digital reconstruction of spinal cord neuron morphologies, including axons and dendritic processes, remains a difficult and laborious task, in large part due to the use of tissue sectioning, which makes tracing long distance axons and complex dendritic arbors difficult. In order to circumvent the issues associated with tissue sections, we have utilized optical tissue clearing techniques and lightsheet imaging to gather 3D images of the whole murine spinal cord. We have started mapping the neural circuitry of V2a interneurons, a pre-motor population in the spinal cord that regulates left-right rhythmicity in hindlimb locomotion, diaphragm contraction, and forelimb reaching, and has been implicated in motor recovery following spinal cord injury. However, the relatively high density of V2a interneurons in the spinal cord has hindered morphological characterization of individual neurons. In this study, we tested a two-component labeling method to sparsely label V2a interneurons. Using a Chx10-Cre mouse line, a Cre-dependent “activator” plasmid expressing FlpO delivered in a PHP.eB adeno-associated virus (AAV) construct, and a Flp-dependent “reporter” plasmid expressing a membrane-bound tdTomato fluorescent tag delivered in a separate PHP.eB AAV construct, we observed robust and sparse labeling of V2a interneurons throughout cleared whole spinal cords

using lightsheet microscopy. V2a interneuron morphologies were traced in the 3D lightsheet images using Imaris, as well as the Single Neurite Tracer plugin for FIJI. Traced V2a morphologies will be quantified and categorized for the creation of an online 3D spinal cord atlas and morphology database.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Program #/Poster #: 496.01

Topic: I.04. Physiological Methods

Support: NSF Grant 31871087
NSF Grant 31925017
NIH Grant 1U01NS120824

Title: Improved norepinephrine sensors shine lights on *in vivo* noradrenergic activities

Authors: *J. FENG¹, H. DONG¹, J. E. LISCHINSKY², J. ZHOU³, H. XIE⁴, G. CUI³, D. LIN², Y. LI¹;

¹Peking Univ., Sch. of Life Sci., Beijing, China; ²Neurosci. Inst., New York Univ. Grossman Sch. of Med., New York, NY; ³Neurobio. Lab., NIH/NIEHS, Research Triangle Park, NC;

⁴Dept. of Automation, Tsinghua Univ., Beijing, China

Abstract: Norepinephrine (NE) is a key biogenic monoamine neurotransmitter involved in a wide range of physiological processes. However, its precise dynamics and regulation remain poorly characterized. With the structural similarity and promiscuous patterns inside our brain for norepinephrine and dopamine, it is indispensable to tease apart these two monoamines during behaviors *in vivo*. Previously, we have developed prototype GPCR activation-based NE (GRAB_{NE}) sensors with either high sensitivity or high selectivity to NE. Here, we further optimized and characterized improved GRAB_{NE} sensors (NE2m and NE2h) with higher responses. They exhibited both higher sensitivity and selectivity to NE compared with prototypes. Viral-expression of improved GRAB_{NE} sensors was able to detect optogenetically and behaviorally triggered NE release in the locus coeruleus (LC) and hypothalamus of freely moving mice. Specifically, dual color fiber photometry recording of a transgenic mouse expressed both NE2m and jRGECO1a reveals NE and calcium dynamics during sleep and wake. Moreover, cortex-wide, real-time, dual-color imaging of NE2m and jRGECO1a reveals spatiotemporally dynamics of noradrenergic and calcium activity in awake and anesthetized mice. In sum, the improved GRAB_{NE} family provides robust tools for spatiotemporally monitoring *in vivo* NE dynamics with high sensitivity and specificity in both physiological and pathological processes.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Support: NIH Grant (1U01NS120824)
NIH BRAIN Initiative (NS103558)
Beijing Municipal Science & Technology Commission (Z181100001318002)

Title: Next-generation GRAB sensors for deciphering serotonin dynamics in vivo

Authors: *F. DENG¹, J. WAN¹, G. LI¹, H. DONG¹, X. XIA¹, Y. WANG¹, X. LI¹, H. XIE³, C. ZHUANG³, L. LIU¹, Y. YAN², Y. LI²;

¹Peking Univ., ²Sch. of Life Sci., Peking Univ., Beijing/Haidian/100871, China; ³Dept. of Automation, Tsinghua Univ., Beijing/Haidian/100084, China

Abstract: Serotonin (5-HT), a phylogenetically conserved monoamine neurotransmitter, plays important roles in a plethora of important physiological processes and is associated with many neurological diseases, such as addiction, anxiety and depression. To better understand the serotonergic system, it is critical to monitor 5-HT dynamics with high sensitivity. Some genetically encoded GFP-based sensors have sprouted out recently, while sensitivities of these sensors are limited by either modest fluorescence changes or low affinity. Moreover, these green 5-HT sensors cannot be combined with other widely used green indicators or blue-light excited optogenetic tools due to spectral overlap. Based on the GPCR Activation Based (GRAB) strategy, we optimized green fluorescent 5-HT sensors and developed novel red fluorescent 5-HT sensors (GRAB_{5HT}). All of them show good membrane trafficking, sub-second kinetics, high spatial resolution with high molecular specificity for 5-HT, and exhibit robust fluorescence increase to physiological 5-HT concentrations. Besides, 5-HT sensors detected optogenetically evoked 5-HT release in mouse whole dorsal cortex by mesoscopic imaging as well as in the BF in freely behaving animals. Dual-color recording of 5-HT sensors with spectral compatible calcium indicators reveals the serotonergic signaling and neuronal activity during sleep/wake cycles and seizure conditions. Moreover, combining the red 5-HT sensor with a green eCB sensor, we uncovered the dynamics of 5-HT and eCB waves during epilepsy in a high spatiotemporal resolution. In sum, these next generation of 5-HT sensors will facilitate our understanding of the serotonergic system in health and diseases.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Topic: I.04. Physiological Methods

Support: NIH Grant 1U01NS120824

Title: High-performance GRAB sensors for spatiotemporally imaging dopaminergic activity in vivo

Authors: *Y. ZHUO, B. LUO, H. DONG, X. YI, J. WAN, R. CAI, T. QIAN, Y. LI; Peking Univ., Beijing, China

Abstract: Dopamine (DA) is a crucial monoamine neurotransmitter involved in many physiological and pathological processes, and the ability to directly monitor DA dynamics is essential for understanding its physiological functions. Here, by tapping into different dopamine receptor subtypes from various species, we developed new generation green fluorescent GRAB_{DA} sensors (DA3m and DA3h) with improved signal-to-noise (SNR), sensitivity, selectivity as well as diverse pharmacology properties. Compared with previously published DA2m, the improved GRAB_{DA} sensors exhibited superior kinetics, robust SNR and photostability in culture cells, in mouse acute brain slices and in vivo. The improved DA3m could monitor cortical DA changes with good selectivity in isoflurane-induced anesthesia. The DA3h reports spatially resolved DA dynamics in the motor cortex of mice during forced running. In parallel, we further optimized red dopamine sensors (rGRAB_{DA}) with different affinities, distinct pharmacology profiles, higher brightness and larger fluorescent increase. Multiplexed imaging reveals foot shock-elicited DA signaling followed by endocannabinoids (eCB) transient in the basolateral amygdala, and DA-cAMP association during mating behavior in the nucleus accumbens. Thus, these new DA sensors provide an extended toolbox for multifaceted *in vivo* DA imaging under a variety of complex behavior contexts, and therefore critical for understanding diverse aspects of dopamine biology.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Support: NSF Grant 31871087
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Title: A toolkit of highly selective and sensitive genetically encoded neuropeptide sensors

Authors: *H. WANG¹, T. QIAN¹, Y. YAN², S. FU², Y. ZHAO¹, Y. ZHUO¹, C. WU¹, T. OSAKADA³, P. CHEN⁴, H. REN², B. LUO¹, H. DONG¹, S. XIE¹, L. GENG¹, L. MEI³, G. LI¹, L. WU¹, Y. WEI¹, Y. JIANG³, W. QIAN², L. CHEN², C. TANG², D. LIN³, J. ZHOU⁴, Y. LI¹; ¹Peking Univ. Sch. of Life Sci., Beijing, China; ²Peking Univ., Beijing, China; ³New York Univ. Sch. of Med., New York, NY; ⁴Univ. of Sci. and Technol. of China, Hefei, China

Abstract: Neuropeptides are key signaling molecules in the endocrine and nervous systems that regulate many critical physiological processes, including energy balance, sleep and circadian rhythms, stress, and social behaviors. Understanding the functions of neuropeptides *in vivo* requires the ability to monitor their dynamics with high specificity, sensitivity, and spatiotemporal resolution; however, this has been hindered by the lack of direct, sensitive and non-invasive tools. Here, we developed a series of GRAB (G protein-coupled receptor activation-based) sensors for detecting somatostatin (SST), cholecystokinin (CCK), corticotropin-releasing factor (CRF), orexin (OX), substance P (SP), neuropeptide Y (NPY), neurotensin (NTS), and vasoactive intestinal peptide (VIP). These fluorescent sensors utilize the corresponding GPCRs as the neuropeptide-sensing module with the insertion of a circular-permuted GFP as the optical reporter. This design detects the binding of specific neuropeptides at nanomolar concentration with a robust increase in fluorescence. We used these GRAB neuropeptide sensors to measure the spatiotemporal dynamics of endogenous neuropeptides release, including electrical stimulation-evoked release in acute brain slices, stressful experiences induced release and sleep-wake cycles regulation *in vivo* using 2-photon imaging and fiber photometry in mice. Furthermore, we also developed second-generation green fluorescent neuropeptides sensors with larger response and brightness compared to previous versions after linker and cpEGFP optimization. Together, these new sensors establish a robust toolkit for studying the release, function, and regulation of neuropeptides under both physiological and pathophysiological conditions.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Title: A genetically encoded sensor for detecting histamine dynamics

Authors: *H. DONG, M. LI, T. QIAN, Y. YAN, Y. LIN, C. LIU, Y. LI;
Sch. of Life Sci., Peking Univ., Beijing, China

Abstract: Histamine (HA) is a biogenic amine that serves as an essential signaling molecule in the immune, digestive, and nervous systems. It is involved in many critical physiological and pathological processes, including allergy, itch, and sleep-wake regulation. A powerful tool to directly measure extracellular HA in real-time will further enhance our understanding of its functions in complex circuits or conditions. Here, we developed a family of genetically-encoded G-protein-coupled receptor-activation-based HA (GRAB_{HA}) sensors, including HA1h (with high affinity) and HA1m (with medium affinity). GRAB_{HA} sensors show robust fluorescence response, tens or hundreds of nanomolar affinity, good photostability, sub-second kinetics, and high specificity in response to extracellular HA. Using GRAB_{HA} sensors, we observed electrical stimulation-evoked HA release with high spatiotemporal resolution in acute brain slices. Moreover, we recorded HA dynamics using simultaneous fiber photometry in the preoptic area and the medial prefrontal cortex during sleep-wake cycles in freely moving mice. Notably, we found HA release kinetics were distinct during brain-state transition across multiple brain regions. Thus, GRAB_{HA} sensors are specific, sensitive and robust tools for monitoring extracellular HA dynamics in vitro and in vivo, potentially providing more new insights into the roles of the HA in different systems.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 496.06

Topic: I.04. Physiological Methods

Support: NSF Grant 31871087
NSF Grant 31925017
NIH Grant 1U01NS120824

Title: Compartmental neuropeptide release measured using a new oxytocin sensor

Authors: ***T. QIAN**¹, **H. WANG**¹, **P. WANG**², **L. GENG**¹, **L. MEI**³, **T. OSAKADA**³, **L. WANG**¹, **Y. TANG**⁴, **A. KANIA**⁵, **V. GRINEVICH**⁵, **R. STOOP**⁴, **D. LIN**³, **M. LUO**⁶, **Y. LI**¹; ¹Peking Univ., Beijing, China; ²Med. Ctr. for Human Reproduction, Beijing Chao-Yang Hospital, Capital Med. Univ., Beijing, China; ³New York Univ., New York, NY; ⁴Lausanne Univ., Lausanne, Switzerland; ⁵Univ. of Heidelberg, Mannheim, Germany; ⁶Natl. Inst. of Biol. Sci., Beijing, China

Abstract: As a peptide hormone and neuromodulator, oxytocin (OT) plays a critical role in a variety of physiological and pathophysiological processes in both the central nervous system and the periphery. However, the processes that regulate spatial OT release in the brain remain enigmatic. Here, we developed a genetically encoded GPCR activation-based (GRAB) OT sensor called GRAB_{OT1.0}. Using this sensor, we directly visualized stimulation-induced OT release from specific compartments of OT neurons in acute brain slices and discovered that N-type calcium channels predominantly mediate axonal OT release, while L-type calcium channels mediate somatodendritic OT release. In addition, we found that components in the fusion machinery of OT release differ between axon terminals versus somata and dendrites. Finally, we demonstrated the sensor responses to the activation of OT neurons in various brain regions *in vivo* and revealed region-specific OT release during male courtship behavior. Taken together, these results provide key insights regarding the role of compartmental OT release in the control of physiological and behavioral functions.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Program #/Poster #: 496.07

Topic: I.04. Physiological Methods

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The Feng Foundation of Biomedical Research
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Title: Spying on purinergic modulation by constructing GRAB fluorescent sensors

Authors: *Z. WU^{1,2}, H. WANG¹, K. HE³, W. PENG⁴, M. JING⁶, H. LI⁴, Y. CUI⁷, S. PAN⁸, T. LI⁹, Z. YUAN⁷, B. LI⁸, J. DU⁴, M. LUO^{7,6}, M. XU⁵, Y. LI^{1,8,2,6};
¹Sch. of Life Sciences, Peking Univ., Beijing, China; ²PKU-IDG/McGovern Inst. for Brain Res., Beijing, China; ³USC, Los Angeles, CA; ⁴Inst. of Neuroscience, State Key Lab. of Neuroscience, Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sci., Shanghai, China; ⁵Inst. of Neuroscience, State Key Lab. of Neuroscience, Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sci., Shanghai City, China; ⁶Chinese Inst. for Brain Res., Beijing, China; ⁷Natl. Inst. of Biol. Sci., Beijing, China; ⁸Peking-Tsinghua Ctr. for Life Sciences, Acad. for Advanced Interdisciplinary Studies, Peking Univ., Beijing, China; ⁹Inst. of Neuroscience, State Key Lab. of Neuroscience, Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sciences, Shanghai, China, Shanghai, China

Abstract: The purinergic transmitters, adenosine (Ado) and adenosine 5' triphosphate (ATP), are playing important roles in a plethora of physiological processes, including sleep-wake control, learning and memory, cardiovascular activity and immune response. Impaired purinergic signaling is associated with diseases such as pain, seizures, stroke and drug addiction. The ability to directly measure Ado and ATP dynamics with high molecular specificity is essential for understanding their physiological and pathological functions. Here by tapping into human A_{2A}R and P2Y₁ receptors, we developed a toolbox of genetically-encoded GPCR-Activation-Based (GRAB) fluorescent Ado and ATP sensors, GRAB_{Ado1.0} and GRAB_{ATP1.0}. In response to extracellular Ado or ATP, GRAB_{Ado1.0} and GRAB_{ATP1.0} sensors exhibit large increases in fluorescence in multiple cell types, with subcellular resolution, subsecond kinetics, nanomolar to micromolar affinity and good molecular specificity. Using GRAB_{Ado1.0}, we detected the dynamic change of Ado levels during sleep-awake cycles, epileptic seizures and acute hypoxia in mice *in vivo*. Using GRAB_{ATP1.0}, we detected injury induced ATP release, and revealed its correlation with microglia migration after injury in zebrafish. Moreover, we characterized a spatially selective ATP releasing event after systemic inflammation induced by lipopolysaccharide (LPS) using two-photon imaging in living mice. Furthermore, by simultaneously monitoring Ado and ATP dynamics during sleep-wake cycles, we unraveled different dynamic patterns of Ado and ATP. In sum, the development of purinergic GRAB sensors provides critical genetically-encoded imaging probes for interrogating Ado and ATP modulation in both physiological and pathological processes with molecular specificity.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Program #/Poster #: 496.08

Topic: I.04. Physiological Methods

Support: NIH Grant R01-GM139850-01
NIH Grant R21-DA051193-01A1

Title: Opto-mass: a high-throughput engineering platform for genetically encoded fluorescent sensors enabling all optical *in vivo* detection of monoamines and opioids

Authors: *M. RAPPLEYE¹, A. GORDON-FENNELL¹, D. C. CASTRO², A. K. MATARASSO¹, C. A. ZAMORANO¹, S. J. WAIT¹, J. D. LEE¹, J. C. SIEBART¹, A. SUKO¹, N. SMITH¹, J. MUSTER¹, K. A. MATREYEK³, D. M. FOWLER¹, G. D. STUBER¹, M. R. BRUCHAS¹, A. BERNDT¹;

¹Univ. of Washington, Seattle, WA; ²Mallinckrodt Inst. of Radiology, Washington Univ., St. Louis, MO; ³Dept. of Pathology, Case Western Reserve Univ., Cleveland, OH

Abstract: Fluorescent sensor proteins are instrumental for detecting biological signals *in vivo* with high temporal accuracy and cell-type specificity. However, engineering sensors with physiological ligand sensitivity and selectivity is time and resource-intensive because their performance is assayed through individual mutagenesis *in vitro* to assess their performance. The vast mutational landscape proteins constitute is a hindrance to sensor development. This is particularly true for sensors that require mammalian host systems to be screened. Here, we developed a novel high-throughput engineering platform that functionally tests thousands of variants nearly simultaneously in HEK293T cells. We showcase the capabilities of our platform, called Optogenetic Microwell Array Screening System (Opto-MASS), by engineering monoamine and opioid *in vivo* capable optogenetic sensors within weeks. We screened over 13,000 mammalian cells expressing a dopamine sensor library using our platform to identify an improved variant. The improved variant, called dMASS^{3A}, has a 1.6-fold improved response saturating conditions of dopamine compared to the parent scaffold *in vitro*. The increase in dynamic range comes with no loss of molecular specificity or baseline brightness. dMASS^{3A} was expressed *in vivo* in Vgat-cre mouse dorsal medial striatum and nucleus accumbens. Using fiber photometry, dMASS^{3A} detected sucrose concentration-dependent dopamine transients *in vivo* in the nucleus accumbens. To expand the applications of Opto-MASS, we functionally screened over 23,000 mammalian cells expressing opioid sensor variants to identify an improved variant called μ MASS^{2A}. μ MASS^{2A} had a ~4.6 fold and ~3.8 fold greater response to 500 nM and saturating concentrations of [D-Ala², N-MePhe⁴, Gly-ol]-enkephalin (DAMGO) compared to the parent construct, respectively. μ MASS^{2A} was expressed in nucleus accumbens of Penk-cre mice and detected dose-dependent morphine administration *in vivo* using fiber photometry. The Opto-MASS addresses the need for improved methods to construct optogenetic sensors. Traditional techniques screen sensors one by one, Opto-MASS presents a method to rapidly develop *in vivo* capable optogenetic sensors by functional screening of thousands of sensor variants expressed in mammalian cells. We showcased our platforms versatility by optimizing monoamine and opioid sensors, highlighting the ability to engineer optogenetic sensors for neurotransmitters with diverse physiological roles.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Program #/Poster #: 496.09

Topic: I.04. Physiological Methods

Title: New Generation Sensors for Caspase Activation and Mitochondrial Superoxide in Live Cell Microscopy

Authors: *D. BEACHAM, C. OON, B. MOHR, B. MANDAVILLI, Y.-Z. HU, J. YANG;
Thermo Fisher Scientific, Eugene, OR

Abstract: Neural cell health and stress readouts are critical indicators of altered or impaired function in normal and diseased states of excitable cells, and work has been underway to develop improved small molecule sensor dyes compatible with traditional imaging and High Content Analysis (HCA) interrogation of apoptotic and mitochondrial stress pathways.

The CellEvent™ Caspase Green dye effectively reports caspase activation, but suffers complications in assay configuration when attempting to multiplex with the Green Fluorescent Protein (GFP), calcein, or other 488 laser line tools in fluorescence microscopy. Here, we describe the testing and functional characterization of a new candidate molecule for measuring apoptosis in living cells. Our sensor is comprised of a fluorogenic reporter dye that is liberated from a DEVD peptide substrate by caspase activation, but operates in the Texas Red, 590nm excitation band, with an emission peak near 610 nm, permitting easy multiplex with GFP or calcein stained neurons in both traditional and HCA microscopy configurations. This reagent is named CellEvent™ Caspase Red Dye and will be benchmarked for signal strength and specificity against CellEvent™ Caspase Green in standard models of apoptosis on cell lines and differentiated neurons. Photostability and toxicity comparisons are made, and DMSO-free dry down formulations of both red and green dye species are demonstrated.

Similarly, mitochondrial superoxide accompanying cell stress is probed in microscopy with the MitoSOX™ Red Mitochondrial Superoxide Indicator dye, which localizes to mitochondria and reports superoxide generation, ignoring other Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). This dye has an unusually long Stokes' shift, requiring specialized microscopy and HCA filters that excite at 405nm, and capture emission at 610nm for specific superoxide detection. This unconventional spectroscopic profile prevents the dye's use on many imaging platforms and promotes phototoxicity. To this end, our team has produced a dye with the same level of specificity for superoxide that will operate in one of the traditional fluorescence microscopy channels. Our candidate dye, here named MitoSOX™ Green Mitochondrial Superoxide Indicator also localizes to mitochondria of live cells and selectively reports superoxide generation, while ignoring other ROS and RNS species in ex vivo testing. With an Excitation/Emission profile in the GFP/FITC microscopy channel, a series of comparative studies in immortalized and neural cells are shown, highlighting photostability, specificity and signal amplitude from the dye.

Disclosures: **D. Beacham:** A. Employment/Salary (full or part-time);; Thermo Fisher Scientific. **C. Oon:** A. Employment/Salary (full or part-time);; Thermo Fisher Scientific. **B.**

Mohr: A. Employment/Salary (full or part-time); Thermo Fisher Scientific. **B. Mandavilli:** A. Employment/Salary (full or part-time); Thermo Fisher Scientific. **Y. Hu:** A. Employment/Salary (full or part-time); Thermo Fisher Scientific. **J. Yang:** A. Employment/Salary (full or part-time); Thermo Fisher Scientific.

Poster

496. Imaging of Neural Activity and Neurotransmitters

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Topic: I.04. Physiological Methods

Support: NIH Grant R01GM139850-01
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ISCRM Fellowship 2020

Title: Oros: a comprehensive suite of fast and sensitive optogenetic H₂O₂ sensors for the in situ monitoring of redox signaling and oxidative stress in neurons.

Authors: ***J. D. LEE**^{1,2,3}, **Y. WANG**², **A. NGUYEN**², **C. NEISWANGER**⁴, **S. S. SCHATTAUER**⁴, **F. YEBOAH**^{5,3}, **S. ZUNIGA**², **S. WAIT**^{1,2,3}, **M. RAPPLEYE**^{2,3}, **S. BREMNER**^{6,3}, **C. CHUN**^{2,3}, **K. EVITTS**^{2,3}, **A. S. T. SMITH**^{7,3}, **D. L. MACK**^{8,3}, **J. E. YOUNG**^{5,3}, **C. I. CHAVKIN**⁴, **A. BERNDT**^{2,3};

¹Mol. Engin. and Sci., ²Bioengineering, ³Inst. of Stem Cell and Regenerative Med., ⁴Pharmacol., ⁵Lab. Med. and Pathology, Univ. of Washington, Seattle, WA; ⁶Bioengineering, Univ. of Washington, Seattle, WA; ⁷Physiol. and Biophysics, ⁸Rehabilitative Med., Univ. of Washington, Seattle, WA

Abstract: H₂O₂ is a key endogenous Reactive Oxygen Species (ROS) in mammalian systems, and recent studies report its roles in neurobiology, including synaptic plasticity, excitability, and receptor signaling. Traditionally, H₂O₂ has also been viewed as a critical marker for oxidative stress as its accumulation was often associated with brain aging and neurodegenerative disorders. Unfortunately, currently available H₂O₂ probes display limitations in terms of sensitivity, brightness, and spatiotemporal resolution which poses a significant challenge for accurate intracellular H₂O₂ monitoring. To address this, we aimed to develop optogenetic tools and methods to monitor both transient (e.g. H₂O₂ in redox signaling event) and steady-state (e.g. basal H₂O₂ level as a disease phenotype) intracellular H₂O₂ in diverse biological contexts. Through structure-guided engineering, we developed oROS, a sensitive and fast H₂O₂ optogenetic sensor suite comprised of oROS-G, a GFP-based green sensor for dynamic range, oROS-Gr, a ratiometric sensor for long-term or non-continuous monitoring of intracellular H₂O₂, and oROS-HT, a JF-635 (Janelia Fluor) conjugated HaloTag based far-red sensor for brightness and multicolor imaging. oROS sensors exhibit exceptional sensitivity that capture immediate H₂O₂ level rise when intracellular Glutaredoxin and Thioredoxin redox enzymes were inhibited

by 1 μ M of Auranofin. Their exceptional on-kinetics enabled visualization of intracellular diffusion of H₂O₂, demonstrating its capability for real-time monitoring of ROS levels. oROS-G (3-fold $\Delta F/F_0$ at sensor saturation) enabled monitoring of H₂O₂ signal induced by biased agonistic activation of opioid receptors that has been investigated for its role in opioid receptor inactivation and tolerance on brain slice. oROS-Gr enabled continuous monitoring of dynamic H₂O₂ level fluctuation induced by various concentrations of ROS-inducing compound Menadione, portraying a potentially complex and dynamic intracellular redox balancing environment. In addition, oROS-Gr was utilized to show steady-state ROS levels in Alzheimer's disease models using human-induced pluripotent stem cell-derived cortical neurons. oROS-HT is a bright and inverse-response sensor (80% decrease from baseline at sensor saturation) for detecting H₂O₂ signals with minimal noise. We also demonstrated the oROS-HT's multiplexing capability by simultaneously imaging with green sensors like GCaMP8f, revealing the specific Ca²⁺/ROS interplay in cultured neurons. The diverse applicability of oROS sensors provides tailored methods for monitoring the multi-faceted dynamic roles of H₂O₂ in neurobiology.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Burroughs Wellcome Fund Postdoctoral Enrichment Program Award

Title: Chronic dual optical-voltage recordings from hippocampus of awake head-fixed mice.

Authors: N. MASALA¹, G. TARCSAY², H. KHAN³, D. GONZALES³, K. JAYANT³, *L. A. EWELL²;

¹Univ. Clin. of Bonn, Bonn, Germany; ²Neurobio. Section, Univ. of California - Irvine, Irvine, CA; ³Purdue Univ., Purdue Univ., West Lafayette, IN

Abstract: Sharp wave ripples (SWR) are thought to support memory consolidation. Observed as transient (<200 ms) fast (150 -200 Hz) oscillations in hippocampal field potential (LFP) recordings, SWR occur during restful periods and during reward consumption. To date, most

cellular studies of SWR have utilized high density electrophysiology, which offers unparalleled temporal resolution but is limited with respect to neuron sampling and tracking over extended periods of time. Thus, basic questions about whether there are anatomical patterns to ensembles active during different types of SWR and how those ensembles evolve over days of experience are poorly understood. Thus, we have developed a novel method in which we record local field potentials (LFPs) and calcium activity from the same population of CA1 neurons across days. A flexible, transparent electrode array (32 channels) is placed on the surface of hippocampus, which facilitates 2-photon imaging of CA1 through the electrode array and planar LFP recordings from stratum oriens. In preliminary experiments we have successfully measured CA1 place cell activity (GCaMP) and theta oscillations (LFP) during running. Current experiments are underway to measure population activity during reward-related and rest-related SWR, over days of exposure to a novel track.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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NIH U01 U01NS113294
Klingenstein-Simons Award
Welch Foundation Q-2016-20190330

Title: Jedi-1p: fast and photostable genetically encoded voltage indicators optimized for one-photon imaging

Authors: *X. LU¹, Y. WANG⁴, Z. LIU², Y. GOU⁶, S. YANG³, D. JAEGER⁵, F. ST-PIERRE⁶;
¹Systems, synthetic and physical biology, ²Electrical and Computat. Engin., ³Chem. and Biomolecular Engin., Rice Univ., Houston, TX; ⁴Neurosci., ⁵Biol., Emory Univ., Atlanta, GA; ⁶Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Understanding the connection between behaviors and underlying neural activities has been a long-pursued goal of neuroscience yet remains challenging. A critical technology gap is the lack of tools that can faithfully report rapid neuronal electrical dynamics such as high-frequency oscillations from large and genetically defined populations of neurons with sub-millisecond temporal resolution. An emerging technology for noninvasive monitoring membrane potential is voltage imaging using Genetically Encoded Voltage Indicators (GEVIs) -- light-emitting protein indicators that transform membrane voltage into fluorescence output. When

combined with advanced microscopy technologies, GEVIs offer immense potential to report brain-wide voltage dynamics at high spatiotemporal resolution in genetically defined cell types, with extra benefits of long-term to permanent expression *in vivo*.

Here, we present our efforts to fill in the gap between the demand for robust neuron imaging and the imperfect performance of existing GEVIs. First, we developed a platform for high-throughput screening of GEVI libraries to improve the kinetics, sensitivity, brightness and photostability of the sensors simultaneously. Then, we deployed the screening pipeline to evolve indicators of the Accelerated Sensor of Action Potential (ASAP) family with structure and evolutionary studies-based library design strategies. The best indicator identified during our screening, which we named Jellyfish-derived Electricity-reporting Designer Indicator (JEDI), is 2 times brighter, 6 times faster and more photostable than its parental indicator ASAP2s. JEDI was characterized *in vitro* with voltage clamp and showed more than 2-fold of improvement over ASAP2s in $\Delta F/F_0$ in response to action potentials. We also benchmarked JEDI *in vivo* and demonstrated JEDI's capability in reporting brain-wide activity in awake behaving mice within single-trial with mesoscopic imaging. We anticipate that these engineering and benchmarking efforts together with our dissemination resources will ultimately promote broader utilities of GEVIs for imaging voltage dynamics in the brains of behaving animals.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Topic: I.04. Physiological Methods

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Helen Hay Whitney Fellowship to L.Z.F.
Merck fellowship from the Life Science Research Foundation to D.W.C

Title: All-optical electrophysiology with improved genetically encoded voltage indicators reveals interneuron network dynamics *in vivo*

Authors: *H. TIAN¹, H. C. DAVIS¹, J. D. WONG-CAMPOS¹, L. Z. FAN⁴, P. PARK², B. GMEINER³, S. BEGUM¹, C. A. WERLEY⁵, G. B. BORJA⁵, H. SHAH⁵, J. JACQUES⁵, Y. QI², V. PAROT¹, K. DEISSEROTH⁶, A. E. COHEN²;

¹Harvard Univ., ²Chem. and Chem. Biol., ³Harvard Univ., Cambridge, MA; ⁴Bioengineering Dept., Stanford Univ., Palo Alto, CA; ⁵Q-state Biosci., Cambridge, MA; ⁶Stanford, Stanford, CA

Abstract: All-optical electrophysiology can be a powerful tool for studying neural dynamics *in vivo*, as it offers the ability to image and perturb membrane voltage in multiple cells

simultaneously. The “Optopatch” constructs combine a red-shifted archaerhodopsin (Arch)-derived genetically encoded voltage indicator (GEVI) with a blue-shifted channelrhodopsin actuator (ChR). We used a video-based pooled screen to evolve Arch-derived GEVIs with improved signal-to-noise ratio (QuasAr6a) and kinetics (QuasAr6b). By combining optogenetic stimulation of individual cells with high-precision voltage imaging in neighboring cells, we mapped inhibitory and gap junction-mediated connections, in vivo. Optogenetic activation of a single NDNF-expressing neuron in visual cortex Layer 1 significantly suppressed the spike rate in some neighboring NDNF interneurons. Hippocampal PV cells showed near-synchronous spikes across multiple cells at a frequency significantly above what one would expect from independent spiking, suggesting that collective inhibitory spikes may play an important signaling role in vivo. By stimulating individual cells and recording from neighbors, we quantified gap junction coupling strengths. Together, these results demonstrate powerful new tools for all-optical microcircuit dissection in live mice.

Disclosures: **H. Tian:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending. **H.C. Davis:** None. **J.D. Wong-Campos:** None. **L.Z. Fan:** None. **P. Park:** None. **B. Gmeiner:** None. **S. Begum:** None. **C.A. Werley:** None. **G.B. Borja:** None. **H. Shah:** None. **J. Jacques:** None. **Y. Qi:** None. **V. Parot:** None. **K. Deisseroth:** None. **A.E. Cohen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent pending.

Poster

496. Imaging of Neural Activity and Neurotransmitters

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Topic: I.04. Physiological Methods

Support: Howard Hughes Medical Institute (HHMI)

Title: Far-red chemigenetic indicators for neuronal activity in living animals

Authors: ***H. FARRANTS**¹, E. R. SCHREITER²;

¹Howard Hughes Med. Institute, Janelia Res. Campus, Ashburn, VA; ²Howard Hughes Med. Institute, Janelia Farm Res. Campus, Ashburn, VA

Abstract: Live optical imaging has been vital for understanding the dynamics of cells in behaving animals. For example, calcium indicators built around fluorescent proteins are routinely used to report on neuronal activity. Far-red fluorescent indicators would allow deeper imaging in tissue and easier multiplexing, but we lack bright, naturally occurring far-red fluorophores. We will present new far-red fluorescent “chemigenetic” calcium indicators using engineered proteins and synthetic Janelia-Flour dyes for simultaneous red and far-red fluorescent multiplexed imaging of neuronal activity in zebrafish larvae in vivo.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

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Program #/Poster #: 496.15

Topic: I.04. Physiological Methods

Title: Greent-ec: a novel biosensor for calcium imaging in the extracellular space

Authors: *A. J. IDZIAK¹, A. VALIENTE GABIOUD², O. GRIESBECK², V. U. NÄGERL¹;
¹Interdisciplinary Inst. for Neurosci., Univ. de Bordeaux/CNRS, Bordeaux, France; ²Max-Planck-Institut für Biologische Intelligenz, Martinsried, Germany

Abstract: Calcium is one of the most important intracellular messengers in the brain, controlling key signaling pathways and cell functions like neuronal excitability, neurotransmitter release and synaptic plasticity. The calcium concentration is very low at rest inside cells (100 nM), but it can increase transiently to micromolar levels during Ca²⁺ influx from the extracellular space (ECS), where the concentration is more than 10,000 times higher. While the spatio-temporal dynamics of intracellular calcium are intensely studied by fluorometry, very little is known about calcium in the ECS. This knowledge gap is mostly because existing calcium indicators (based on small organic molecules or fluorescent proteins) have a very high calcium binding affinity, which let them detect changes in the (sub)micromolar range, but render them insensitive to changes in calcium in the ECS, where they would be saturated. We report on the properties of a novel Ca²⁺ sensor designed to have an affinity in the low millimolar range. It is based on the calcium-binding protein TroponinC and mNeonGreen as fluorescent protein. The genetically encoded sensor is targeted to the plasma membrane, exposing its calcium-sensing domain to the extracellular side, where it can detect calcium specifically in the ECS. We virally expressed the sensor in organotypic hippocampal brain slices and checked its functionality by 2-photon time-lapse imaging. Pressure-injecting or bath-applying solutions with different calcium concentrations, we characterized its sensitivity and response time to forced changes in extracellular calcium. To check the ability to report physiological changes in calcium concentration, we electrically stimulated afferent fibers (Schaffer collaterals) and looked for changes in sensor fluorescence in the CA1 target region. Unexpectedly, we detected clear signal increases after stimulation, with rise times on the order of hundreds of milliseconds and decay times of seconds. Several lines of evidence indicate that the signals reflect extrusion of calcium from active neurons and its delayed dissipation in the ECS after spontaneous and stimulated neuronal activity. In sum, the novel sensor (GreenT-EC) has calcium binding and fluorescent properties that make it suitable for imaging changes in extracellular calcium concentration. It is potentially a groundbreaking tool to study calcium dynamics and its regulation in the ECS.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 496.16

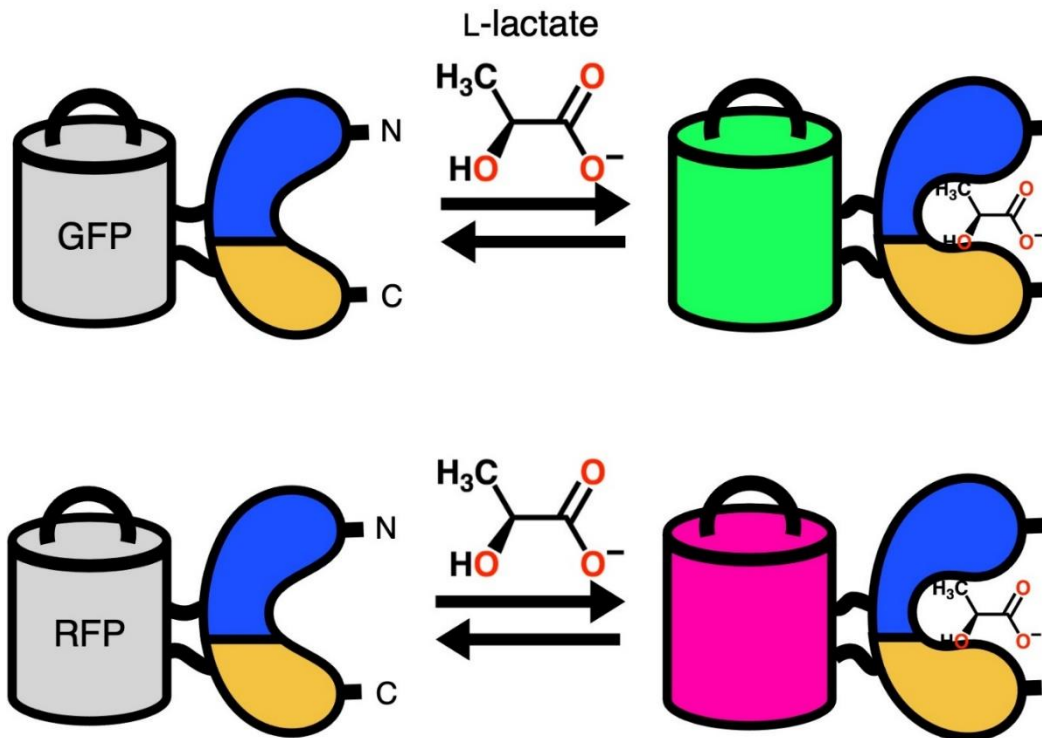
Topic: I.04. Physiological Methods

Support: JSPS Grant 21K14738
JSPS Grant 19H05633

Title: LACCO series: Genetically encoded fluorescent biosensors for extracellular and intracellular L-lactate

Authors: *Y. NASU, Y. KAMIJO, S. HARIO, G. N. T. LE, R. E. CAMPBELL;
Univ. of Tokyo, Tokyo, Japan

Abstract: Organisms on Earth produce biologically-useful energy by catabolizing glucose to pyruvate which, in the absence of oxygen, is normally converted into L-lactate. Traditionally, L-lactate has been considered “waste” by-products of glucose metabolism. However, growing evidence suggests that L-lactate is better considered a biological “fuel currency”, that can be shuttled from cell-to-cell and plays a central role in the energy supply for organisms. For example, the astrocyte-to-neuron lactate shuttle (ANLS) hypothesis proposes that astrocytes metabolize glucose to produce L-lactate which is then released to the extracellular environment and taken up by neurons. In neurons, L-lactate is converted to pyruvate which is fed into the tricarboxylic acid cycle (TCA) for production of adenosine triphosphate (ATP) which provides energy necessary to sustain heightened neural activity. The ANLS hypothesis remains a controversial mystery, with recent reports of evidence both for and against it. Fluorescent proteins (FPs) have been proven to be versatile scaffolds for development of biosensors (protein constructs that change their fluorescence intensity in response to changes in the environment). Specifically, GCaMP, a calcium ion (Ca^{2+}) sensor based on green FP (GFP) and Ca^{2+} sensing domains, has been widely employed to monitor neural activities in live model organisms. In addition to GCaMP, various FP-based biosensors for non- Ca^{2+} target have been developed. However, few sensors have sensitivity as high as GCaMP, hampering their wide application *in vivo*. Herein, we show that directed protein evolution can enable the engineering of FP-based biosensors for an important metabolite L-lactate with high sensitivity comparable to GCaMP. This study provides a powerful new optical toolbox, LACCO series (Figure), for the investigation of extracellular and intracellular L-lactate in neurons and astrocytes. We anticipate that the LACCO series will play a central role in investigations of L-lactate shuttles, including the controversial ANLS hypothesis.



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Poster

496. Imaging of Neural Activity and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 496.17

Topic: I.04. Physiological Methods

Support: NIH Grant R01-GM139850-01
 NIH Grant R21-DA051193-01A1
 NSF GRFP DGE-2140004
 Herbold Fellowship (2021)

Title: Ensembled machine learning algorithms to direct the engineering of calcium indicators for neuronal imaging

Authors: *S. WAIT, J. D. LEE, M. RAPPLEYE, A. ASECIO, A. SUKO, A. BERNDT;
 Univ. of Washington, Seattle, WA

Abstract: Real-time optical interrogation of neuronal activity can be achieved optogenetically using genetically encoded fluorescent indicators (GEFIs). GEFIs link fluorescent reporters to

naturally occurring proteins that interact with neurotransmitters to create a tool that's fluorescent output relies on the presence of the neurotransmitter of interest. To meet the needs of experimentalists, the proteins that comprise the GEFI can be engineered to alter the overall tool's fluorescent response, speed of response output, and sensitivity. However, *GEFIs are complex proteins with multiple dynamic states, making their engineering both intellectually and experimentally taxing*. Here, we investigate machine learning as a mechanism to aid in expedited and data-driven GEFI engineering. We developed an ensemble of three regression models that learn from empirically derived mutational libraries to provide predictions of mutations that dictate overall protein function. With this ensemble, we chose to direct the engineering of GCaMP, a commonly used GEFI that increases fluorescence intensity in response to calcium. We sought to modify the sensor's dynamic range and kinetics with the data found in pre-published mutation libraries. We supplemented a novel library of GCaMP sequences and allowed the ensemble to formulate predictions of each mutant's fluorescent and kinetic potentials. We cloned mutations the ensemble predicted would be most influential into the GCaMP protein. The fluorescent and kinetic properties intrinsic to each mutant were quantified *in vitro* in the HEK293 WT cell line via epifluorescent microscopy. We tested all mutations in triplicate and developed a deep-learning based cell segmentation pipeline to bolster the number of cells analyzed. We found that ~50% of tested variants displayed qualities that reflected predictions made by the ensemble. Within this assay, we discovered mutations that led to 4x greater fluorescent response and 22x faster kinetics than jGCaMP7s. In neurons, our highest performing mutations achieved a <2000% increase in fluorescence and displayed single spike fidelity. The biophysical properties of these newly identified mutants provide promise for improved calcium recording ability *in vivo* with clear distinctions of single spike events. Furthermore, the speed of discovery glaringly outperforms traditional GEFI engineering methods. These findings illustrate the competency of machine learning to supplement scientific understanding and hone optogenetic mutation efforts. With this data-driven platform, we aim to generate mutational libraries for underdeveloped GEFIs to encourage accelerated engineering.

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Poster

496. Imaging of Neural Activity and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 496.18

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH - R01NS102870
NIH - K25NS083754
American Heart Association
Washington University Department of Radiology
McDonnell Center for Systems Neuroscience

Title: Dual fluorophore imaging system to acquire calcium, metabolic and hemodynamic activity

Authors: *X. WANG¹, J. PADAWER-CURRY², B. KIM³, A. BICE⁴, Z. P. ROSENTHAL⁵, J.-M. LEE⁶, A. Q. BAUER⁴;

¹Washington University, St. Louis, Saint Louis, MO; ²Optical Radiology, Washington Univ. at St. Louis, Saint Louis, MO; ³Washington Univ. In St. Louis, Saint Louis, MO; ⁴Washington Univ. in St. Louis, Saint Louis, MO; ⁵Neurol., Washington Univ. In St Louis, Saint Louis, MO; ⁶Dept Neurol, Washington Univ. Med. Sch., Saint Louis, MO

Abstract: In the healthy brain, local changes in blood flow and brain energy utilization are coupled to changes in local neural activity, phenomenon described as neurovascular coupling (NVC) and neurometabolic coupling (NMC). NVC and NMC evolve during development, and are altered under anesthesia, and other conditions affecting the central nervous system disrupt individual or multiple pathways involved in NVC. Understanding how different central nervous system diseases affect different components of NVC will enable linking of changes in neural or metabolic dysfunction to changes in hemodynamic signaling upon which functional neuroimaging methods rely. Genetic engineering techniques in mice have provided new opportunities for extending wide-field optical imaging methods to direct measures of neural activity. Fluctuations in calcium concentration can be imaged and visualized using fluorescent, genetically encoded calcium indicators (GECIs). However, the excitation/emission spectra of commonly used GECIs (e.g. GCaMP) overlap with those of flavin adenine dinucleotide (FAD), an endogenous measure of oxidative metabolism. Red-shifted GECIs allow for parallel imaging of calcium and FAD events and have advantages for in vivo imaging due to reduced tissue scattering and absorption. In addition, unlike GCaMP, excitation spectrum of red-shifted GECI does not overlap with Channelrhodopsin-2(ChR2) spectrum, which makes optogenetics targeting possible. Six mice (7-8 months of age) expressing the red-shifted GECI jRGECO1a with cranial window were used for experimentation. A custom light engine consisting of 470nm, 530nm and 625nm LEDs illuminated the skull. Diffuse reflected light for optical intrinsic signal imaging and fluorescence emission were collected by a lens, split by a 580nm dichroic and sampled by two CMOS cameras. We developed a dual-fluorophore, wide field imaging system for rapid, simultaneous mapping of calcium, metabolic and hemodynamic activity in awake mice. As a proof-of-concept, we report stimulus-evoked and spontaneous patterns of activity across hemoglobin, FAD and jRGECO1a in awake and anesthetized animals. Contrast-specific patterns of activity differ across state and frequency range examined, providing complementary information along the NVC pathway. This flexible hardware platform allows for integrating optogenetic stimulation for all optical neural circuit interrogation and readout, and for examining the interaction between excitatory and inhibitory neurons and measures of cortical excitatory activity during photostimulation of parvalbumin inhibitory interneurons.

Disclosures: X. Wang: None. J. Padawer-Curry: None. B. Kim: None. A. Bice: None. Z.P. Rosenthal: None. J. Lee: None. A.Q. Bauer: None.

Poster

496. Imaging of Neural Activity and Neurotransmitters

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Program #/Poster #: 496.19

Topic: I.04. Physiological Methods

Support: NIH UF1 NS116241
NIH R01 DC000566
NSF BCS-1926676
NSF CBET-1631912

Title: Grintrode: a neural implant for simultaneous two-photon imaging and extracellular electrophysiology in head-fixed or freely moving mice

Authors: *C. MCCULLOUGH¹, D. RAMIREZ-GORDILLO², G. FUTIA¹, M. HALL³, A. K. MORAN², E. GIBSON¹, D. RESTREPO²;

¹Bioengineering, ²Cell and Developmental Biol., ³Neurosci. Machine Shop, Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: In vivo imaging and electrophysiology are powerful tools to explore neuronal function that each offer unique complementary information with advantages and limitations. Capturing both data types from the same neural population would allow researchers to take advantage of the capabilities of both modalities and further understand how they relate to each other. Here we present a head-mounted neural implant suitable for in vivo two-photon imaging of neuronal activity with simultaneous extracellular electrical recording in head-fixed or freely moving animals. A GRIN lens-based head-mounted neural implant with extracellular electrical recording provided by tetrodes on the periphery of the GRIN lens was chronically implanted. The design of the neural implant allows for recording from head-fixed animals, as well as freely moving animals by coupling the imaging system to a coherent imaging fiber bundle. We demonstrate simultaneous two-photon imaging and extracellular electrophysiology of neural activity in awake head-fixed, and freely moving mice. The use of tetrodes for multisite extracellular electrophysiology enables the isolation of single unit electrical activity. Using the collected information, we perform correlation analysis to reveal positive correlation between optical and electrical recordings. Simultaneous recording of optical and electrical activity from overlapping neuronal populations provides complementary information from each modality. Designs that are capable of recording from freely moving animals allow for the investigation of neural activity underlying a broader range of behavioral paradigms.

Disclosures: C. McCullough: None. D. Ramirez-Gordillo: None. G. Futia: None. M. Hall: None. A.K. Moran: None. E. Gibson: None. D. Restrepo: None.

Poster

497. Network Computation I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 497.01

Topic: I.06. Computation, Modeling, and Simulation

Support: (NIMH) - R01MH118487

Title: Modeling the role of neuronal subpopulations in establishing spatial temporal patterns of activity across the cortical surface during visual decision making

Authors: *S. SHARMA¹, A. A. KIANI¹, T. I. NETOFF², G. M. GHOSE¹;

¹Dept. of Neurosci., ²Dept. of Biomed. Engin., Univ. of Minnesota, MINNEAPOLIS, MN

Abstract: How do populations of neurons interact with each other at different spatial scales during information processing and decision making? Despite the abundance of interest focused on cortical spatiotemporal patterns and their implication in conditions such as epilepsy, the dynamics underlying these patterns remain elusive, since measures such as excitation-inhibition balance within local circuits and long range connectivity between such circuits are difficult to directly and simultaneously measure. To address these challenges, we applied local circuit models (Kiani, Netoff, Ghose; 2021), each containing a population of excitatory, inhibitory and pyramidal cells, to LFP recordings from electrodes spanning a 4 mm² region of area V4 obtained while the monkeys performed a difficult visual detection task. We initially validated the model on single channel recordings, demonstrating its ability to consistently fit LFP recordings ($r > 0.83$) across electrodes (N up to 87). We found that variation of only two parameters were required to explain spatial variations across electrodes: the inhibition acting on pyramidal cells, and external input. We found that the correlations between inferred excitatory cell populations were strongest ($r = 0.93$ between nearby electrodes) and the broadest ($\lambda = 4.4$ mm), and correlations between inferred pyramidal cell populations the weakest and narrowest ($r = 0.8$ and $\lambda = 3.0$ mm). Our results are consistent with V4 decorrelating the input arising from earlier visual areas, which in our model was strongly and broadly correlated ($r = 0.87$ and $\lambda = 3.3$ mm). Additionally, the strength and breadth of correlations among excitatory interneurons may spatially diffuse top-down modulatory signals associated with phenomena such as spatial attention. Given the strong correlations between inferred neuronal subpopulations across large distances, our results suggest that simple local circuit models coupled with broad connectivity are capable of capturing the spatiotemporal complexity local field potentials across the cortical surface. Going forward, we will use the spatial correlations inferred by our model to investigate the role of lateral connectivity in the emergence of spontaneous rhythmic oscillations across the V4 and their relevance to perceptual decision making.

Disclosures: S. Sharma: None. A.A. Kiani: None. T.I. Netoff: None. G.M. Ghose: None.

Poster

497. Network Computation I

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 497.02

Topic: I.06. Computation, Modeling, and Simulation

Support: NIMH Grant R01MH118487

Title: Inferred states and parameters in a neural mass model can predict task performance on a trial by trial basis from neural recording data

Authors: *A. KIANI¹, G. M. GHOSE², T. I. NETOFF³;

²Neurosci., ¹Univ. of Minnesota, Minneapolis, MN; ³Dept Biomed Eng, University of Minnesota, Minneapolis, MN

Abstract: Inferred states and parameters in a neural mass model can predict task performance on a trial by trial basis from neural recording data Kiani, AA¹, Netoff, TI², Ghose GM¹. Department of Neuroscience, University of Minnesota². Department of Biomedical Engineering, University of Minnesota Contact e-mail: kiani006@umn.edu Neural recording techniques such as LFP, measure population-level activity reflecting neural information processing, as well as cognitive and behavioral states. Connecting these signals to underlying processes, however, is a difficult task with current methods. Neural mass modeling has risen as an alternative approach to addressing these issues by adopting a minimalistic view of neural circuitry. Recently, we developed a physiologically informed neural mass model, coined self-consistent intrinsically oscillating microcircuit (SCIOM), that produces realistic intrinsic oscillations and evoked transients (Kiani, Netoff, Ghose; 2021) using subpopulations of excitatory, inhibitory and pyramidal cells. In this study, we design a model inversion method via an unscented Kalman-filter to assimilate model states (excitatory, inhibitory, and pyramidal) and parameters (coupling coefficients between the subpopulations) to V4 LFP data acquired from monkeys while they performed a visual detection task. Although the model requires a narrow balance between excitation and inhibitory, the magnitude of IPSPs makes the model particularly sensitive to inhibitory couplings. Accordingly, PCA analysis revealed that variations in inhibitory couplings were particularly important in accounting for LFP variability over 40 minute sessions. To study the behavioral contribution of specific subpopulations, we then examined how well the inferred populations predicted performance using a cross-validated linear discriminator. Pyramidal estimates most reliably predicted performance at a rate of 76%, while excitatory (68%), inhibitory (60%) estimates and inferred afferent inputs (70%) were less reliable. These estimates are consistent with the notion that V4 circuitry contributes to shape perception, since the inferred outputs (pyramidal) predict behavior better than inputs. Our results also suggest that variations in local inhibitory coupling constrain reliability of V4 pyramidal cells to accurately convey shape information. In future studies, we will validate our model by comparing inferred pyramidal estimates with existing single unit spike data and examine whether they are capable of explaining our previous results regarding the presence of precise and reliable detection signals in particular neurons (Weiner, Ghose; 2014).

Disclosures: A. Kiani: None. G.M. Ghose: None. T.I. Netoff: None.

Poster

497. Network Computation I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 497.03

Topic: I.06. Computation, Modeling, and Simulation

Support: NIMH109548

Title: Cross-frequency coupling in a macroscopic model of collective neural activity

Authors: *Y. QIN¹, A. SHEREMET²;

¹Univ. of Florida, PO BOX 116580, ²Engin. Sch. for Sustainable Infrastructure and Envrn. (ESSIE), Univ. of Florida, Gainesville, FL

Abstract: Collective neural activity, i.e., spatiotemporal patterns of activity that entrain a large subset of the neural population, such as oscillations, propagating waves, Turing patterns, spiral waves and others, is macroscopic with respect to cell scale, in the sense that the details of the behavior of any single cell have no detectable expression in its dynamics. In physics, the evolution of large-scale systems has been traditionally modeled in the framework of statistical physics, or its thermo-/hydrodynamic limits, which represent the physical system as a continuum. These approaches have been successfully used to investigate phenomena such as phase transitions, that bear little resemblance to microscopic dynamics.

The application of such models to brain activity, however, poses a fundamental challenge: there are no neurons, as "cell individual", in these models. These models do not describe pairwise communication between neurons, or deterministic interconnections between small groups of neurons. If brain activity depends crucially on the dynamics of cell individuals, rather than populations, these models might not be of much use.

However, measurements reveal intriguing macroscopic features of brain activity such as strong nonlinear deformation of the theta rhythm in rats running at high speed, cross-frequency coupling between theta and gamma rhythms, and cross-scale coupling in transient processes such as sharp waves and ripples, or slow waves and spindles. These features are similar to those observed in large-scale systems such as wave turbulence, where they have been successfully studied using statistical models. Here, we present an investigation into the properties of a macroscopic model of collective activity and demonstrate that cross-frequency coupling is a fundamental phenomenon that originates in the stochastic and nonlinear character of macroscopic dynamics.

Disclosures: Y. Qin: None. A. Sheremet: None.

Poster

497. Network Computation I

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Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 497.04

Topic: I.06. Computation, Modeling, and Simulation

Support: NIMH109548

Title: A macroscopic description of collective neural activity based on the microscopic Hodgkin-Huxley model of a neuron.

Authors: *A. SHEREMET, Y. QIN;
Univ. of Florida, Univ. of Florida, Gainesville, FL

Abstract: Collective activity is defined by the emergence of spatiotemporal patterns of activity that entrain a large subset of the neural population, such as oscillations, propagating waves, Turing patterns, spiral waves and others. Collective activity is macroscopic with respect to the cell size, in the sense that many details of cell-scale dynamics, such as the exact shape of the action potential of any given single cell, are ignorable. In the cortex, collective activity is mesoscopic, with characteristic scales of 0.1 - 10 mm and 5 - 500 ms. While one could conceivably attempt to model collective activity by integrating a cell-scale model (e.g., the Hodgkin-Huxley equations) for every participating neuron, the large number of cells involved and the lack of accurate information about the individual cells and the structure and characteristics of the interneuronal connections, make this an impossible task. It is also an unnecessary task, because the details of the behavior of a single cell have no detectable expression in collective activity. In physics, the evolution of large scale systems has been traditionally modeled in the framework of statistical physics, or its thermo-/hydrodynamic limits, which represent the system as a continuum. This approach was used successfully to explain phenomena such as phase transitions, that are fundamentally macroscopic in nature and bear little resemblance to microscopic dynamics.

Here, we present a statistical formulation of collective activity dynamics and explore further macroscopic simplifications. Starting from the dynamical equations of Hodgkin and Huxley, we formulate a Fokker-Plank-type statistical model describing evolution of the distribution of the neural population in the parameter space of the Hodgkin-Huxley model. In the long-wave limit we reduce the dimensionality of the Fokker-Plank representation to a small number of state variables that are observable at macroscopic scale (e.g., through LFP measurements). The resulting model is similar in functionality to a hydrodynamic description of gas. We compare the model with other existing formulations of the same type (e.g., Wilson-Cowan models), and present a preliminary analysis of the linear properties of the model.

Disclosures: A. Sheremet: None. Y. Qin: None.

Poster

497. Network Computation I

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Program #/Poster #: 497.05

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH 1RF1DA055665

Title: Combined mechanistic and input-output modeling of the hippocampus: Decoding memory categories from hippocampal spikes

Authors: *X. SHE, T. W. BERGER, D. SONG;
USC, USC, Los Angeles, CA

Abstract: The overarching goal of this project is to develop a novel modeling paradigm inspired by the generative adversarial network (GAN) that synergistically combines both mechanistic and input-output (machine learning) modeling techniques to build a large-scale biologically realistic model of the hippocampus that is functionally indistinguishable from the real hippocampus. To achieve this goal, we build a double-layer, multiple temporal-resolution classification models for decoding memory categories from hippocampal spikes. The model takes spiking activities as input signals and binary cognitive variables (e.g., visual memory categories) as output signals and represents the input-output mapping with a double-layer ensemble classifier. In the first layer, to solve the underdetermined problem caused by the small sample size and the very high dimensionality of input signals, B-spline functional expansion and $L1$ -regularization are used to reduce dimensionality and yield sparse model estimations. A wide range of temporal resolutions of neural features is included by using a large number of classifiers with different numbers of B-spline knots. Each classifier serves as a base learner to classify spatiotemporal patterns into the probability of the output label with a single temporal resolution. In the second layer, another $L1$ -regularized logistic classifier takes outputs of first-layer classifiers as inputs to generate the final output predictions. This classifier serves as a meta-learner that fuses multiple temporal resolutions to classify spatiotemporal patterns of spikes into binary output labels. Machine learning techniques such as bagging and nested cross-validation are utilized to reduce model estimation variances and avoid overfitting. We test this decoding model with synthetic data and experimental data recorded from rodents and human subjects performing memory-dependent behavioral tasks. Results show that this method can effectively avoid overfitting and yield accurate prediction of output labels with small sample size. The decoding model also provides signature functions that represent spatiotemporal characteristics of spike patterns most relevant to the memory categories. This model provides a powerful tool for understanding how memory information such as visual memory categories is encoded in spikes, and how such encoding evolves as hippocampal activities propagate along with hippocampal circuits. It will also be used as a component of the discriminative model for validating the biologically-realistic generative model of the hippocampus.

Disclosures: X. She: None. T.W. Berger: None. D. Song: None.

Poster

497. Network Computation I

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Program #/Poster #: 497.06

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH 1RF1DA055665

Title: Combined mechanistic and input-output modeling of the hippocampus: Full-scale biologically realistic neuronal network model of hippocampus

Authors: G. J. YU, C. XU, M. BIENKOWSKI, T. W. BERGER, *D. SONG;
USC, Los Angeles, CA

Abstract: The overarching goal of this project is to develop a novel modeling paradigm inspired by the generative adversarial network (GAN) that synergistically combines both mechanistic and input-output (machine learning) modeling techniques to build a full-scale realistic model of the hippocampus that are functionally indistinguishable from the real hippocampus. The present work focuses on the construction of a mechanistic spiking neuronal network model of hippocampus. Hippocampal function has been shown to be organized topographically along multiple anatomical axes, and there is evidence indicating that the functional organization is due to the topographical organization of the connectivity in hippocampus. With recent advances in anatomical and imaging methods, mesoscale connectivity data is becoming increasingly accurate as the targets or inputs to specific cell types can be selectively investigated. Understanding of hippocampal anatomy has remained limited to critical studies from investigations from multiple decades ago. Using this starting point, we are incorporating data obtained from traditional and modern methods to construct a highly realistic mesoscale and microscale connectivity for the neuronal network model. Currently, the model includes all excitatory connections of the canonical feedforward trisynaptic pathway. Connections were organized topographically following mesoscale anatomical constraints that had been characterized in vivo. Microscale constraints included the numbers of synapses that are received from each input, their laminar distribution across the dendrites, and the properties of their postsynaptic potentials. The connections included the medial/lateral entorhinal perforant path projection to dentate gyrus and CA3, the dentate mossy fiber projection to CA3, the ipsilateral CA3 to CA3 associational projection, and the ipsilateral CA3 Schaffer collateral projection to CA1. The full-scale connectivity represents 112 000 entorhinal neurons, 1 200 000 dentate granule cells, 250 000 CA3 pyramidal cells, and 380 000 CA1 pyramidal cells. Neurons were represented using reduced morphology multi-compartment models. Using both random and grid cell input, the model has been used to investigate the successive spatiotemporal transformations that were performed by the hippocampal subfields. The sparse connectivity generated spatially correlated activity though pairwise spike correlations remained low (1-5%). This biologically-realistic model will serve as the generative model of the hippocampus that will be validated and modified with a discriminative model that uses an input-output model structure.

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Poster

497. Network Computation I

Location: SDCC Halls B-H

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Program #/Poster #: 497.07

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant 1RF1DA055665

Title: Combined mechanistic and input-output modeling of the hippocampus: Deep multi-input multi-output model of spike train transformation across brain regions

Authors: ***B. MOORE**, D. SONG;
USC, Los Angeles, CA

Abstract: The overarching goal of this project is to develop a novel modeling paradigm inspired by the generative adversarial network (GAN) that synergistically combines both mechanistic and input-output (machine learning) modeling techniques to build a large-scale biologically realistic model of the hippocampus that is functionally indistinguishable from the real hippocampus. To achieve this goal, we are developing an updated multi-input multi-output (MIMO) model that can provide an invariant representation of the input-output nonlinear dynamics that underlie spike transformations across different hippocampal regions during spatial navigation. This MIMO takes the form of a convolutional neural network (CNN) with a shallow layer of convolution to capture temporal dynamics and multiple fully connected layers with skip connections in a DenseNet style to capture nonlinearities. Compared with our previous shallow, double-layer MIMO model, this model is capable of capturing arbitrary forms of temporal dynamics and arbitrarily high-order nonlinearities. More importantly, the temporal filters and hidden layers can be shared by all inputs and outputs, and form a population-level invariant representation of the input-output function. Such a MIMO model enables the comparison of neural nonlinear dynamics between different datasets, e.g., dataset recorded from animals and dataset simulated with the full-scale realistic model, without relying on the one-to-one correspondence between recorded and simulated neurons. Therefore, it provides a powerful tool for functional validation of the full-scale realistic model with experimental data as the ground truth. We have tested this MIMO model with data simulated with a second-order Volterra kernel based spiking neuronal network model. Results show that we are able to obtain accurate recovery of the ground-truth kernels, probability of spiking in the output neuron, and output spiking activity. Current work is focused on using shared temporal filters in shallow layers of the deep MIMO model to enable a more parsimonious, population-invariant model structure that takes advantage of latent representations of large-scale neural input-output nonlinear dynamics. This deep MIMO model will be used as a component of the discriminative model for validating the biologically-realistic generative model of the hippocampus.

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Poster

497. Network Computation I

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Program #/Poster #: 497.08

Topic: I.06. Computation, Modeling, and Simulation

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Title: Brain stimulation effects on seizure dynamics through whole brain modeling for drug-resistant epilepsy

Authors: *B. DOLLOMAJA¹, P. TRIEBKORN², J. SCHOLLY³, F. BARTOLOMEI⁴, H. WANG⁵, V. K. JIRSA⁶;

¹Inst. de neurosciences des systemes, Marseille, France; ²Inst. de Neurosciences des Systemes, Marseille, France; ³APHM, Epileptology and Clin. Neurophysiol. Department, Timone Hospital, Marseille, France, Marseille, France; ⁴INSERM & Inst. De Neurosciences Des Systè, Marseille, France; ⁵Inst. De Neurosci. Des Systemes, INSERM U1106, Aix-Marseille Univ., Marseille, France; ⁶Inst. De Neurosciences Des Systemes UMR1106, Marseille, France

Abstract: Brain stimulation is a useful clinical practice that is based on empirical trial and error, but with very little knowledge about the mechanistic effects. Personalized virtual brain models can help us better understand how electrical stimulation affects the brain. We used this approach to model brain stimulation for drug-resistant epilepsy diagnosis.

First, we used The Virtual Brain platform to build personalized brain models based on patient-specific MRI, dMRI and CT-scan. Next, we used a phenomenological model that captures seizure dynamics, called the Epileptor. We extended this model for brain stimulation effects on seizure onset. We hypothesized, based on empirical human data and present literature, an accumulatory effect of brain stimulation. This accumulation is region-specific, and when reaching a certain threshold, it can give rise to seizures.

In our clinical practice, brain stimulation is applied in drug-resistant epileptic patients in different brain regions with varying parameters (amplitude, frequency, pulse width, location) prior to brain surgery. This is done using implanted SEEG (StereoElectroEncephaloGraphy) electrodes which can stimulate and record local brain activity. When stimulation induces a seizure at a certain location, that area is considered to be epileptogenic. Stimulating systematically across zones and across parameters can help in diagnosing the epileptogenic zones.

We validated our model against empirical recordings where brain stimulation was applied. We did this for 8 patients so far, each of them having at least one spontaneous and one stimulated seizure. We found that by changing *only* stimulation parameters and keeping the virtual brain model unchanged, we could reproduce the various spatiotemporal seizure dynamics observed empirically. Thus, the *same* brain model can elicit different seizure dynamics, which we also observe clinically. We could then explore the system beyond the limited stimulation parameters (on average 10 empirical stimulation trials per patient). We found that the most determinant factors for brain stimulation effects are: stimulation *site*, stimulation *intensity*, brain *connectivity* and brain *state*.

These results point out that personalized brain modeling informed from patient-specific data has predictive power over the optimal stimulation parameters (location, intensity) that induce seizures. This can save clinical time and cost. Ultimately, this allows us to have a deeper understanding of brain stimulation effects in drug-resistant epilepsy.

Disclosures: **B. Dollomaja:** None. **P. Triebkorn:** None. **J. Scholly:** None. **F. Bartolomei:** None. **H. Wang:** None. **V.K. Jirsa:** None.

Poster

497. Network Computation I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 497.09

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EU H2020 Virtual Brain Cloud 826421
Human Brain Project SGA2 785907
Human Brain Project SGA3 945539
ERC Consolidator 683049
German Research Foundation SFB 1436 (project ID 425899996)
SFB 1315 (project ID 327654276)

Title: Decoding spatial patterns of amyloid-beta PET using TVBase - a tool for mapping biological knowledge onto a 3D brain atlas

Authors: ***L. MARTIN**^{1,2}, **L. STEFANOVSKI**^{1,2}, **K. BÜLAU**^{1,2}, **A. KODAMULLIL**³, **M. JACOBS**³, **M. HOFMANN-APITIUS**³, **P. RITTER**^{1,2,4};

¹Brain Simulation Section, Berlin Inst. of Hlth. at Charité - Universitätsmedizin Berlin, Berlin, Germany; ²Dept. of Neurol. with Exptl. Neurol., Charité - Universitätsmedizin Berlin, Berlin, Germany; ³Dept. of Bioinformatics, Fraunhofer Inst. for Algorithms and Scientific Computing SCAI, Sankt Augustin, Germany; ⁴Bernstein Ctr. for Computat. Neurosci. Berlin, Berlin, Germany

Abstract: Biomedical knowledge about the brain increases daily with a rapidly growing number of scientific publications. While this informational plethora is not mere comprehensible by human beings, recent developments in computational information science aim to make this knowledge programmatically accessible by literature mining. Utilizing the results of these linguistic methods for systematically constraining computational models of the brain may unravel their full potential for biomedical research. We developed the semantic meta-analysis platform TVBase (unpublished) that projects biomedical knowledge of 32 million PubMed

articles onto a common 3D brain atlas for neuroimaging. The literature-mining platform *SCAIView* (<https://academia.scaiview.com>) was used to extract well defined biomedical concepts and their associations with brain anatomy in the literature. By querying a concept, the association strength with co-occurring anatomical terms was calculated and projected onto a standard brain in MNI space. We therefore created a unique transformation matrix that links 870 anatomical terms to voxel coordinates. With TVBase we mapped the anatomical literature associations of 37 common Alzheimer's Disease (AD) symptoms to decode spatial information of amyloid-beta (Abeta) PET from 1127 subjects provided by the ADNI database. Systematic similarities between spatial distribution patterns were assessed between individuals with AD, mild cognitive impairment (MCI) and healthy controls (HC). Further, spatial similarity between symptom maps and Abeta PET predicted neuropsychological outcomes of the Mini-Mental State Examination (MMSE), the AD Assessment Scale (ADAS) and the Neuropsychiatric Inventory (NPI). Results were compared against shuffled PET data and random TVBase maps. A mixed model ANOVA shows significant main effects of spatial similarity for all diagnostic groups ($\eta^2 = .24$) and for all 37 symptom maps ($\eta^2 = .34$). Pairwise t-test showed that similarity of Abeta PET with brain maps of "episodic memory", "short-term memory" and "comprehension" differentiated best between AD and HC. Linear regressions showed a positive relationship between spatial similarity and neuropsychological symptom severity (NPI: $R^2 = .03$, ADAS: $R^2 = .13$, MMSE: $R^2 = .10$, $p < .01$). TVBase extracts region-specific information about biomedical concepts from the literature to support knowledge-based translational multi-scale approaches of computational neuroscience. It allows for hypothesis-free neuroimaging pattern interpretation, hypothesis generation, as well as applications in personalized medicine and will be openly available as a python library.

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Poster

497. Network Computation I

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Topic: I.06. Computation, Modeling, and Simulation

Support: This research was conducted with the support of the Ontario Brain Institute (POND, PIs: Anagnostou/Lerch), funded in part by the Government of Ontario. CIHR Grant PJT 168980

Title: An automated multimodal pipeline for pediatric neuroimaging data to generate robust model inputs for TheVirtualBrain

Authors: *L. ROKOS¹, N. FRAZIER-LOGUE³, J. WANG³, S. L. BRAY⁵, M. TAYLOR^{6,2}, R. MCINTOSH³, K. SHEN⁴;

¹Inst. of Med. Sci., ²Med. Imaging and Psychology, Univ. of Toronto, Toronto, ON, Canada;

⁴Simon Fraser Univ., ³Simon Fraser Univ., Burnaby, BC, Canada; ⁵Univ. of Calgary, Univ. of Calgary, Calgary, AB, Canada; ⁶Diagnos. Imaging, The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: TheVirtualBrain (TVB, thevirtualbrain.org) is a neuroinformatics platform that is able to create individualized brain network models based on structural and functional neuroimaging data. Utilizing TVB to model brain dynamics in children can help characterize typical, healthy developmental trajectories. However, there are notable challenges in pediatric neuroimage processing including small brain size and head motion. The TVB-UKBiobank pipeline, an automated and open-source multimodal magnetic resonance imaging (MRI) processing pipeline, was developed to generate the model inputs required by TVB (Frazier-Logue et al., 2022). In order to test the pipeline's functionality for processing data from children across early development, four multimodal neuroimaging pediatric datasets were used. The datasets included data collected by Dr. Signe Bray (controls), Dr. Margot Taylor (children born very preterm and controls), the Province of Ontario Neurodevelopmental Network (children diagnosed with various neurodevelopmental disorders and controls), and the Calgary Preschool MRI dataset (controls). Specifically, T1-weighted, resting or passive viewing state functional MRI, and diffusion-weighted MRI data from children ($N=80$) between the ages of 4 and 8 were tested. The pipeline introduces new support for user-specified, age-specific templates for brain extraction and registration. Additionally, the pipeline generates detailed quality control reports that were used to assess subject processing outcomes and evaluate the pipeline's robustness. In addition to previously tested healthy aging and clinical adult populations, the scope of this pipeline has been extended to support pediatric populations and thus large datasets across the entire lifespan. Importantly, the pipeline offers greater accessibility to model brain network dynamics in early development, supporting investigations around brain maturation and individual differences that predict varying outcomes for children.

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Poster

497. Network Computation I

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Program #/Poster #: 497.11

Topic: I.06. Computation, Modeling, and Simulation

Support: CIHR
NSERC

Title: Structural connectivity between the memory and oculomotor systems changes with age

Authors: A. KHOSLA^{1,2}, N. MAZLOUM-FARZAGHI^{1,2}, J. WANG⁴, A. R. MCINTOSH^{5,1,2,4}, J. D. RYAN^{1,2,3}, *K. SHEN^{5,1};

¹Rotman Res. Inst., Baycrest, Toronto, ON, Canada; ²Psychology, ³Psychiatry, Univ. of Toronto, Toronto, ON, Canada; ⁴Biomed. Physiol. and Kinesiology, ⁵Inst. for Neurosci. and Neurotechnology, Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Memories influence how we view the world by guiding our eye movements. Investigations of structural connectivity (SC) in macaques suggest that memory-guided eye movements may be mediated by indirect anatomical connections between the memory and oculomotor systems. The SC between the memory and oculomotor systems has yet to be examined in humans. Of particular interest are the connections between hippocampus (HC) and the frontal eye fields (FEF), regions critical to memory processing and oculomotor control, respectively. To address this gap, we examined the SC between the HC and FEF in humans. Memory-guided visual behavior also changes with age and may be due to concomitant changes in SC between the memory and oculomotor systems. We therefore also examined whether there are age-related changes in the SC between HC and FEF with age. Using diffusion-weighted imaging data from the Cambridge Centre for Aging and Neuroscience cohort (18-87 years, N = 640), whole-brain SC was estimated using probabilistic tractography. SC was calculated as the probability of connection (or ‘weights’) between 420 regions of interest. The SC matrices were thresholded to reduce the likelihood of false positive connections and matrices were excluded from further analyses if they contained region(s) with no connections (remaining N = 492). To examine the SC between HC and FEF in humans, we computed the average SC matrix of all participants aged 18-37 years (N = 77). There were no direct connections between the HC and FEF and instead, as in macaques, a set of brain regions that indirectly connected the HC and FEF. These pathways involved somatomotor regions, central regions, frontal medial cortex, dorsal prefrontal cortex, anterior thalamus, putamen, and caudate. Using a partial least squares analysis on the full dataset, we correlated participants’ ages with the subset of connections between HC and FEF. The analysis revealed one significant latent variable showing a moderate correlation between SC and age ($r \sim 0.5$, $p < .001$). Age-related changes in SC were predominantly between HC and the intermediary regions while few reliable changes between the intermediary regions and FEF were detected. Our findings show that, in humans, the HC and FEF are structurally connected via a set of polysynaptic pathways. These anatomical connections offer a potential neural substrate for the interaction between the memory and oculomotor systems, and for the declining influence of memory on viewing with age.

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Poster

497. Network Computation I

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Program #/Poster #: 497.12

Topic: I.06. Computation, Modeling, and Simulation

Support: European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3)

Title: Entropy, free energy, symmetry and dynamics in the brain

Authors: V. JIRSA, *H. SHEHEITLI;
Aix-Marseille Univ., Marseille, France

Abstract: Neuroscience is home to concepts and theories with roots in a variety of domains including information theory, dynamical systems theory, and cognitive psychology. Not all of those can be coherently linked, some concepts are incommensurable, and domain-specific language poses an obstacle to integration. Still, conceptual integration is a form of understanding that provides intuition and consolidation, without which progress remains unguided. This work is concerned with the integration of deterministic and stochastic processes within an information theoretic framework, linking information entropy and free energy to mechanisms of emergent dynamics and self-organization in brain networks. We identify basic properties of neuronal populations leading to an equivariant matrix in a network, in which complex behaviors can naturally be represented through structured flows on manifolds establishing the internal model relevant to theories of brain function. We propose a neural mechanism for the generation of internal models from symmetry breaking in the connectivity of brain networks. The emergent perspective illustrates how free energy can be linked to internal models and how they arise from the neural substrate.

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Poster

497. Network Computation I

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Topic: I.06. Computation, Modeling, and Simulation

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Title: The Virtual Brain Ontology: A new computational tool translating multiscale, multimodal biological entities to brain network models

Authors: *J. COURTIOL^{1,2}, L. STEFANOVSKI^{1,2}, K. BÜLAU^{1,2}, L. K. MARTIN^{1,2}, P. RITTER^{1,2};

¹Charité-Universitätsmedizin Berlin, Berlin, Germany; ²Berlin Inst. of Hlth. at Charité - Universitätsmedizin Berlin, Berlin, Germany

Abstract: Brain modeling and simulation play an increasing role in the development of new diagnostic and therapeutic solutions. Theoretical concepts built into simulation technologies such as The Virtual Brain, allow the computation of patient-specific brain models serving as *in silico* platforms for clinical hypothesis testing, by manipulation of model parameters. However, the wide spectrum of computational models available and the significant number of parameters governing their dynamics and structure, makes it difficult, or even impossible, to systematically compare modeling results to identify the general principles - a precondition for the successful translation into clinical tools.

To bridge this gap, we have developed The Virtual Brain Ontology (TVB-O): the first Web Ontology Language (OWL2) ontology that formalizes the mathematical framework at the core of TVB, providing a valuable resource for annotation of large-scale brain network model (BNM) components, but going beyond simple annotation of terms and entities, and relationships. We demonstrate the power of our approach by relating, in a standardized 3D brain space, TVB-defined mathematical models to multiscale, multimodal biological information, by identifying the overlapping entities that are not clearly visible from the model equations. This mapping from the TVB brain regions is done to the TVBase (TVB knowledge base adapter) brain regions, that empowers the integration of literature-derived biological knowledges into a BNM.

TVB-O is providing a new modeling tool for the general neuroscientific community, including clinicians, that paves the way for a better understanding of the underlying mechanisms that are involved in the pathophysiology of specific diseases, and bears the exciting potential to provide guidance for drug discovery strategies.

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Poster

497. Network Computation I

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Topic: I.06. Computation, Modeling, and Simulation

Support: NSERC
Simon Fraser University Faculty Recruitment Grant

Title: Signal complexity of local field potentials during sleep reflects both sleep stage and learning

Authors: A. ZAHEDIFARD¹, K. SHEN², *A. MCINTOSH^{1,2};

¹Biomed. Physiol. and Kinesiology, ²Inst. for Neurosci. and Neurotechnology, Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Brain signal complexity, as measured using multiscale entropy, is thought to reflect information processing capacity. Studies have shown an increase in the multiscale entropy of brain signals in humans performing cognitive tasks, and this change may support more accurate and stable behavior. Studies have also shown a decrease in signal complexity with decreased wakefulness. However, neuroplasticity during slow wave sleep is considered an important mechanism for learning and memory consolidation. Multiscale entropy, then, may be able to capture the effects of sleep-dependent neuroplasticity in brain signals. Here, we tested the hypothesis that signal complexity varies as a function of sleep stage and learning. Using data from two different datasets collected from sleeping rats before and after a motor learning task (Eckert et al 2020; Lemke et al 2021), we computed multiscale entropy from local field potentials (LFPs) recorded in hippocampus or M1 during sleep. LFPs were considered separately for slow-wave (SWS) or non-REM (NREM) and rapid eye movement (REM) stages of sleep. For each stage type, data were epoched into 4000 ms durations and multiscale entropy was calculated for each epoch. This resulted in a minimum of 65 epochs (max = 950) across sleep stages (SWS/NREM vs REM) and condition (pre- vs post- task). Using a data-driven multivariate Partial Least Squares analysis, we compared multiscale entropy across sleep stages and conditions. The first latent variable in both datasets showed a main effect that differentiated sleep stages ($p < 0.001$), where multiscale entropy was higher in finer scales and lower in coarser scales in REM sleep. The second latent variable showed a contrast between pre- and post-learning sleep but the effect was inconsistent across datasets. In hippocampal LFPs, multiscale entropy was lower at both the finest and coarsest scales in the post-learning condition ($p = 0.013$) while we observed the opposite effect in M1 LFPs that did not reach significance ($p = 0.13$). Future work is needed to determine the effects of differences in experimental design, performance, as well as regional differences across the brain. Our scale-dependent findings suggest that brain signal complexity may be a useful measure of the neuroplasticity that occurs in sleep.

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Poster

497. Network Computation I

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Topic: I.06. Computation, Modeling, and Simulation

Support: eBRAIN-health 101058516
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Human Brain Project SGA2 785907
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ERC Consolidator 683049
German Research Foundation SFB 1436 (project ID 425899996)
SFB 1315 (project ID 327654276)

Title: Decoding endophenotypes of psychosis by combining computational semantics and multi-omics databases into a knowledge-driven model

Authors: *K. BÜLAU^{1,2}, L. STEFANOVSKI^{1,2}, L. MARTIN^{1,2}, K. DHINDSA^{1,2}, A. KODAMULLIL³, N. KOUTSOULERIS⁴, P. RITTER^{1,2,5};

¹Brain Simulation Section, Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Berlin, Germany; ²Dept. of Neurol. with Exptl. Neurol., Charité - Universitätsmedizin Berlin, Berlin, Germany; ³Fraunhofer Inst. for Algorithms and Scientific Computing SCAI, Sankt Augustin, Germany; ⁴Dept. of Psychiatry and Psychotherapy, Ludwig-Maximilians-University, Munich, Germany; ⁵Bernstein Ctr. for Computat. Neurosci. Berlin, Berlin, Germany

Abstract: Little is known about the molecular mechanisms that lead to the development of psychosis. A former study (1) found a pattern of gray matter volume changes on structural magnetic resonance imaging (sMRI) that predicts the transition of high-risk patients to psychosis with great accuracy. However, the “black box” pattern deduced by machine learning did not offer any mechanistic explanation as to the origin of this pattern and therefore no insights into possible preventive treatments.

With the new semantic mapping tool TVBase (unpublished) we aim to decode this pattern using 3D gene maps created from literature mining. These maps display the cumulated knowledge about spatially distinct associations of each gene (pathway) in the brain. Genes of interest were selected using a knowledge graph (KG) specific to psychosis - following methods for NeuroMMSig KG for neurodegenerative diseases (2).

We used a support vector regression (SVR) model to reconstruct the sMRI pattern with gene maps as features. By employing a leave-one-site-out cross-validation, as well as minimum redundancy and maximum relevance forward feature selection we found 78 gene maps that were essential for the reconstruction. The model was validated against a randomly permuted null model. Overall, the model achieved an adjusted $R^2 = .25$ significantly outperforming the null model ($\Delta = 10.47$, $p < .001$), indicating a specific reconstruction of the sMRI pattern. More importantly, the molecular role of the feature gene maps could be interpreted with the well-established STRING database (3). Selected genes were clustered with the Markov Cluster Algorithm and could be robustly linked to pathways associated with neural growth, inflammation, glutamatergic and dopaminergic signaling. High-ranking feature genes were also prevalent in a proteomic analysis of a blood sample from the same cohort used to construct the sMRI pattern.

This new approach combines computational semantics with a multi-omics database into a knowledge-driven model to mechanistically interpret an sMRI pattern derived from multimodal machine learning predicting psychosis in high-risk patients. This leads to new testable hypotheses about the mechanisms leading to the onset of psychosis. Follow-up studies testing these hypotheses in-silico, e.g., using brain simulations with the neuroinformatics platform The Virtual Brain (TVB), or in-vitro to find potential therapeutic interventions, are being under development.

References:

(1) Koutsouleris et al., 2021 - doi:10.1001/jamapsychiatry.2020.3604

(2) Domingo-Fernández et al., 2017 - doi: 10.1093/bioinformatics/btx399

(3) Szklarczyk et al., 2020 - doi: 10.1093/nar/gkaa1074

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Poster

497. Network Computation I

Location: SDCC Halls B-H

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Program #/Poster #: 497.16

Topic: I.06. Computation, Modeling, and Simulation

Support: Ontario Graduate Scholarship Award
PREVENT-AD Research Group

Title: Longitudinal sex differences in episodic memory-related brain activity and behaviour in older adults with a family history of Alzheimer's disease

Authors: *A. D. SAMSON^{1,2}, S. RAJAGOPAL³, S. PASVANIS³, A. R. MCINTOSH^{1,2,4,5}, M. N. RAJAH^{3,6};

¹Rotman Res. Inst., Toronto, ON, Canada; ²Psychology, Univ. of Toronto, Toronto, ON, Canada; ³Centre for Cerebral Imaging, Douglas Hosp. Res. Ctr., Montreal, QC, Canada; ⁴Inst. of Neurosci. and Neurotechnology, ⁵Biomed. Physiol. and Kinesiology, Simon Fraser Univ., Burnaby, BC, Canada; ⁶Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Females make up two-thirds of those diagnosed with Alzheimer's disease (AD) and have been demonstrated to be disproportionately more susceptible to AD neuropathologies when compared to males. However, why this is the case remains unclear. To best capture AD progression, it has been suggested studies focus on longitudinal brain and cognitive changes in older adults at high risk of developing AD. Episodic memory decline is a prominent cognitive feature of the early stages of late-onset sporadic AD. Therefore, in the present study, we analyzed longitudinal sex differences in episodic memory-related brain activity and its correlation with memory performance in healthy older adults with at least one first-degree family member with an AD diagnosis. Having a family history of the disease increases one's risk of AD by 30 percent. Participants were recruited for the PREVENT-AD program in Montreal, Canada (N = 192, 140 females and 52 males; $M_{\text{age at baseline}} = 63.56 \pm 4.89$ years; $M_{\text{education}} = 14.95 \pm 3.51$ years) and were observed at two different time points (baseline and 2-year follow-up). Analysis of the neuropsychological data, using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), indicated females outperformed males on immediate memory, language, delayed memory, and total RBANS scores at baseline however only females demonstrated a significant within group decrease in RBANS subtest scores over time. The greatest decrease was seen in females' RBANS language performance scores suggesting that females at risk of AD experience greater declines in language abilities compared to males. The

behavioural results from the task functional magnetic resonance imaging (fMRI) paradigm indicated a significant decrease in the ability to successfully detect new objects as new (correct rejections) during the retrieval phase of the episodic memory task in both males and females, however, on average females outperformed males across all visits on this behaviour. Using behavioural-based multivariate partial least squares of the longitudinal task fMRI, we observed sex similarities in brain activity-behaviour correlations during the episodic memory task at baseline in superior temporal and middle frontal regions. But at follow-up, we observed sex differences in brain-behaviour correlations in anterior cingulate and orbitofrontal regions during encoding and retrieval of object-location memory and recollection of new objects. Our findings provide a new perspective on sex differences in brain-behaviour relationships in people at high risk of developing AD.

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Poster

497. Network Computation I

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Program #/Poster #: 497.17

Topic: I.06. Computation, Modeling, and Simulation

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Computation of underlying data has been performed on the HPC for Research cluster of the Berlin Institute of Health
EU H2020 Virtual Brain Cloud 826421
Human Brain Project SGA2 785907
Human Brain Project SGA3 945539
ERC Consolidator 683049
German Research Foundation SFB 1436 (project ID 425899996)

Title: Enhancing the Diagnosis of the Alzheimer's Disease Spectrum with Whole-Brain Simulations and Machine-Learning

Authors: ***L. STEFANOVSKI**^{1,2}, **K. DHINDSA**^{1,2}, **L. MARTIN**^{1,2}, **K. BÜLAU**^{1,2}, **P. RITTER**^{1,2,3};

¹Brain Simulation Section, Berlin Inst. of Hlth. at Charité – Universitätsmedizin Berlin, Berlin, Germany; ²Dept. of Neurol. with Exptl. Neurol., Charité – Universitätsmedizin Berlin, corporate member of Freie Univ. Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; ³Bernstein Ctr. for Computat. Neurosci. Berlin, Berlin, Germany

Abstract: Despite achievements in the development of early biomarkers and novel techniques as machine learning (ML), the diagnosis of Alzheimer's Disease (AD) remains challenging. A recent approach showed as proof-of principle how simulated brain activity, based on an amyloid PET informed brain network model (1) constructed with The Virtual Brain software (www.thevirtualbrain.org), can improve the differential diagnosis between AD, mild cognitive impairment (MCI) and controls (2). We validated our former studies (1, 2) that were based on a small subset of 33 subjects through a larger cohort data set comprising 1127 participants with amyloid PET (AV-45 tracer) at baseline visit from the Alzheimer's Disease Neuroimaging Initiative (ADNI). We simplified our former approach by using the standard parcellation of Desikan-Killiany (DK) in contrast to the previously used Glasser parcellation. Due to the reduction of regions, the simulation time was reduced by a factor around 4 to 5. regional amyloid burden is translated into a change in excitability of a neural mass model (1). This allows for the simulation of virtual local field potentials (LFPs) in 84 brain regions, which have been shown to express different dynamics in AD and controls (1,2). These LFPs will be further used for ML of the diagnostic groups significant memory concern (SMC), early and late MCI, AD, and controls. A test set with 335 subjects with another amyloid tracer, Florbetaben, is used as technical validation handled as a second modality. We simulated virtual LFPs for 232 controls, 189 SMC, 339 early MCI, 198 late MCI, and 170 AD patients. The previously reported phenomenon of spectral bistability in the AD group (1) was reproducible with the smaller DK parcellation, again also showing LFP slowing in AD and individually distinct bifurcations in parameter space, leading to crucially different dynamic behaviors, which has formerly been shown to classify between groups (2). The averaged LFP peak frequency across parameter space differed significantly between all diagnostic groups ($p < 0.001$). This study aims to reproduce our former work with more accurate diagnostic categories meanwhile less computational effort, enabling computing a larger sample size and qualifying the approach to be translated into a clinical application for PET informed individualized brain simulation that augments dementia classification.

References:

(1) Stefanovski et al. 2019 - doi:10.3389/fncom.2019.00054

(2) Triebkorn et al. 2022. - doi:10.1002/trc2.12303

Data from ADNI - see adni.loni.usc.edu for complete investigator list at:

http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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Poster

497. Network Computation I

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Program #/Poster #: 497.18

Topic: I.06. Computation, Modeling, and Simulation

Title: Functional lateralization in Alzheimer's disease (AD): TheVirtualBrain (TVB) Computational Approach

Authors: *Y. WANG¹, L. M. ARBABYAZD², A. R. MCINTOSH³, P. RITTER⁴, V. K. JIRSA², A. J. SOLODKIN¹;

¹UT Dallas Cognition and Neurosci. Grad. Program, UT Dallas, Dept. of Neurosci., Richardson, TX; ²Inst. De Neurosciences Des Systemes UMR1106, Marseille, France; ³Inst. for Neurosci. & Neurotechnology, Simon Fraser Univ., Vancouver, BC, Canada; ⁴Charite, Univ. Med. Berlin, Charite, Univ. Med. Berlin, Berlin, Germany

Abstract: ObjectiveTVB is a multiscale approach that uses neuroimaging data to create brain dynamic models cataloging biophysical parameters and producing empirical states. In this framework, the brain is considered a complex system whose local dynamics depend on critical attractors (the critical point where the brain reverses between spatiotemporal patterns). Previously, we detected a linear increase of the critical point in a limbic subnetwork from normal subjects to those with mild cognitive impairment (aMCI) or AD. In this study, we explore if these effects are lateralized between the left and right hemispheres.**Methods**We built dynamic network models of 77 subjects (16 AD, 35 aMCI, 16 normal (NC), and 10 supernormal (SNC) controls) using imaging data from the Sydney MAS database. Under low noise conditions, simulated LFP oscillations at the critical bifurcation point emerged in the right and left posterior cingulate gyrus (PCG). Laterality indexes were calculated based on the morphometry of the simulated signals (amplitude, frequency, and phase). In addition, model-free analyses focused on weights of DTI-derived interhemispheric tracts and metaconnectivity (MC). The latter assessed spatiotemporal properties of the fluctuating networks by measuring time-varying similarities of dynamic *edges* derived from dynamical functional connectivity matrices. Significance between groups was done via permutation statistics. **ResultsTVB:** **1.** All Laterality metrics were biased to the right hemisphere **2.** Lateralization for amplitude and frequency of LFP oscillations showed an inverted quadratic trend across groups, with the highest values in AD and the largest differences in the gamma frequency domain **3.** The phase delay of theta oscillations between hemispheres were shorter in healthy controls compared to aMCI and AD but did not reach statistical significance.**Model-free:** **1.** There was a linear decrease in the weights of interhemispheric structural connections from SNC to AD **2.** MC showed a significant reduction in the temporal stability of specific functional links, connecting the PCG with the rest of the limbic subnetwork. The largest decrease of MC was seen in the connectivity between left and right PCG. **Interpretation**This work presents converging mechanistic evidence on the progressive deterioration of the essential role of the PCG from the readiness of the system to change between states (criticality) to the high integration represented by the patterns of connectivity between hemispheres. Furthermore, as brain laterality patterns changed across groups, we suggest these metrics could aid in predicting those NC or aMCI subjects who may convert to AD in the near future.

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Poster

497. Network Computation I

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 497.19

Topic: I.06. Computation, Modeling, and Simulation

Support: DARPA W911NF1820264
NSF CRCNS Japan-US 2113096
NIH R21-NS11361
USC Research Enhancement Fellowship
Consejo Nacional de Ciencia y Tecnología (CONACYT-Mexico)

Title: The translation of in-air movement to on-ground locomotion of a tendon-driven quadruped through adaptive learning

Authors: *T. FANELLE¹, D. URBINA-MELÉNDEZ¹, S. CHAKRAVARTHI RAJA², A. MARJANINEJAD¹, F. J. VALERO-CUEVAS³;

¹Dept. of Biomed. Engin., ²Dept. of Electrical and Computer Engin., USC, Los Angeles, CA;

³Biomed. Engin., USC, La Crescenta, CA

Abstract: The ability to take a known skill and adapt it to a novel task is fundamental to lifelong learning (Kudithipudi et al., 2022). Here we show in hardware that the knowledge a quadruped gains by babbling and refining movement in-air is beneficial to further learning when transitioning from movement in-air to on-ground (Marjaninejad, Urbina-Melendez, et al., 2019b; Marjaninejad, 2021). The quadruped creates an implicit model of its own kinematics by undergoing five minutes of motor babbling and training an artificial neural network (ANN) to produce the inverse kinematics through usage of the General-to-Particular (G2P) autonomous learning algorithm (Marjaninejad, Urbina-Melendez, et al., 2019a; Sun et al., 2019). By feeding a set of desired kinematics into this ANN we produce a set of motor activations that the model predicts will result in those kinematics; the error between desired and actual kinematics obtained from the activations can then be used to refine the model with a few-shot learning approach. The robot went through eight refinements of in-air movement and was then placed in contact with the ground to continue training. Because the quadruped is first trained in air, the model predicted lower muscle activations than the ones really needed to overcome contact with the ground. To counteract this, a velocity compensation term based on the positional and cumulative error of each trial is added to the desired kinematics (Marjaninejad, Urbina-Melendez, et al., 2019b) resulting in an increased muscle activation at points of previously too low activation and decreased muscle activation at points of previously too high activation. Critically, this velocity compensation term is used as the connecting link between the quadruped's knowledge of in-air movements and the final explorations to complete the translation to on-ground locomotion. The current results of this study show the usefulness of transferring learned in-air movements to on-ground locomotion (Kudithipudi et al., 2022), but also highlight the need for systems to be able to adapt as they learn in order to transfer learned skills to novel situations (Marjaninejad, Urbina-Melendez, et al., 2019b). Acknowledgments: The authors acknowledge the contributions of Irie Cooper, Yifan Xue, and Jan Lao.

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Poster

497. Network Computation I

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Program #/Poster #: 497.20

Topic: I.06. Computation, Modeling, and Simulation

Support: ANR-20-CE45-0005-01 (Brain-Net)

Title: Unsupervised classification of vocal and speech patterns with artificial spiking neural networks

Authors: *S. POKALA, M. LION, B. YVERT;
Univ. Grenoble Alpes, Inserm, U1216, Grenoble Inst. Neurosciences, Grenoble, France

Abstract: A fundamental task that human beings do on a daily basis is pattern recognition and the human brain performs this very efficiently. Taking general inspiration from the human brain, Artificial Neural Networks (ANNs) are being developed to mimic this ability. Deep Neural Networks (DNNs) based on global learning with backpropagation are making strides in reproducing this ability and are successfully used for various static and sequential pattern recognition applications. However, they come with their limitations - they usually require training on large datasets and heavy computations. An alternative, aimed at addressing these issues, are Spiking Neural Networks (SNNs) which are neuromimetic and use more biologically plausible event-based neurons with evolving membrane potentials that emit spikes. They need fewer data to train thanks to local learning rules and can be implemented on low-power neuromorphic hardware. They are thus the next generation of AI, but, require dedicated architectures. Therefore, developing new SNN architectures dedicated for complex pattern recognition tasks is crucial. In this context, we developed a potentially energy-efficient SNN with Spike-Timing-Dependent-Plasticity (STDP) for unsupervised classification of vocal and speech patterns. We built a network with Low Threshold Spiking (LTS) neurons. Two encoding methods were tested to transform the audio data into spike trains - i) ‘time-to-first-spike’ encoding of spectrograms and ii) Short Term Plasticity (STP) based encoding of MELs. We assessed the performance of our network on synthetic spectrotemporal patterns mimicking vocal spectrograms and a real vowel dataset which allowed us to evaluate the network’s output with respect to the known ground truth. Such a network could successfully classify several different patterns and remained very robust when noise was added to alter the patterns. The network was also tested on a minipig vocalization dataset for which the ground truth is unknown but estimated by an expert. The ability to classify vocal patterns with a rather simple SNN architecture opens new possibilities for fully unsupervised complex spectrotemporal pattern detection with SNNs that are compatible with low-power hardware.

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Poster

497. Network Computation I

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Topic: I.06. Computation, Modeling, and Simulation

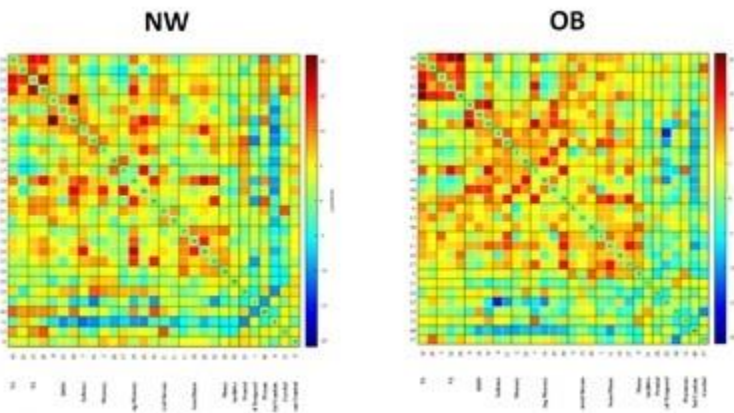
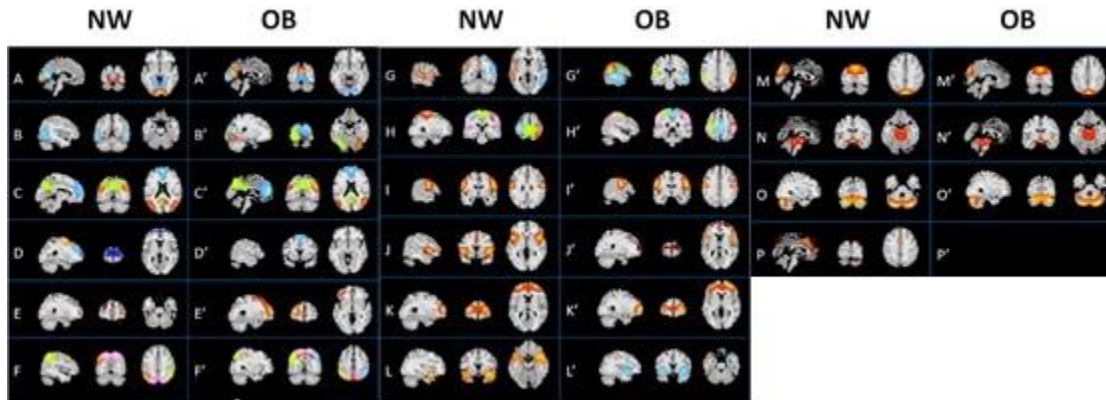
Support: FONCICYT/37/2016, I000/667/2016

Title: Resting state mapping of infants, dependence with BMI

Authors: *B. DE CELIS ALONSO¹, M. ANTONIO DE LA ROSA², S. HIDALGO-TOBÓN³, P. DIES SUÁREZ⁴, E. BARRAGÁN LÓPEZ⁵, B. LÓPEZ MARTÍNEZ³, P.-W. SO⁶;

¹Fac. of Physical and Mathematical Sci., Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; ²Fac. of Physical and Mathematical Sci., Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; ⁴Imagenology Dept., ⁵Neurol. Dept., ³Hosp. Infantil de México Federico Gómez, CDMX, Mexico; ⁶Dept. of Neuroimaging, Inst. of Psychiatry, Psychology and Neurosci., King's Col. London, London, United Kingdom

Abstract: **Aim:** Search for differences in Resting State networks when comparing an infant population of obese and normo-weighted subjects. **Methods:** 126 children with ages between 7 and 9 years were classified into two BMI groups Obese OB and Normo-weight NM). All subjects were righthanded, had no present or previous neurological disorders and were not depressed. MR resting state and anatomical imaging was performed. **Results:** 16 (NW) and 15 (OB) different networks were found for both groups (**Figure 1**). Different connectivity between groups was also found (**Figure 2**). Figure 1. Resting Networks for NW and OB groups. A corresponds to Visual 1, B to visual 2, C Default Mode Network, D Salience, E Memory, F Working Memory, G ventral Stream, H Senso-Motor, I Motor, J Auditive, K Frontoparietal, L Medial Temporal, M Precuneus, N Thalamic-Caudate, O Cerebellar, P Executive Control. In Figure 2 we can observe different connectivities between the different networks. Discussion and Conclusions. Both groups presented different connectivity “strength”. In figure 2, the OB group had larger strengths for both memory networks as well as both visual networks. From Figure 1 we can see that all networks, even if similar in shape, covered different regions, like for the default mode, auditive, salience, ventral stream or medial temporal. An extraordinary result is that the executive control network was not found in the OB group. All these findings show a completely different regulation of behaviors mediated by BMIs.



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Poster

497. Network Computation I

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Program #/Poster #: 497.22

Topic: I.06. Computation, Modeling, and Simulation

Support: Howard Hughes Medical Institute
 DFG EXC-Number 2064/1, Project number 390727645

Title: Connectome constrained simulations with task optimization lead to accurate predictions of tuning properties in the fruit fly visual system

Authors: *J. K. LAPPALAINEN^{1,3}, F. D. TSCHOPP³, S. PRAKHYA³, M. MCGILL^{4,3}, A. NERN³, K. SHINOMIYA³, S.-Y. TAKEMURA³, E. GRUNTMAN³, J. H. MACKE², S. C.

TURAGA³;

¹Machine Learning in Sci., Tübingen Univ., Tübingen, Germany; ²Machine Learning in Sci., Tübingen Univ., Tübingen, VA; ³HHMI Janelia Res. Campus, Ashburn, VA; ⁴Computation and Neural Systems, Caltech, Pasadena, CA

Abstract: How can connectomic reconstructions of neural circuits be incorporated into models of neural computation? While connectomic reconstructions and transcriptomic profiling can suggest the relative strengths of synaptic connections and their signs, electron microscopy cannot be used to infer single neuron dynamical properties like time constants and resting potentials, essential for simulating a neural circuit. We hypothesize that these unknown parameters can be estimated by using machine learning techniques to optimize a biophysics and connectome constrained simulation to perform a task. We built a recurrent dynamical model with 44,228 neurons representing 721 visual columns and 64 cell types in the first two stages of the *Drosophila* visual system, combining connectivity constraints with estimates of synapse signs. We use simplified point neuron models with threshold-linear passive dynamics for the voltage, and graded release synapses. Since this circuit is known to compute visual motion, we optimize the simulation to compute optic flow in a computer vision dataset. We characterized the tuning properties of individual cell types and their variability across 50 task-optimized models. Impressively, the predictions of our model match the known on and off selectivity properties of all cell types in the circuit for which such data are available. Notably, networks correctly predict motion selectivity of well-known elementary motion detector neurons.

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Poster

497. Network Computation I

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Program #/Poster #: 497.23

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH/ NIDCD R01DC018227 (AF)
NIH/ NINDS Brain Initiative 1UF1NS115779 (AF and GLC)

Title: Temporal progression through metastable coding states during decision-making in the mouse gustatory cortex

Authors: ***L. LANG**, G. LA CAMERA, A. FONTANINI;
Stony Brook Univ., Stony Brook, NY

Abstract: The mouse gustatory cortex (GC) is involved in taste-guided decision-making in addition to sensory processing. Rodent GC exhibits metastable ensemble dynamics during ongoing and stimulus-evoked activity, but how these dynamics might evolve in the context of a

taste-based decision-making task remains unclear. Here we employed analytical and modeling approaches to i) extract metastable dynamics in ensemble spiking activity recorded from GC of mice performing a perceptual decision-making task; ii) investigate the computational mechanisms underlying GC metastability in this task; and iii) establish a relationship between GC dynamics and behavioral performance. Our results show that activity in GC during perceptual decision-making is metastable and that this metastability may serve as a substrate for sequential encoding of sensory, abstract cue, and decision information over time. Our model of GC captures the neural dynamics and behavioral performance observed experimentally and offers testable predictions about GC network structure and function. Perturbations of the model indicate that boosting inhibition during different coding epochs differentially impacts network performance and suggest an explanation for a counterintuitive effect of GC optogenetic silencing on mouse behavior.

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Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.01

Topic: I.06. Computation, Modeling, and Simulation

Support: EU Horizon 2020, Specific Grant Agreement No. 945539 (Human Brain Project SGA3).

Title: Autoalign rat: spatially anchoring rat brain histology to the waxholm rat brain atlas using deep neural networks.

Authors: *H. CAREY, G. CSUCS, M. PUCHADES, J. G. BJAALIE;
Univ. of Oslo, Oslo, Norway

Abstract: The rat offers many benefits over the mouse for experimental neuroscience, though the popularity of the mouse has surged in recent years due to an ever-growing genetic toolbox. This toolbox is now being expanded to rats and will surely lead to an explosion in rat brain data. To prepare for this we must build tools to spatially anchor rat brain data into a common reference space, enabling comparison and meta-analysis of anchored data. To this end we have created AutoAlign, a deep learning toolbox which spatially anchors rat brain histology to the Waxholm Space atlas of the rat brain (RRID: SCR_017124). AutoAlign can anchor rat brain histology cut coronally, sagittally, and horizontally, here we compare the accuracy of the algorithm to humans across each of these planes. While anchoring a whole-brain dataset would take an anatomist many hours, AutoAlign achieves this in seconds. AutoAlign is compatible with the QUINT workflow, including QuickNII (RRID: SCR_016854), allowing predictions to be modified by users, and VisuAlign (RRID: SCR_017978) enabling the atlas to be warped and deformed to

anchored sections. AutoAlign Rat is freely available as both a Python package and web application.

Disclosures: H. Carey: None. G. Csucs: None. M. Puchades: None. J.G. Bjaalie: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

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Program #/Poster #: 498.02

Topic: I.07. Data Analysis and Statistics

Support: This project has received funding from the European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3).

Title: Quint workflow for brain-wide quantification of rodent models: new functionality for high-throughput studies

Authors: *S. C. YATES¹, B. N. GURDON², G. CSUCS¹, N. E. GROENEBOOM¹, M. A. PUCHADES¹, C. KACZOROWSKI², J. BJAALIE¹;

¹Univ. of Oslo, Oslo, Norway; ²The Jackson Lab., Bar Harbor, ME

Abstract: Sectioned material from brains of rodent models is used to explore the cellular and molecular composition in the normal brain and in models of disease mechanisms. The QUINT workflow was developed to support standardized atlas-based analysis of sectioned tissue without the need for coding ability. It is shared on the EBRAINS Atlas Services as a suite of open-source tools that can be flexibly combined to meet the needs of diverse projects (ebrains.eu). This includes tools for atlas-registration, feature extraction and quantification in regions defined by a reference atlas of the brain. New functionalities have been added to the QUINT workflow to meet the needs of a variety of projects, including a novel Alzheimer's disease project involving a large population of genetically diverse mice: the AD-BXD panel (Neuner et al, Neuron 2019, PMID: 30595332). This includes QCAAlign: a tool for assessing the quality of section images, as well as the quality of the registration of atlas to sections as performed with the registration tools. By supporting systematic assessment of histological material, QCAAlign allows the removal of damaged sections, and the post-processing of results according to strict criteria. In addition, QCAAlign makes it easier for users to explore the atlas hierarchy and to decide on a customized hierarchy level for the investigation. As a proof-of-concept, the QCAAlign tool was applied to data from the Alzheimer's project to detect and remove sections with more than 30% damage. It was also used to assess the quality of the atlas-registration before and after application of nonlinear refinements to the registration. To summarize, QCAAlign tackles challenges posed by high-throughput studies and expands the scope of the QUINT workflow for comprehensive analysis.

Disclosures: S.C. Yates: None. B.N. Gurdon: None. G. Csucs: None. N.E. Groeneboom: None. M.A. Puchades: None. C. Kaczorowski: None. J. Bjaalie: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.03

Topic: I.07. Data Analysis and Statistics

Title: Neuropedia: an online tool for depositing and sharing digital brain maps.

Authors: ***J. HECKSHER-SØRENSEN**, J. PERENS, J. LERCKE SKYTTE, U. ROOSTALU, C. GRAVESEN SALINAS;
Gubra ApS, Hørsholm, Denmark

Abstract: Light-sheet fluorescence microscopy (LSFM) in combination with whole organ immunolabelling has made it possible to visualize intact mouse brains with single cell resolution. However, the price for this level of detail comes in form of enormous datasets that often challenges extraction of quantitative information. One approach for analyzing whole brain data is to align the scanned brains to a reference brain atlas. Having a fixed spatial reference provides each voxel of the sample brains with x-, y-, z-coordinates from which it is possible to obtain anatomical information on the observed fluorescence signal. An additional and important benefit of aligning light sheet data to a reference brain is that the aligned data provides a digital map of gene expression or cell counts which can be deposited in databases or shared with other scientists. To facilitate sharing of digital brain maps we have developed an open access data base called NeuroPedia (<https://www.neuropedia.dk/>). This enables researchers to perform virtual neuroscience by overlaying maps derived from either drug induced neural activity, gene expression, transgenic expression and connectivity.

Disclosures: **J. Hecksher-Sørensen:** A. Employment/Salary (full or part-time); Gubra ApS. **J. Perens:** A. Employment/Salary (full or part-time); Gubra ApS. **J. Lercke Skytte:** A. Employment/Salary (full or part-time); Gubra ApS. **U. Roostalu:** A. Employment/Salary (full or part-time); Gubra ApS. **C. Gravesen Salinas:** A. Employment/Salary (full or part-time); Gubra ApS.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.04

Topic: I.07. Data Analysis and Statistics

Support: NIH R24-MH-114793

Title: The Brain Image Library- a brain microscopy resource

Authors: M. KENNEY¹, G. HOOD¹, A. WETZEL¹, L. TUIITE¹, I. CAO-BERG¹, M. BRUCHEZ², A. M. WATSON³, I. VASYLIEVA³, *A. ROPELEWSKI¹;

¹Pittsburgh Supercomputing Ctr., ²Dept. of Chem., Carnegie Mellon Univ., Pittsburgh, PA; ³Ctr. for Biologic Imaging, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The Brain Image Library (BIL) is a public resource serving the neuroscience community by providing a persistent centralized repository for brain microscopy data. The BIL framework is supported by rigorous metadata standards and DOI indexing ensuring that thousands of contributed microscopy datasets are findable, accessible, interoperable, and reusable (FAIR).

Here we describe BIL, the data contained within, how to get started using BIL data, and how to contribute your own research data to BIL. We also describe the BIL analysis ecosystem that provides an integrated computational and visualization system to explore, visualize, and access BIL data in-place without downloading it. Furthermore, we describe our innovative on-demand data transformation approach that services scaled or full-resolution voxel-level data requests in a variety of common visualization and analysis formats.

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Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.05

Topic: I.07. Data Analysis and Statistics

Support: IARPA 2017-17032700004

Title: NeuVue: a platform for high-throughput connectomics proofreading for iarpa microns dataset

Authors: *D. XENES, L. KITCHELL, P. RIVLIN, R. BRODSKY, H. GOODEN, J. JOYCE, D. LUNA, R. NORMAN-TENAZAS, D. RAMSDEN, K. ROMERO, V. ROSE, W. R. GRAY RONCAL, B. A. WESTER;

Applied Physics Lab., Johns Hopkins Univ., Laurel, MD

Abstract: NeuVue is a software platform created for large-scale proofreading of the “Minnie65” connectomics dataset generated on the Intelligence Advanced Research Projects Activity (IARPA) Machine Intelligence from Cortical Networks (MICrONS) program. The NeuVue platform provides a robust web-based interface for proofreaders to collaboratively view, annotate, and edit the segmentation and connectivity data generated from the electron

microscopy image data. The NeuVue interface provides a number of quality-of-life features that streamline complex editing operations such as splitting and merging objects in dense nanoscale segmentation. A backend queuing service organizes proofreader tasks into specific task types and thereby increases proofreader throughput by scoping proofreader actions to simpler, atomic operations on the data. A collection of analytical dashboards, data visualization tools, and Application Program Interface (API) provides real-time assessment of proofreading activities. NeuVue leverages cloud resources and production-quality features such as load-balancing and auto-scaling to enable proofreaders to simultaneously access and edit data on the platform. NeuVue is currently powered by well-supported open-source community tools such as Neuroglancer, PyChunkedGraph, and CAVE (Connectome Annotation Versioning Engine), and could point to other tools and data sources to pull image and segmentation data and post proofreading updates.

Proofreading on the MICrONS dataset utilizing the NeuVue platform has yielded over 40,000 edits distributed across two petavoxels of neuroimaging data, including correctively splitting over 10,000 falsely merged neurons from multi-cell objects. Forty-four proofreaders of various skill levels have cumulatively logged 3,300 proofreading hours, making a significant impact on the underlying connectivity of a large percentage of the 75,000+ neurons in the volume. With continued development on the platform and proofreader model, and integration of semi-automated and automated error detection and error correction methods, we believe that high-throughput proofreading will be achievable for any large-scale connectomics datasets of the future.

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Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

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Program #/Poster #: 498.06

Topic: I.07. Data Analysis and Statistics

Support: IARPA Grant D16PC00004

Title: A scalable and modular automated pipeline for stitching of large electron microscopy datasets

Authors: *G. MAHALINGAM¹, R. TORRES¹, D. KAPNER¹, E. T. TRAUTMAN³, T. FLISS¹, S. SESHAMANI⁴, E. PERLMAN⁵, R. YOUNG¹, S. KINN¹, J. BUCHANAN², M. M. TAKENO⁶, W. YIN, Esq.⁷, D. J. BUMBARGER⁶, R. GWINN⁹, J. K. NYHUS¹, E. LEIN⁸, S. J. SMITH⁷, C. REDD¹, K. KHAIRY¹⁰, S. SAALFELD¹¹, F. C. COLLMAN⁷, N. M. DA COSTA⁷; ²Neural Coding, ¹Allen Inst. for Brain Sci., Seattle, WA; ³Scientific Computing, Howard Hughes Med. Inst., Ashburn, VA; ⁴Synapse Biol., Allen Inst., Seattle, WA; ⁵Yikes LLC, Baltimore, MD;

⁶Neural Coding, ⁸Human Cell Types, ⁷Allen Inst. For Brain Sci., Seattle, WA; ⁹Epilepsy surgery and functional neurosurgery, Swedish neuroscience institute, Seattle, WA; ¹⁰Developmental Neurobio., St. Jude Children's Res. Hosp., Memphis, TN; ¹¹Max Plank Inst., Dresden, Germany

Abstract: Serial-section electron microscopy (ssEM) is the method of choice for studying macroscopic biological samples at extremely high resolution in three dimensions. In the nervous system, nanometer-scale images are necessary to reconstruct dense neural wiring diagrams in the brain, so called connectomes. In order to use this data, consisting of up to 10^8 individual EM images, it must be assembled into a volume, requiring seamless 2D stitching from each physical section followed by 3D alignment of the stitched sections. The high throughput of ssEM necessitates 2D stitching to be done at the pace of imaging, which currently produces tens of terabytes per day. To achieve this, we present a modular volume assembly software pipeline ASAP (Assembly Stitching and Alignment Pipeline) that is scalable to datasets containing petabytes of data and parallelized to work in a distributed computational environment. The pipeline is built on top of the Render services used in the volume assembly of the brain of adult *Drosophila melanogaster* fly_brain. It achieves high throughput by operating on the meta-data and transformations of each image stored in a database, thus eliminating the need to render intermediate output. ASAP is modular, allowing for easy incorporation of new algorithms without significant changes in the workflow. The entire software pipeline includes a complete set of tools for stitching, automated quality control, 3D section alignment, and final rendering of the assembled volume to disk. ASAP has been deployed for continuous stitching of several large-scale datasets of the mouse visual cortex and human brain samples including one cubic millimeter of mouse visual cortex (Yin et al. 2020) at speeds that exceed imaging. The pipeline also has multi-channel processing capabilities and can be applied to fluorescence and multi-modal datasets like array tomography.

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Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.07

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF NeuroNex Technology Hub 1707356
NSF NeuroNex 2 2014862

Title: Improved downstream neuron segmentation with SWiFT alignment and Local Shape Descriptors

Authors: ***V. THIYAGARAJAN**¹, A. SHERIDAN², A. WETZEL⁵, T. M. BARTOL, Jr.³, J. M. MENDENHALL¹, J. YANCEY³, J. CARSON⁶, T. J. SEJNOWSKI⁴, K. M. HARRIS¹, U. MANOR²;

¹Ctr. for Learning and Memory, Univ. of Texas at Austin, Austin, TX; ²Waitt Advanced Biophotonics Core, ³CNL-S, ⁴Salk Inst., La Jolla, CA; ⁵Pittsburgh Supercomputing Ctr., Pittsburgh, PA; ⁶Texas Advanced Computing Ctr., Austin, TX

Abstract: In 3D electron microscopy, alignment is indispensable for downstream tasks including annotation, segmentation, reconstruction, and analysis. We explored the effects of improved alignment on automatic segmentation for three densely-annotated EM volumes. We trained a 3D-UNet to learn voxel-wise direct neighbor affinities for neuronal boundary detection, along with Local Shape Descriptors (LSDs) as an auxiliary learning task. LSDs are 10-dimensional embeddings that encode local spatial statistics of the object to be segmented, and have been shown to increase accuracy of affinity-based methods. Ground truth labels, along with original alignment of the EM images, were generated manually in RECONSTRUCT and used to train the baseline model. All models were trained on A100 GPUs from Lonestar6 at Texas Advanced Computing Center (TACC). A re-alignment of the series was generated using the Signal Whitening Fourier Transform Image Registration (SWiFT-IR) technique, which is robust to typical image distortions and defects. The original labeling was transformed to overlay onto the re-aligned stack, which were used together to train an identical model. Inference was done on both the original and re-aligned series which contained each of the annotated training volumes. Segmentations were obtained using standard post-processing techniques, and then evaluated. Preliminary results show that the predicted affinities are significantly better on the re-aligned volume compared to those generated on the original alignment, even by the baseline model. The affinities are less prone to false negatives when encountering oblique membranes within a section, resulting in fewer merge errors in the segmentation. These results suggest that with improved alignment, LSDs infer with greater confidence the local spatial features of the target object. This leads to more accurate affinities and subsequent segmentations.

Disclosures: **V. Thiyagarajan:** None. **A. Sheridan:** None. **A. Wetzel:** None. **T.M. Bartol:** None. **J.M. Mendenhall:** None. **J. Yancey:** None. **J. Carson:** None. **T.J. Sejnowski:** None. **K.M. Harris:** None. **U. Manor:** None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

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Program #/Poster #: 498.08

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH P41GM103712
NSF DBI-1707356
NSF DBI-2014862

Title: Alignem-swift: open-source software for aligning electron micrographs using signal whitening fourier transforms

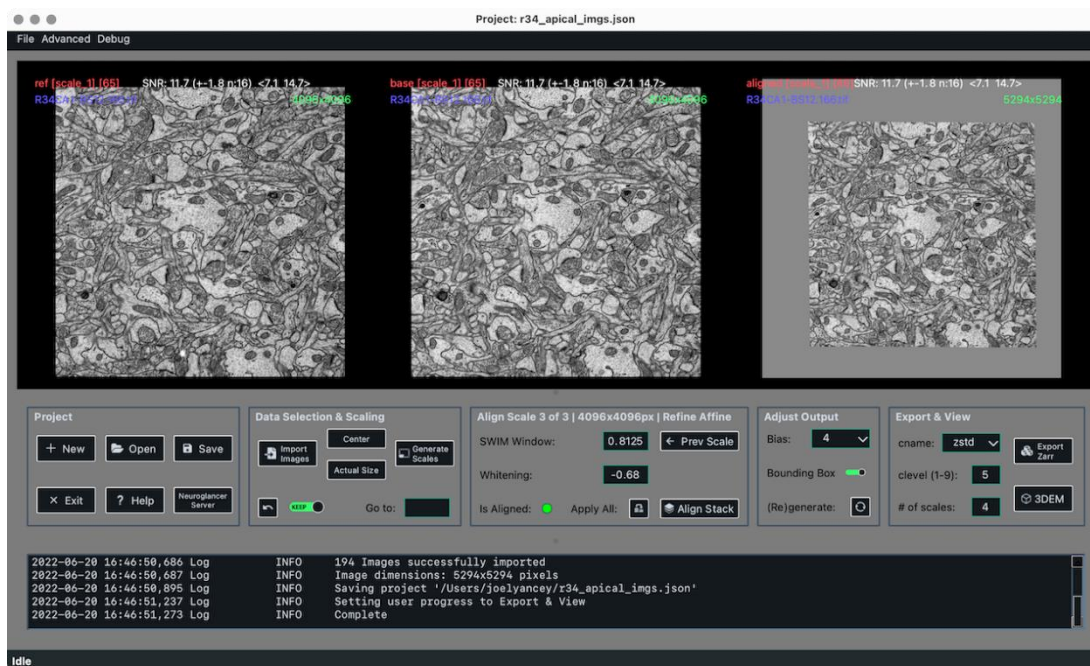
Authors: *J. G. YANCEY¹, T. M. BARTOL¹, A. WETZEL², J. CARSON³, J. M. MENDENHALL⁴, V. THIYAGARAJAN⁴, M. KUWAJIMA⁴, K. M. HARRIS⁴, T. J. SEJNOWSKI¹;

¹Computat. Neurobio. Lab., Salk Inst., La Jolla, CA; ²Pittsburgh Supercomputing Ctr., Pittsburgh, PA; ³Texas Advanced Computing Ctr., Austin, TX; ⁴Ctr. for Learning and Memory, Univ. of Texas at Austin, Austin, TX

Abstract: We have built an intuitive graphical user interface for aligning serial section electron micrographs (ssEM) using Signal Whitening Fourier Transforms (SWiFT). *AlignEM-SWiFT* is a graphical extension of *SWiFT-IR*, a proven suite of image registration programs developed by computer scientist Arthur Wetzel at the Pittsburgh Supercomputing Center. The *SWiFT-IR* approach achieves high precision image matching but requires specific mathematical understanding that limits its accessibility.

AlignEM-SWiFT is a complete EM alignment solution able to generate scale image hierarchies, compute affine transforms, generate aligned images using multi-image rendering, generate model images using remodeling, and create alignments with a global 3D coordinate system based on projections through multiple sections. Alignments can be exported to several scaled, chunked, and compressed file formats including Zarr. It features an embedded Neuroglancer viewer for instantaneous volumetric rendering within the application window. Our application circumvents shell scripting by generalizing low-level computer instructions and forging useful high-level abstractions. The control panel adjusts to user needs based on a checkpoint mechanism for tracking project completeness. It also has a terminal-like output display for monitoring running processes and a detachable project inspector. Users new to EM alignment will benefit from on-board documentation, descriptive warning dialogs, and instructive tooltips. Advanced controls and debugging features are available in the menubar. We are developing support for user-defined alignment scenarios or "recipes" comprised of interchangeable alignment modules or "ingredients".

AlignEM-SWiFT is deployed and available to the community via the 3DEM.org Workbench at the Texas Advanced Computing Center (TACC). This work is part of an effort to integrate with other open-source EM technologies being developed at TACC. Our integrated 3DEM analysis platform will include tools for segmentation, annotation, reconstruction, and tomography.



Disclosures: J.G. Yancey: None. T.M. Bartol: None. A. Wetzel: None. J. Carson: None. J.M. Mendenhall: None. V. Thiyagarajan: None. M. Kuwajima: None. K.M. Harris: None. T.J. Sejnowski: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.09

Topic: I.06. Computation, Modeling, and Simulation

Support: NIM Grant MH124566

Title: An AI Extended Solution for Performing Integrated 3D Neuro Microstructure and Nanostructure Analysis of Expansion Microscopy Data

Authors: *M. L. HEAL¹, *N. J. O'CONNOR¹, A. L. WILLSON¹, A. RODRIGUEZ¹, N. ROUSSEL¹, B. J. EASTWOOD¹, B. AN², K. LEUNG³, J. KANG³, M. SCHROEDER⁴, Y. LEE^{3,5}, E. S. BOYDEN³;

¹MBF Biosci., Williston, VT; ²Neurosci., ³MIT, Cambridge, MA; ⁴Brain and Cognitive Sci., MIT Grad. Brain and Cognitive Sci., Cambridge, MA; ⁵Howard Hughes Med. Inst., Cambridge, MA

Abstract: Expansion microscopy (ExM) is a groundbreaking technique to visualize biological structures at nanoscale resolution. By isotropically expanding biological tissues more than ten-

fold their physical size, researchers can overcome limitations of diffraction, and image messenger RNAs (mRNAs), proteins, and other biomolecules and sub-cellular structures with a variety of traditional and contemporary light microscopy techniques. More than just a method to circumvent electron microscopy for ultrastructural analysis, ExM has a myriad of advantages - including the ability for repeated hybridization, repeated antibody staining, and repeated multi-channel imaging. To enable thorough investigation of tissues processed and imaged with ExM methods, we're presenting new software tools in NeuroLucida 360 that can be used to perform comprehensive and complex 3D neuromorphological reconstruction and analysis of the spatial distribution of nanoscale populations (mRNAs, proteins, etc.) and their proximity to neuronal and vascular structures (somas, axons/dendrites, spines, synapses, vessels) across dozens of imaging channels from repeated imaging rounds. The new toolset will include automatic alignment and assembly of repeated ExM images into a single 3D registered dataset. This is a necessary and computationally-intensive step in analyzing ExM data - a task that our software will do without requiring the user to have any programming experience. Robust quantification of the size, proximity, and distribution of sub-cellular structures visible in ExM data is made easy in NeuroLucida 360 with puncta detection tools that utilize machine-learning (ML) algorithms. Further software advancements include dendritic spine modelling enhancements, new ML-based axon/dendrite tracing algorithms, and integrated methods to model and quantify interactions between neurons, puncta, and the cerebral microvasculature. With innovative ExM and NeuroLucida 360, researchers can fully explore the spatial transcriptome and proteome of the central nervous system on the micro and nanoscale. Together, these technologies will be an invaluable system for developing and evaluating novel treatments against neurodevelopmental, neuropsychiatric, neurodegenerative, neuroimmune, and neurological diseases.

Disclosures: **M.L. Heal:** A. Employment/Salary (full or part-time); MBF Bioscience. **N.J. O'Connor:** A. Employment/Salary (full or part-time); MBF Bioscience. **A.L. Willson:** A. Employment/Salary (full or part-time); MBF Bioscience. **A. Rodriguez:** A. Employment/Salary (full or part-time); MBF Bioscience. **N. Roussel:** A. Employment/Salary (full or part-time); MBF Bioscience. **B.J. Eastwood:** A. Employment/Salary (full or part-time); MBF Bioscience. **B. An:** None. **K. Leung:** None. **J. Kang:** None. **M. Schroeder:** None. **Y. Lee:** None. **E.S. Boyden:** None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.10

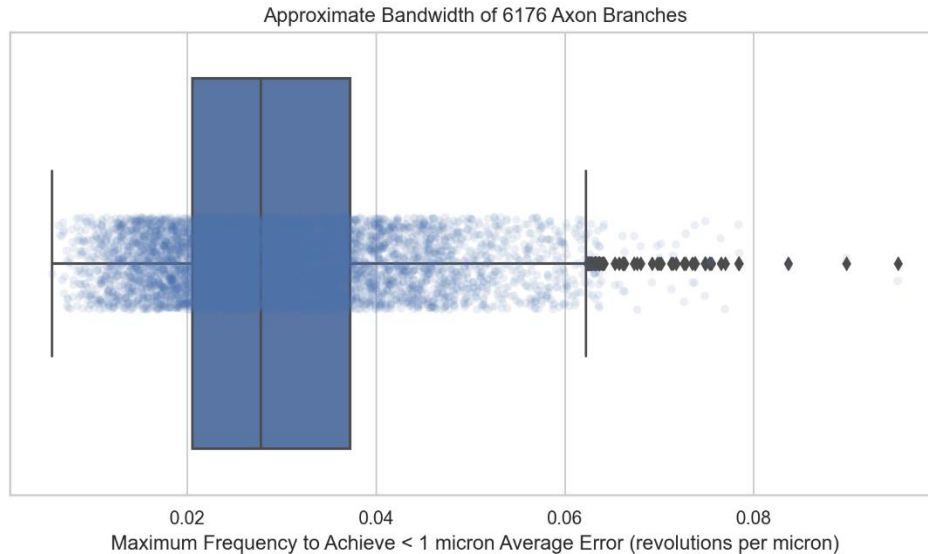
Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant 1RF1MH121539-01

Title: Nyquist Sampling Rate for Projection Neuron Reconstruction

Authors: *T. ATHEY, J. VOGELSTEIN, M. MILLER;
Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract:



Advances in tissue preparation and microscopy have made it possible to image entire mammalian brains at sub-micron resolution. The international neuroscience community is using these tools to generate comprehensive atlases of neuron subtypes, which involves tracing the morphology of thousands of neurons. Neuron tracing protocols vary between labs, however, and there are no quantitatively based guidelines on how neurons should be traced. Neurons are traced by placing points periodically along their processes, i.e. they are discretely sampled, so we study neuron sampling from the perspective of the Nyquist-Shannon sampling theorem. This theorem states that a bandlimited signal can be perfectly reconstructed from a uniform sampling of, at least, half the frequency of the signal's maximum bandwidth. We estimated the spatial bandwidth of projection neuron axons in mice that were traced as part of the Janelia MouseLight project. We fit a finite cosine series representation to these neuron traces using least squares and determined how many cosine terms were needed to achieve an average error below one micron, which is the resolution of the underlying image. On a dataset of 6176 axon branches, we found that 99% of the branches could be approximated to sub-micron accuracy using spatial frequencies below 1/15 revolutions per micron (see figure). Applying the Nyquist theorem, we conclude that projection neuron axons should be traced by placing points at a spacing of no more than 30 microns, to capture the underlying axonal geometry. We note that our analysis employs uniform sampling theory, while work in nonuniform sampling theory has shown that random additive sampling schemes, i.e. random spacing between sample points, can further reduce aliasing and thus may allow for even sparser sampling schemes. Further, other types of neuronal branches, such as dendrites or interneuron axons, may have different spatial frequency content, and therefore require different sampling schemes. Applying our approach to other cell types would be an interesting line of future work.

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Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.11

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R01NS39600
NIH Grant R01NS86082
NIH Grant U01MH114829
NIH Grant RF1MH128693

Title: A Universal Conversion Service of Digital Reconstructions into the SWC Standard Community Format

Authors: ***B. LJUNGQUIST**¹, K. MEHTA¹, J. OGDEN¹, S. NANDA¹, R. G. ASCOLI¹, L. NG², G. A. ASCOLI¹;

¹Ctr. for Neural Informatics, Structures & Plasticity, Krasnow Inst. for Advanced Study, and De, George Mason Univ., Fairfax, VA; ²Allen Inst. For Brain Sci., Seattle, WA

Abstract: Branching morphology of neural arbors (axons, dendrites, and glial processes) is a central and dynamic field of study in neuroscience. Thousands of labs worldwide routinely collect and openly share digital reconstructions of neural morphology from a broad variety of research designs, experimental techniques, imaging modalities, and tracing software. This heterogeneity has resulted in the proliferation of a diversity of file formats to represent digital reconstructions. The SWC format has emerged as the most broadly recognized and used format in the community, with a rich ecosystem of related software for tracing, visualizing, analyzing, and modeling neural morphology. It is non-proprietary, open-source, and readable by both humans and machines. However, conversion to this format from others is challenging, as there hitherto exists no universal converter and several formats are not publicly described.

Furthermore, multiple variants of the SWC format definition and interpretation have emerged, causing occasional misunderstandings and confusion among researchers. We present here a standardized specification for the SWC file format describing minimum requirements and optional extensions. We have made the SWC file format specification publicly available, following and promoting FAIR principles, and initiated version management to encourage communal development. Additionally, we have developed xyz2swc, a universal conversion service that imports different reconstruction formats and exports them as a standardized SWC file. It supports as input a total of 29 different formats and 55 format variations covering the majority of reconstruction software programs. The xyz2swc service also provides the functionality to verify and correct non-standard SWC files to ensure that they meet the proposed

standard specification. It is built on a modular programming structure that wraps together existing open-source converters wherever possible, is operating system and programming language independent, and requires no local software installation. The xyz2swc service is made freely available as a web application with a user-friendly graphical interface. The accompanying Application Programming Interface (API) also allows programmatic access to the service for easy integration into existing workflows and large batch conversions. The presented service and proposed standard aim to promote efforts for data sharing and facilitate compatibility between software programs.

Disclosures: B. Ljungquist: None. K. Mehta: None. J. Ogden: None. S. Nanda: None. R.G. Ascoli: None. L. Ng: None. G.A. Ascoli: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

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Program #/Poster #: 498.12

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant R01NS39600
NIH Grant U01MH114829
NIH Grant RF1MH128693
NIH Grant R01NS86082

Title: Neuromorpho.org at sweet 16: sharing neural reconstructions in the big data era

Authors: *C. TECUATL, B. LJUNGQUIST, P. MARAVER, K. BIJARI, M. A. AKRAM, G. A. ASCOLI;

Ctr. for Neural Informatics, Structures, & Plasticity and Bioengineering Dept., George Mason Univ., Fairfax, VA

Abstract: NeuroMorpho.Org is a centrally curated inventory of digitally reconstructed neurons and glia associated with peer-reviewed publications. To date, this continuously updated resource constitutes the largest collection of publicly accessible 3D neural reconstructions and associated metadata, with more than 180,000 cells from 91+ animal species contributed by over 900 laboratories worldwide. Tracings can be browsed, searched, and downloaded by brain region, cell type, experimental condition, morphological features, and many other criteria. Scientific applications include statistical analysis, computational modeling, visualization, and classification. Data reuse increases the research impact of the original contributors' reports as quantified by over 1000 citations derived from secondary utilization of files shared through NeuroMorpho.Org. The database content has more than doubled in the last five years, prompting a gradual transition from largely manual processing to an increasingly automated workflow. The implementation of Paperbot, a semi-autonomous literature crawler that leverages a deep learning classifier to identify papers describing new reconstructions, reduced the time needed for this step

by 70%. A systematic computerized pipeline ensures format conversion and standardization, including removal of overlapping points, engulfed side branches, and other common idiosyncrasies. A custom-designed metadata management portal enabling smooth annotation with controlled vocabularies recently integrated a machine-learning module to suggest appropriate terms extracted from the article text along with a relevancy score. Furthermore, the deployment of an automated ingestion system now allows for daily release of datasets, decreasing the mean access delay from 3 months to 2 weeks. Finally, we have added new analytic functions such as similarity search, which enables fast morphological comparison of hundreds of thousands of neural reconstructions, and summary reporting, which allows data selection and grouping based on user-defined filters.

Disclosures: C. Tecuatl: None. B. Ljungquist: None. P. Maraver: None. K. Bijari: None. M.A. Akram: None. G.A. Ascoli: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

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Program #/Poster #: 498.13

Topic: I.07. Data Analysis and Statistics

Support: NSF Grant DGE1745016
NSF Grant ECCS-2044785
NSF Grant CMMI-1953323
Pennsylvania Infrastructure Technology Alliance (PITA)
Pennsylvania Manufacturing Fellows Initiative (PMFI)

Title: Quantitative assessment of *in vitro* neurite morphological development

Authors: *A. S. LIAO¹, W. CUI², Y. J. ZHANG¹, V. A. WEBSTER-WOOD¹;
¹Mechanical Engin., ²Biomed. Engin., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The axons and dendrites of neurons exhibit extensive arborization. The morphology of these processes enables the cells to connect with neighboring cells, resulting in the important neural functions of intercellular signaling and information processing. The development of neurites occurs in multiple stages. For *in vitro* rat hippocampal neurons, a model system for studying neurite polarization, the development occurs in 5 key stages [1]: (1) formation of lamellipodia, (2) extension of select lamellipodia to become neurites, (3) rapid growth of one neurite, which differentiates into the axon, (4) extension of remaining neurites to form dendrites, and (5) continued maturation of the axons and dendrites. Although these growth stages have been qualitatively described, the neurite morphology has not been quantitatively assessed to distinguish between the specific days *in vitro* (DIV) during which developmental milestones are expected.

To monitor neurite development, primary rat hippocampal neurons were cultured for 6 DIV and

imaged using brightfield microscopy at 0.5, 1, 2, 3, 4, and 6 DIV, which correspond to Stages 2, 3, and 4. The neurites in the images were semi-automatically traced using NeuronJ [2], an ImageJ plugin. We developed an automated system that can use the neurite trace data from NeuronJ to quantitatively characterize neurite morphology using both commonly used neurite morphometrics (degree, number of neurites, total length, tortuosity) and newly defined morphometrics based on the Change Point Test (CPT) [3] (distance between change points, relative turning angle, number of change points). The CPT is a statistical analysis to identify locations in which there was a significant directional change in a path initially developed for assessing animal walking trajectories. Using the novel CPT-based metrics with the common metrics, we can describe both the morphology of neurons at different time points and how the neurite trajectory behaved in an unconstrained *in vitro* environment.

Of the morphometrics used, total neurite length, number of endpoints, number of change points, and the average distance between change points were significantly different between 0.5, 1.5, and 4 DIV, which are the approximate time points that rat hippocampal neurons are expected to reach Stage 2, 3, and 4, respectively. Our findings indicated that these morphometrics can be useful for quantitatively describing neuron development *in vitro* and potentially for assessing directional changes in neurites in the future.

References: [1] Dotti *et al. Journal of Neuroscience*. (1988). [2] Meijering *et al. Cytometry*. (2004). [3] Byrne *et al. Animal Behaviour*. (2009).

Disclosures: A.S. Liao: None. W. Cui: None. Y.J. Zhang: None. V.A. Webster-Wood: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

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Program #/Poster #: 498.14

Topic: I.06. Computation, Modeling, and Simulation

Support: NIDCD RO1 DC013798
NIDCD RO1 DC008846
CTSI(Pilot Award), Wallace H. Coulter Foundation

Title: Image Based Analysis of the Otoliths Macula in the Rat

Authors: J. M. TELISCHI¹, F. RACITI², T. J. ROBOHN³, *R. SANGALETTI², S. RAJGURU⁴;

¹Univ. of Miami Miller Sch. of Med., Miami, FL; ²Otolaryngology, ³Biomed. Engin., Univ. of Miami, Miami, FL; ⁴Biomed. Engin. and Otolaryngology, Univ. of Miami, Coral Gables, FL

Abstract: In the vestibular system, the sensory epithelia of the otolith endorgans present a distinctive cellular organization. Different zones of the macula exhibit regional diversity in number and type of hair cells and afferent innervations and morphology. Our lab utilizes a rat model to detail the sensitivity of the vestibular epithelia to various stimuli, such as infrared

radiation or sound, and the role of different cell types and synaptic inputs. Hence, it is pivotal to accurately characterize the morphology of the sensory epithelium. Additionally, a detailed spatial characterization of the maculae could provide further information on how the otoliths encode spatiotemporal properties of head movement. Vestibular morphological analysis is typically performed through labor-intensive manual processing of confocal images. Here, we present a semi-automated approach to detail the morphological map of the otolith endorgans in a rat model. The vestibular endorgans were collected from several cohorts of rodents, some of them exposed to different levels of noise including blast trauma. In whole mount preparations of utricular and saccular specimens, the hair cell bundles were labeled using antibodies against phalloidin. High resolution images were obtained and analyzed via a custom MATLAB script. Image segmentation and thresholding were employed for a semi-automated determination of vestibular hair cell quantity, distribution, and stereociliary bundle orientation. Image analysis using the semi-automated approach was extended to complete a map of the rat maculae including morphometric data and hair cell distribution. In all the samples analyzed, vestibular hair cells present in the neuroepithelia were successfully identified using custom thresholds with image filters based on size, cell boundaries and fluorescence intensity. The epithelial disruption observed in blast exposed samples did not affect the accuracy of the code. Details of macular differentiation, including the localization of the line of polarity reversal, were also determined by analysis of cell orientation measured as direction of the vector from the center of the cuticular plate to the center of the kinocilium. The MATLAB-based approach represents an efficient tool for analyzing regional specializations in otoconial maculae and significantly reduces the time required for quantification. Detailing the cellular and synaptic architecture of the neuroepithelium is important for studies of various vestibular pathologies and for understanding the efficacy of potential therapeutic targets. Semi-automated tools such as those presented here could provide higher throughput for such work.

Disclosures: J.M. Telischi: None. F. Raciti: None. T.J. Robohn: None. R. Sangalatti: None. S. Rajguru: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

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Program #/Poster #: 498.15

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R01NS085211
NIH Grant R21NS093349
NIH Grant R01NS112274
NIH Grant R01NS060910

Title: Boss: beta-mixture unsupervised oligodendrocytes segmentation system

Authors: *E. BAE¹, J. L. ORTHMANN-MURPHY², R. SHINOHARA¹;

¹Dept. of Biostatistics, Epidemiology, and Informatics, ²Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: To develop reparative therapies for multiple sclerosis (MS), we need to better understand the physiology of loss and replacement of oligodendrocytes, the cells that make myelin and the target of damage in MS. *In vivo* two-photon fluorescence microscopy allows direct visualization of oligodendrocytes in transgenic mouse models, and promises a deeper understanding of the longitudinal dynamics of replacing oligodendrocytes after damage. However, the task of tracking oligodendrocytes requires extensive human effort and is especially challenging in three-dimensional images. While several models exist for automatically annotating cells in two-dimensional images, few models exist to annotate cells in three-dimensional images and even fewer are designed for tracking cells in longitudinal imaging. Furthermore, the complexity of processes and myelin formed by individual oligodendrocytes can result in the failure of algorithms that are specifically designed for tracking cell bodies alone. Here, we propose a novel beta-mixture unsupervised oligodendrocyte segmentation system (BOSS) that can segment and track oligodendrocytes in three-dimensional images over time that requires minimal human input. We evaluated the performance of the BOSS model on a set of eight images obtained longitudinally. We showed that the BOSS model can segment and track oligodendrocytes similarly to a blinded human observer.

Disclosures: E. Bae: None. J.L. Orthmann-Murphy: None. R. Shinohara: F. Consulting Fees (e.g., advisory boards); Octave Bioscience.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

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Program #/Poster #: 498.16

Topic: I.06. Computation, Modeling, and Simulation

Support: OD023847/NH/NIH HHS/United States
OD026585/NH/NIH HHS/United States
OD023872/NH/NIH HHS/United States

Title: Modeling and quantifying spatial arrangement of unmyelinated axons in peripheral nerves

Authors: *A. SHEMONTI¹, E. PLEBANI⁴, N. P. BISCOLA⁵, L. A. HAVTON^{5,6,7}, J. R. KEAST⁸, A. POTHEN¹, M. DUNDAR⁴, T. POWLEY², B. RAJWA³;

¹Dept. of Computer Sci., ²Dept. of Psychological Sci., ³Bindley Biosci. Ctr., Purdue Univ., West Lafayette, IN; ⁴Dept. of Computer and Information Sci., Indiana Univ. - Purdue Univ., Indianapolis, IN; ⁵Dept. of Neurol., ⁶Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; ⁷James J. Peters Dept. of Veterans Affairs Med. Ctr., Bronx, NY; ⁸Dept. of Anat. and Physiol., Univ. of Melbourne, Parkville, Victoria, Australia

Abstract: Understanding the relationship between peripheral nerve function and axonal organization requires a comprehensive and quantitative characterization of the neuroanatomy. This is essential for the development of efficient neurostimulation and neuromodulation techniques. The quantitation of axonal organization in the vagus nerve is particularly difficult because most axons are unmyelinated. Moreover, it is unclear how the location of segmented axons would be compared between samples to capture the key characteristics of their spatial organization and recognize abnormalities. State-of-art quantitative neuroanatomy techniques analyzing peripheral nerves focus on the number of axons per unit area and their morphometric properties, such as area, diameter, or shape (Havton et al. *Scientific Reports* 11:23831, 2021). With the support of the NIH SPARC program, in this study, spatial statistics, point-process models, and optimal transport distances were utilized to describe the spatial arrangement of axons and to compute the similarities between these spatial characteristics (in terms of first- and second-order statistics) in different vagus and pelvic nerve cross-sections. We used high-resolution TEM images that have been segmented by a custom-built, high-throughput deep learning system based on a highly modified U-Net architecture (Plebani et al. *Scientific Reports* 12:1198, 2021). We employed an inhomogeneous variant of Ripley's K-function to quantify the axonal patterns, expressed the organizational anisotropy by the sector K-function, and examined pair orientation distribution. We modeled the observed patterns using Strauss-Hardcore and Diggle-Gratton processes. Although it is commonly assumed that the spatial arrangement of myelinated and unmyelinated axons in peripheral nerves is random for biophysical modeling purposes, we showed that the complex axonal organization is inhomogeneous and anisotropic. We also demonstrated that the organizational similarities between nerve cross-sections correspond to the anatomic location of the samples. Using mathematical metrics derived from the solution to the transportation problem, our research demonstrated a novel and innovative method for quantifying similarities between biological patterns. In addition, this research led to the development of a generalizable analysis pipeline for peripheral nerve spatial architecture.

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Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

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Program #/Poster #: 498.17

Topic: I.07. Data Analysis and Statistics

Support: NIMH 1R43MH128076-01

Title: Multi-scale, multi-dimensional deep learning for dense axon detection and tracing

Authors: L. A. GJESTEBY¹, B. S. EASTWOOD², M. G. FAY², N. J. O'CONNOR², J. R. GLASER², C. R. GERFEN³, ***L. J. BRATTAIN**¹;
¹MIT Lincoln Lab., Lexington, MA; ²MBF Biosci., Williston, VT; ³DIRP NIMH, Bethesda, MD

Abstract: A significant, unsolved problem in neuroscience research is the need to accurately and rigorously analyze the diverse and complex nature of axonal fibers that are found in various forms throughout the central nervous system (CNS). Analysis of axonal fibers in CNS tissue of non-transgenic and transgenic animal models as well as in human postmortem CNS tissue holds the promise of novel insights into physiological neural network connectivity patterns as well as into the neuropathological underpinnings of alterations in connectivity associated with human neuropsychiatric and neurological disorders. However, despite imaging advances, detection, tracing, reconstruction, and quantitative analysis of all axonal fibers in 3D microscopic images of CNS areas with high axonal fiber density has been hindered by the lack of adequate image processing capabilities.

The goal of this project is to develop unprecedented functionality for performing automated segmentation, tracing, and analysis of axonal fibers in multi-terabyte sized, three-dimensional (3D) microscopic images of CNS areas, even those with extremely dense axonal fibers. We have developed a machine learning-based, high performance computing pipeline comprising (i) a 2D convolutional neural network (CNN) to detect regions of interest (ROI) for further processing by delineating fibers of passage from terminal fields, (ii) a 3D CNN that detects axonal fiber voxels, (iii) morphological operations that extract axonal fiber centerlines, and (iv) a tracking logic that connects axonal fiber segments across low-intensity gaps and unresolved axonal fiber crossings. The algorithms were trained and tested on images of mouse brain sections in the thalamus acquired with a Leica confocal microscope. The tissue was labeled via cortical injection with recombinant adeno-associated virus expressing tdTomato and imaged at 20x magnification with a lateral size of 581.25 μm^2 and z-depth of 35 μm at 0.69 $\mu\text{m}/\text{slice}$. Our 2D CNN detector first detects the general regions of fibers of passage and terminal fields. Then, our 3D axon segmentation algorithm performs axon tracing in the selected fiber regions. Our initial performance shows effective tracing capability of fibers based on visual inspection and quantitative metrics including Dice and centerline-Dice. Further work is being pursued on varicosity detection and analyses such as density and centrality.

Disclosures: **L.A. Gjestebj:** None. **B.S. Eastwood:** A. Employment/Salary (full or part-time);; MBF Bioscience. **M.G. Fay:** A. Employment/Salary (full or part-time);; MBF Bioscience. **N.J. O'Connor:** A. Employment/Salary (full or part-time);; MBF Bioscience. **J.R. Glaser:** A. Employment/Salary (full or part-time);; MBF Bioscience. **C.R. Gerfen:** None. **L.J. Brattain:** None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.18

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF CRCNS #1822517
NSF CRCNS #2112862
NIMH #MH117488
California NanoSystems Institute

Title: Single serotonergic axons: From a convolutional neural network for trajectory analysis to a neuroscience-inspired dropout in machine learning

Authors: *P. MADINEI, K. C. MAYS, S. JANUSONIS;
Dept. of Psychological & Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: The brain serotonergic axons (fibers) are quintessential “stochastic” axons in the sense that their individual trajectories are best described as sample paths of a spatial stochastic process. These fibers are present in high densities in virtually all regions of vertebrate brains; more generally, they appear to be an obligatory component of all nervous systems on this planet (from the dominating arthropods to such small phyla as the kinorhynchs). In mammals, serotonergic fibers are nearly unique in their ability to robustly regenerate in the adult brain, and they have been strongly associated with neural plasticity. We have recently developed several experimental approaches to trace individual serotonergic fibers in the mouse brain (Mays et al., 2022). To further advance the theoretical analyses of their stochastic properties (e.g., the increment covariance structure), we developed a convolutional neural network (CNN) that performs high-throughput analysis of experimental data collected with sub-micrometer resolution. In contrast to a recently developed mesoscale platform that can separate large-caliber fiber segments from the background on the whole-brain scale (Friedmann et al., 2020), our microscale model prioritizes the accuracy and continuity of individual fiber trajectories, an essential element in downstream stochastic analyses. In particular, it seamlessly integrates information about the physical properties of serotonergic fibers and high-resolution experimental data to achieve reliable, fully-automated tracing of trajectories in brain regions with different fiber densities. This 3D-spatial information supports our current theoretical frameworks based on step-wise random walks (Janusonis & Detering, 2019) and continuous-time processes (Janusonis et al., 2020). In a complementary approach, we also investigated whether the structure of the serotonergic fibers may provide useful information for machine learning architectures. Specifically, we studied whether dropout, a standard regularization technique in artificial neural networks, can be matched or improved by virtual serotonergic fibers moving through CNN layers (endowed with the Euclidean metric) and triggering spatially correlated dropout events.

Disclosures: P. Madinei: None. K.C. Mays: None. S. Janusonis: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.19

Topic: I.07. Data Analysis and Statistics

Title: Spine Classification in Dendritic Spine Counter Plugin for ImageJ

Authors: *M. VOLOSHIN¹, J. PARATO²;

¹Mighty Data, Inc., Weaverville, NC; ²Natural Sci., SUNY Empire State, Brooklyn, NY

Abstract: Dendritic Spine Counter is a free ImageJ plugin which allows researchers to semi-automatically count dendritic spines from 2D images. Spines differ qualitatively in morphology, and the population ratios of different types of spines are relevant to research into neurodegenerative disorders, pharmacology, and many other clinical and scientific applications. We have created a workflow in Dendritic Spine Counter that offers researchers an efficient, easy-to-use interface to measure spine characteristics, and to categorize spines into morphological groupings based on each spine's relationships of head diameter, neck diameter, and overall spine length. On-screen measuring tools provide automated assistance to the user while still granting them full manual control over classifying spines into standard categories such as thin, stubby, or mushroom, or into custom user-defined categories. The workflow outputs a spreadsheet containing these measurements and categorizations, with which the researcher can perform subsequent numerical analysis on an application-specific basis. With this software, we hope to aid laboratories studying dendritic spines by reducing the cost, time, effort, and likelihood of human error involved in spine tabulation.

Disclosures: M. Voloshin: None. J. Parato: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.20

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant #2P20GM103432

Title: Validating the efficacy of spinej compared to imaris for dendritic spine structural analysis

Authors: *S. HODGINS, L. T. FLOM, K. L. SANDUM, L. A. VACCARO, A.-C. BOBADILLA;

Univ. of Wyoming, Univ. of Wyoming, Laramie, WY

Abstract: Substance use disorder (SUD) remains a chronic and debilitating condition among millions without definitive relapse treatment. At the center of the mesolimbic pathway, temporary morphological enhancement of nucleus accumbens core (NAcore) dendritic spines, known as transient synaptic potentiation (t-SP), is critical to drive drug seeking. This structural plasticity has been found to correlate with the strength of substance-seeking. In this study, we aim to compare two software programs allowing the measurement of dendritic spine morphology specifically in neuronal ensembles linked to cocaine seeking in the NAcore using a cocaine self-administration model of SUD in 8–10-week-old c-Fos^{iCreER2} Ai14 male and female mice (n=7).

Seeking-ensemble dendrites (n=29) expressing red fluorescent protein (tdTom+) via the targeted recombination in active populations (TRAP) method were analyzed within subjects in Imaris and SpineJ software. Nested t-tests revealed an expected significant difference in spine count, dendrite length, and spine density between Imaris and SpineJ, suggesting that SpineJ's 2D limitation restricts the accuracy of spine quantification. This study emphasizes the importance of periodic evaluation of research tools for accurate analysis, an essential component of the development of reliable SUD treatment.

Disclosures: S. Hodgins: None. L.T. Flom: None. K.L. Sandum: None. L.A. Vaccaro: None. A. Bobadilla: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.21

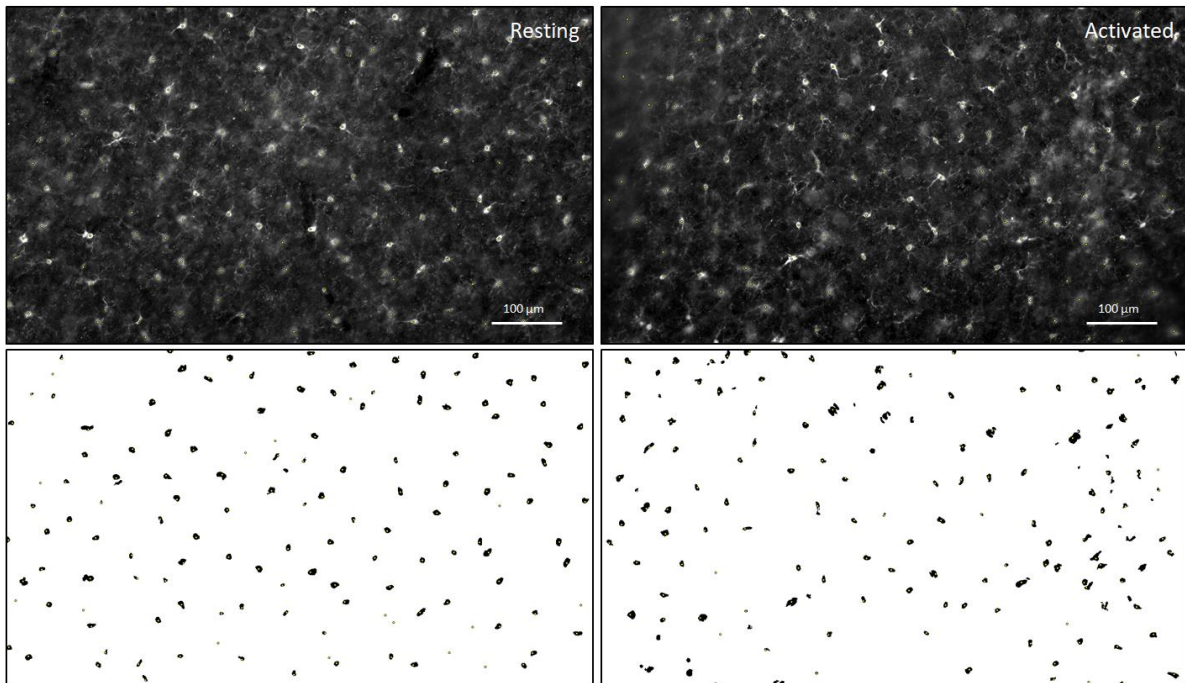
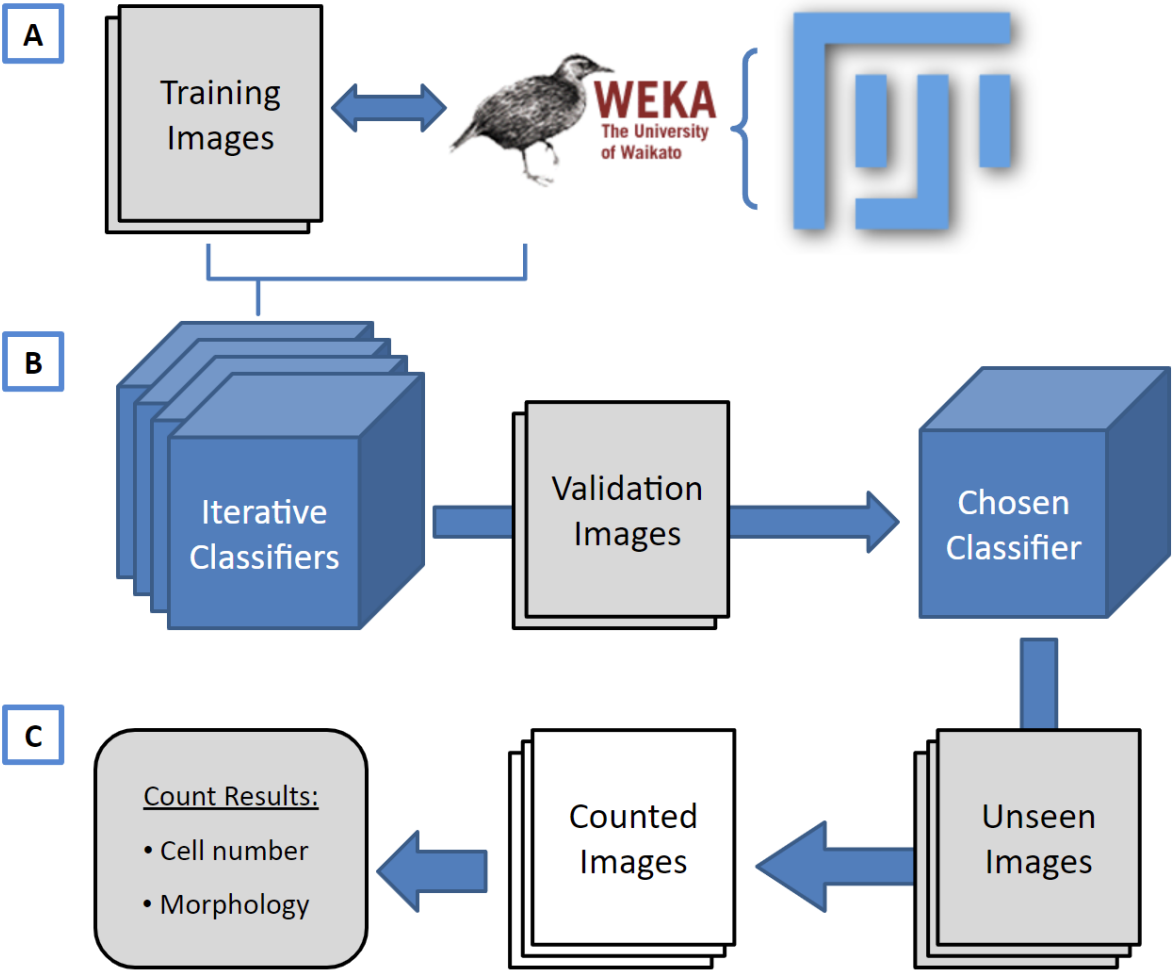
Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R01 MH104131
NIH Grant R01 MH105330
NIH Grant R01 MH087332
NIH Grant R01 DA052209

Title: ACCT: automatic cell counting with Trainable Weka Segmentation

Authors: *T. KATARAS, T. JANG, J. KOURY, H. SINGH, M. KAUL;
Univ. of California, Riverside, Riverside, CA

Abstract: We introduce a novel tool ACCT: Automatic Cell Counting with Trainable Weka Segmentation which allows for flexible automatic cell counting via object segmentation after user-driven training. ACCT is demonstrated with a comparative analysis of publicly available images of neurons and an in-house dataset of immunofluorescence-stained microglia cells. For comparison, both datasets were manually counted to demonstrate the applicability of ACCT as an accessible means to automatically quantify cells in a precise manner without the need for computing clusters or advanced data preparation. ACCT replicated the increase in microglia found under neuro-immune activating conditions by observer counts and demonstrated precision in diverse neuron images.



Disclosures: T. Kataras: None. T. Jang: None. J. Koury: None. H. Singh: None. M. Kaul: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.22

Topic: I.07. Data Analysis and Statistics

Support: NIMH Grant 5R44MH105091-04

Title: Combining machine learning with stereology: the next generation of unbiased 3D stereology for cell counting

Authors: A. L. WILSON, D. PERUZZI, *A. E. SULLIVAN, N. ROUSSEL, A. D. LEDUC, B. S. EASTWOOD, P. J. ANGSTMAN, J. R. GLASER;
MBF Biosci., Williston, VT

Abstract: With the advent of Cellairus, automated stereology is rising to the forefront of unbiased cell counting by leveraging artificial intelligence to dramatically reduce the labor and expertise involved in manual cell counting. The manual aspects of stereology have been a barrier to wider utilization of unbiased stereology. Cellairus dramatically accelerates stereological cell counting through the use of machine learning to replicate expert human observer judgments about recognizing cells, their location and size. Once the machine learning algorithms are trained, Cellairus identifies cells in 3D volumes throughout 3D brain regions using the same observer criteria as a human expert. Additionally, automation avoids user fatigue and subjectivity by consistently applying the same cell counting criteria throughout the brain. The results can be audited and reviewed for every counting frame site. Cellairus makes stereology easier and faster to perform, without sacrificing accuracy. In Cellairus, 3D image volumes are analyzed using the Optical Fractionator probe combined with novel 3D detection methods to ensure accurate cell detection and unbiased population estimates. Cellairus can be trained to differentiate between different cell types, sub-cellular objects and non-cell objects. To accommodate varying neuron densities in different brain regions we trained machine learning classifiers on both dense and sparse neuron populations. Cellairus uses a patent pending technique to perform true 3D stereological analysis rather than analyzing 2D images collapsed from 3D volumes which reduces accuracy and introduces bias. In this study, we validated the cell counts from the Cortex and Caudate-Putamen in mouse brains by comparing automated stereology results with ground-truth data collected by manual stereology. Coronal brain sections were prepared with two fluorescent labels, DAPI and NeuN. Manual and automated stereology were performed for both wide-field fluorescence and scanning laser confocal microscopy in order to assess the performance of Cellairus across multiple imaging technologies. Population estimates, coefficients of error, false positive, false negative, and true positive detection rates were quantified and compared between cell counting methods and imaging modalities.

Disclosures: **A.L. Wilson:** A. Employment/Salary (full or part-time); MBF Bioscience. **D. Peruzzi:** A. Employment/Salary (full or part-time); MBF Bioscience. **A.E. Sullivan:** A. Employment/Salary (full or part-time); MBF Bioscience. **N. Roussel:** A. Employment/Salary (full or part-time); MBF Bioscience. **A.D. LeDuc:** A. Employment/Salary (full or part-time); aleduc@mbfbioscience.com. **B.S. Eastwood:** A. Employment/Salary (full or part-time); MBF Bioscience. **P.J. Angstman:** A. Employment/Salary (full or part-time); MBF Bioscience. **J.R. Glaser:** A. Employment/Salary (full or part-time); MBF Bioscience.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.23

Topic: I.07. Data Analysis and Statistics

Title: Image Volume Fractionator: A new probe for performing unbiased stereology on cleared tissue

Authors: J. A. MCMULLEN, M. L. HEAL, N. J. O'CONNOR, ***D. PERUZZI**, D. M. HOPPE, J. R. GLASER;
MBF Biosci., Williston, VT

Abstract: Traditional methods for investigating microscopic neuroanatomical specimens require histological sectioning which can introduce artifacts that damage tissue and sever important pathways. Significant work has been performed to develop accurate, unbiased stereological probes that address the issues inherent in using histological sectioning. The introduction of tissue clearing and microscopic imaging techniques have allowed for biological tissue to be studied in its intact form, maintaining the integrity of structures and eliminating the artifacts caused by sectioning. As research using cleared tissue specimens improves and becomes more mainstream, it is important that robust, accurate, and unbiased tools are created for the collection of quantitative data from these images. Stereology is an unbiased statistical sampling method wherein data are collected from a subset of an anatomic region of interest. Specialized equations are then applied to the sampled data to produce statistically unbiased quantitative estimates. Until now, stereology methods were focused on use with sectioned tissue specimens. With the introduction of Stereo Investigator Cleared Tissue Edition, stereology probes have been adapted for use with data from light sheet or confocal microscope images of cleared tissue specimens to produce unbiased stereological measurements. Here we explain and demonstrate a new, innovative probe specifically designed for estimating cell number in large 3D cleared tissue image volumes containing an entire region of interest - the Image Volume Fractionator. This new probe includes proscribed procedural rules and mathematical equations for obtaining accurate and unbiased estimates from cleared tissue specimens.

Disclosures: **J.A. McMullen:** A. Employment/Salary (full or part-time); MBF Bioscience. **M.L. Heal:** A. Employment/Salary (full or part-time); MBF Bioscience. **N.J. O'Connor:** A.

Employment/Salary (full or part-time); MBF Bioscience. **D. Peruzzi:** A. Employment/Salary (full or part-time); MBF Bioscience. **D.M. Hoppes:** A. Employment/Salary (full or part-time); MBF Bioscience. **J.R. Glaser:** A. Employment/Salary (full or part-time); MBF Bioscience.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.24

Topic: I.06. Computation, Modeling, and Simulation

Title: A framework for computing high resolution 3D microscopic volumes

Authors: Z. ASHOURI¹, *J. YESUF², A. SADIKOT², M. KUNZ¹;

¹Digital Technologies, Natl. Res. Council Canada, Ottawa, ON, Canada; ²Neurol. and Neurosurg., Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada

Abstract: Introduction Whole-slide imaging microscopy is more available recently but 3D analysis of such histological images still remains a bottleneck. For generating 3D microscopic volumes of tissue blocks, histology images need to be spatially aligned, which is problematic due to inevitable global and local deformations introduced during processing, especially in free-floating sections. We propose a framework to create high-resolution 3D microscopic volume datasets by correcting and combining micro-scale images. **Methods and Results** Due to the substantial local deformation of free-floating sections, the core method in our proposed framework is the shape alignment between histology and corresponding blockface image. Matching pair-points are manually selected in both images, especially in locations with high confidence and around the border of the tissue. Based on these pair-points, a sparse deformation map is generated. To evenly distribute deformations around the tissue, the map is refined using a thin plate spline interpolation. We tested the method on a series of human subcortical slices sectioned at a thickness of 50um. Stained slides were digitized using the Axioscan 7 multiplex scanner at an in-plane resolution of 0.3um/pixel and downsampled to the resolution of blockface image. The result of one of these registered histology images is show in figure 1. To improve the alignment between the histology images, a feature matching approach will be utilized in combination with a resolution upsampling method and a continuous 3D volume will be generated using interpolation between images. **Conclusions** In this work we have discussed a semi-manual technique to reconstruct 3D histology volumes, with special focus on correcting free floating stained histology deformations using the blockface images. The high-resolution 3D microscopic volume provides advanced analytical opportunities, such as co-registering with other volumetric images (e.g. MRI), or perform spatial 3D analysis. In future work, we plan to improve the procedure by making the point selection semi- or fully automatic.

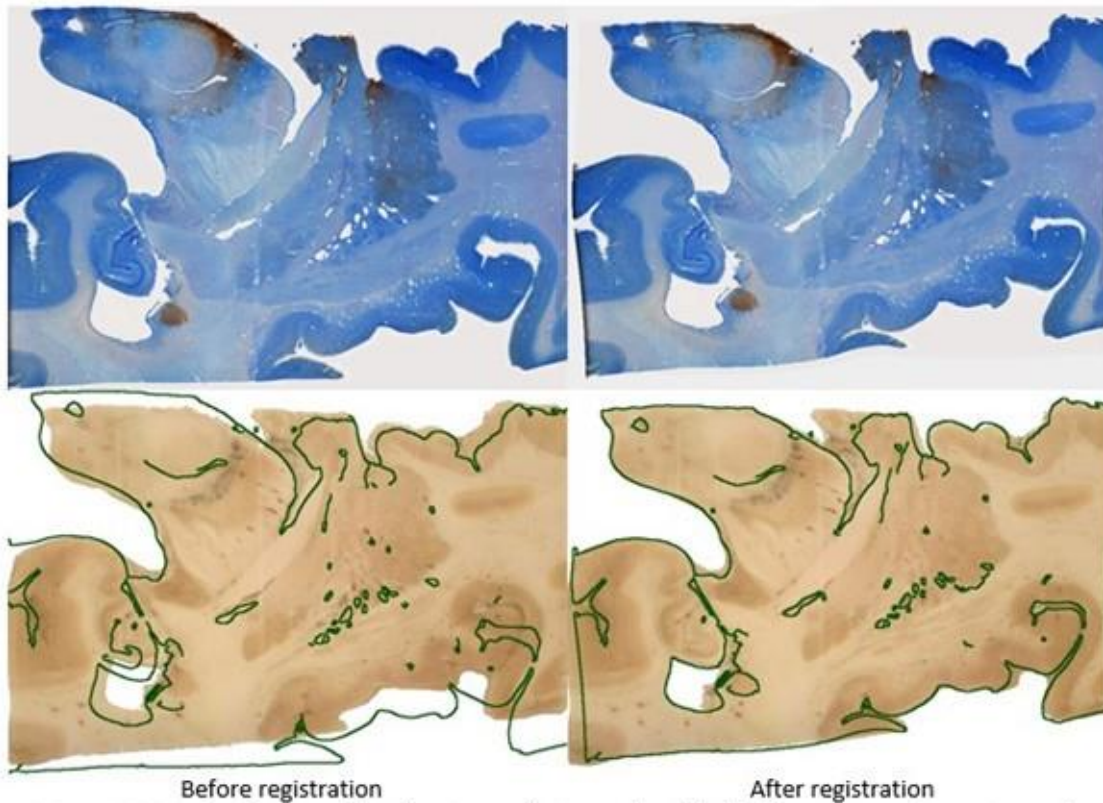


Figure 1. Original and registered histology images (in top row) and the blockface image with superimposed contours of the histology image before and after registration (bottom row)

Disclosures: Z. Ashouri: None. J. Yesuf: None. A. Sadikot: None. M. Kunz: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.25

Topic: I.07. Data Analysis and Statistics

Support: UM Rackham Predoctoral Fellowship
NSF Grant 1707316
NIH Grant 1RF1MH123402
NIH Grant 1RF1MH124611

Title: Petabyte-scale image visualization and annotation on the Brain Image Library with nTracer2

Authors: *L. A. WALKER^{1,2}, W. J. LEE³, M. WEATHERSPOON³, D. CAI^{4,1,5};
¹Biophysics, ²Computat. Med. and Bioinformatics, ³Electrical Engin. and Computer Sci., ⁴Cell and Developmental Biol., ⁵Michigan Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: High speed light microscopy technologies, such as lightsheet imaging, are capable of generating petabytes of data in a single brain image. Handling this data is an ongoing challenge because the data is too large to store or process on a single desktop computer. To address this challenge, we have developed nTracer2, a cloud-based platform to enable visualization and neuron tracing of PB-scale brain images stored in a supercomputer cluster environment. nTracer2 utilizes Google Neuroglancer's web browser-based application for remote client data access. Our platform makes use of HDF5*, a novel schema of the broadly used HDF5 data format which has been optimized for maximum I/O efficiency when accessed through all archival storage systems. This innovation enables smoothly browsing whole-brain image data even through a smartphone. nTracer2 consists of three independent components: a data server that synthesizes the proper views from the raw dataset and streams them to the user end, a database backend that supports parallel data analysis and result curation in the cloud, and a neuron tracing interface that is added to the user's browser. This modular structure allows other developers to integrate third-party functions into nTracer2 as plugins or to reuse different nTracer2 modules in their own platforms. The nTracer2 modules are designed to be scalable to large compute servers to support centralized data access. We are actively collaborating with the Brain Image Library (BIL) to deploy nTracer2 for the visualization of fMOST and other images. With the ability to sustain the maximum data transfer rate from the BIL storage cluster, in our user testing, nTracer2 enabled 5 users to simultaneously visualize the same whole-brain image dataset from different geological locations across the US, with many more users likely achievable. We expect that the nTracer2 modules can be implemented on other BRAIN initiative supported image repositories, such as BossDB, DANDI, and NeMO to make the large image datasets accessible to the public. As a general cloud-based image visualization, annotation, and data management platform, nTracer2 will provide a viable solution to meet the increasing microscopy demands of the scientific community.

Disclosures: L.A. Walker: None. W.J. Lee: None. M. Weatherspoon: None. D. Cai: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.26

Topic: I.07. Data Analysis and Statistics

Support: NIH 1RF1MH123402
NIH 1RF1MH124611

Title: An automated pipeline for mouse whole-brain CCF registration

Authors: *B. DUAN¹, H. DING¹, L. A. WALKER², Y. YAN¹, D. CAI³;

¹Computer Sci., Illinois Tech., Chicago, IL; ²Univ. of Michigan, Ann Arbor, The Univ. of Michigan, Ann Arbor, MI; ³Cell and Developmental Biol., Univ. of Michigan Neurosci. Grad. Program, Ann Arbor, MI

Abstract: Advances in molecular and structure profiling resulted in high-throughput image data generation for whole mouse brains. To compare the neuron anatomy and molecule profiles among animals, brain image datasets are routinely registered onto a reference atlas called Common Coordinate Framework (CCF), which annotates the boundaries of brain domains. To generate the CCF, whole-brain fluorescence images taken from thousands of mice were averaged as a template for anatomy experts to annotate the brain regional boundaries. Registering experimental brain images to this 3D image template makes the CCF annotation readily transferred to the experimental brain. This registration provides an expert-like annotation for each new experiment. While an automated CCF registration pipeline is highly desirable, existing methods normally require human interventions. Here, we present a fully automated CCF registration pipeline in three steps: preprocessing, linear global alignment, and deformable local alignment. The preprocessing step adjusts the resolution and removes the experimental brain's imaging artifacts. Next, the linear global alignment step applies a point cloud registration technique to match the overall orientation and volume. Finally, a deformable alignment is used to locally refine the registration. Instead of transforming an experimental brain to match the CCF, we reversely transformed the CCF to create specific domain annotations for the experimental brain. As such, brain images remain in the raw data format without the resource-consuming transformation process. Structural and morphological quantification under the native tissue conditions also reflects the intrinsic variations, such as brain shape and volume differences among animals. This avoids potential errors caused by forcing the brains of different animals to be deformed to fit the same template. To enable result viewing, the transformed CCF is overlaid as an annotation layer with the raw brain images in nTracer2, a browser-based visualization platform developed in our lab. Our automated CCF registration pipeline is accurate, determined by visual inspections, and efficient, where registering a whole-brain fMOST dataset took ~30 minutes on a 2.09GHZ AMD Ryzen 1950X CPU with ~32 GB of memory peak usage.

Disclosures: B. Duan: None. H. Ding: None. L.A. Walker: None. Y. Yan: None. D. Cai: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.27

Topic: I.07. Data Analysis and Statistics

Support: MRC Grant MC_UP_1201/22

Title: D-lmbmap: a fully automated deep learning pipeline for whole-brain profiling of circuits

Authors: Z. LI¹, Z. SHANG², J. LIU², H. ZHEN², R. N. STURGESS¹, S. ZHONG³, M. H. HASTINGS¹, R. LIN³, *J. REN¹;

¹Neurobiology Div., MRC Lab. of Mol. Biol., Cambridge, United Kingdom; ²Sch. of Software Engin., Xi'an Jiaotong Univ., Xi'an, China; ³Natl. Inst. of Biol. Sci. (NIBS), Beijing, China

Abstract: Recent frontiers of tissue-clearing and light-sheeting microscopy techniques present novel opportunities to achieve high-throughput mesoscale whole-brain connectivity mapping. However, current computational solutions for data analysis are labour-intensive due to the exhaustive manual annotation. Applications for different axonal types are also limited because of heavy customized training. Meanwhile, whole-brain data analysis always requires combining multiple packages and needs secondary development by users. Here we developed D-LMBmap, (Deep-Learning pipeline for Mouse Brain circuitry Mesoscale Automatic Profiling), an end-to-end package providing an integrated workflow containing three modules based on novel deep-learning algorithms for whole-brain connectivity mapping: axon segmentation, brain region segmentation and whole-brain registration. Our axon segmentation pipeline contains automated annotation for selected 3D cubes with axons and junks, data augmentation for increasing diversities and complexities of 3D cubes, nnU-Net based deep transfer learning for training extensible and robust models, and prediction for whole-brain axons. We achieved the axon segmentation of 45 whole brains, with stained serotonin, dopamine, and GABA axons via immunolabeling and viral-genetic approaches. We got superior performance (average CI-Dice: 91.1%, Recall: 92.3%), with more than 15% improvements over state-of-the-arts. Most importantly, our pipeline does not need manual annotation, which is also suitable for axons from different cell types. To quantify axon densities in each brain region, we developed a cross-modality whole-brain 3D registration method through style transfer and brain region constraints. The method considers image differences between collected brains and standard atlas, where the deep model is trained to transfer all brain images with atlas style (e.g., Allen atlas). It automatically segmented 7 major brain regions with an average Dice score of 91.8% for style transferred brains. A multiple constraint unsupervised VoxelMorph-based network is designed for whole-brain registration, which considers both the brain outline and 7 brain regions. Our methods accomplished an average registration Dice of 93.1%. Unlike previous machine learning-based methods, D-LMBmap analyses whole-brain projections in one single workflow without any manual annotation. Each deep models are also extensible to various image modalities. We also developed a user-friendly interface, with no technological barrier. D-LMBmap outperforms existing methods in all three modules in accuracy, speed, generalization and ease of use.

Disclosures: Z. Li: None. Z. Shang: None. J. Liu: None. H. Zhen: None. R.N. Sturges: None. S. Zhong: None. M.H. Hastings: None. R. lin: None. J. Ren: None.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.28

Topic: I.07. Data Analysis and Statistics

Support: NEI Grant F30EY02959
NIMH Grant R44MH119989

Title: Validation of an end-to-end platform for whole-brain differential analysis of regionalized neuronal activity using immediate-early gene products

Authors: R. AZEVEDO^{1,3}, C. LO^{1,3}, F. DENTI⁴, B. SHAHBABA², D. G. WHEELER³, *S. GANDHI^{1,3};

¹Neurobio. and Behavior, ²Statistics, Univ. of California, Irvine, CA; ³Translucence Biosystems, Irvine, CA; ⁴Statistics, Univ. Cattolica del Sacro Cuore, Milan, Italy

Abstract: The combination of optical tissue clearing with light-sheet fluorescence imaging provides a powerful approach for understanding the link between neuronal activity and behavior. Immediate-early gene (IEG) products like Npas4 and cFos provide sensitive, cellular resolution snapshots of recent neuronal activity. When coupled with atlases such as the CCFv3, the number of active neurons can be quantified across hundreds of distinct regions in the mouse brain. Obtaining differential signals from optically cleared, intact brains pose unique challenges. Artifacts specific to 3D imaging can introduce significant error. Furthermore, the hierarchical structure of atlas regions imposes strong interdependence between regional signals. Here we develop a statistical framework designed specifically to tackle these unique challenges. We apply this framework to identify regional signatures of cFos and Npas4 activated when dark-adapted mice are exposed to light. Differential analysis reveals both expected and surprising patterns of IEG expression. For example, both Npas4 and cFos quantification reveal that visual areas in mouse cortex are prominently activated by the animal's exposure to light. In contrast, cFos and Npas4 expression levels are not similarly modulated in other regions of the central visual pathway. Thin section immunohistochemistry of IEG products was used to validate regional signatures obtained through whole brain analysis. Together, these analysis tools are combined into BrainQuant3D, a scalable platform for whole-brain image segmentation and analysis that transforms differential signals into publication-ready figures.

Disclosures: R. Azevedo: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems. C. Lo: None. F. Denti: None. B. Shahbaba: None. D.G. Wheeler: A. Employment/Salary (full or part-time):; Translucence Biosystems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems. S. Gandhi: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems.

Poster

498. Computational Tools and Other Resources: Microscopy and Imaging

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 498.29

Topic: I.06. Computation, Modeling, and Simulation

Title: Assessment of preclinical developmental neuropathology using image analysis

Authors: *M. STAUP¹, T. MASINDE¹, A. ŻURAW², E. RAMAKER⁴, J. LENSEN³, V. PICCICUTO³, O. MENDES¹;

¹Charles River, Durham, NC; ²Charles River, Frederick, MD; ³Charles River, 's-Hertogenbosch, Netherlands; ⁴North Carolina State Univ., Raleigh, NC

Abstract: The assessment of toxicological effects in the developing brain is a regulatory requirement for the pharmaceutical, industrial and agrochemical industries. These data are used, in conjunction with histopathology, to evaluate developmental neurotoxicity. Current methodology relies on a limited number of manual linear morphometric measurements that are intrinsically associated procedural variability and bias. We present an automated solution using digitally scanned PND 21/22 and PND 71/73 rat brain samples. A GLP (Good Laboratory Practices)-validated commercial artificial intelligence-based image analysis platform (Visiopharm®) was used to train a convolutional neural network (CNN)-based supervised deep learning model to automatically detect and measure relevant brain regions: neocortex, caudate putamen, corpus callosum, hippocampus and cerebellum. This method is highly reproducible and precise. It increases the number of quantitative endpoints collected, decreases analytical turn-around time, and improves the ability to detect morphometric changes in homologous sections, providing an invaluable regulatory-compliant asset for decision-making across industries.

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Poster

499. Development and Application of Optogenetic Tools

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 499.01

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH grant R01MH075957
HHMI

Title: Structure-guided design of pump-like channelrhodopsins with properties enabling markedly improved optogenetic control in the brain

Authors: *Y. KIM¹, P. Y. WANG², E. F. X. BYRNE², Y. JO², C. RAMAKRISHNAN², S. QUIRIN², H. E. KATO³, K. DEISSEROTH⁴;

¹Stanford Univ., Palo Alto, CA; ²Stanford Univ., Stanford, CA; ³Univ. of Tokyo, Tokyo, Japan; ⁴Stanford, Stanford, CA

Abstract: The recently discovered pump-like channelrhodopsins (PLCRs), including ChRmine and KCRs, exhibit puzzling properties (unusually-large photocurrents, extreme light-sensitivity, and exclusive ion selectivity to monovalent cations) that have opened up new opportunities in optogenetics. Although PLCRs have gained broad interest and application in neuroscience research (since Marshel et al., *Science* 2019), little is known about the molecular mechanisms by which these unusual channelrhodopsins operate. Structural mechanisms and structure-guided engineering of channel conduction, light sensitivity, and speed in this family of proteins would likely lead rapidly to creation of powerful new resources for optogenetics. Here we present several designed PLCRs based on our recently-published 2.0 Å resolution cryo-electron microscopy structure of ChRmine (Kishi et al., *Cell* 2022). The structure reveals novel architectural features including the retinal binding pocket, ion conduction pathways and putative selectivity filters, which enabled us to engineer variants with red-shifted action spectra, faster- and slower-closing kinetics, and markedly-changed ion selectivity. Our structure-based design of PLCRs will open the door to diverse applications in neuroscience and point the way toward further structure-guided creation of novel channelrhodopsins for optogenetic applications across biology.

Disclosures: **Y. Kim:** None. **P.Y. Wang:** None. **E.F.X. Byrne:** None. **Y. Jo:** None. **C. Ramakrishnan:** None. **S. Quirin:** None. **H.E. Kato:** None. **K. Deisseroth:** None.

Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.02

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH New Innovator Program
Arnold and Mabel Beckman Foundation
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Title: A kinetic-optimized CoChR variant with enhanced high-frequency spiking fidelity

Authors: ***X. BI**, C. BECK, Y. GONG;
Duke Univ., Duke Univ., Durham, NC

Abstract: Channelrhodopsins are a promising toolset for noninvasive optical manipulation of genetically identifiable neuron populations. Existing channelrhodopsins have generally suffered from a tradeoff between two desired properties: fast channel kinetics and high photosensitivity. Such a tradeoff hinders spatiotemporally precise optogenetic activation during both one-photon and two-photon photostimulation. Furthermore, the simultaneous use of spectrally separated genetically encoded indicators and channelrhodopsins has generally suffered from non-negligible crosstalk in photocurrent or fluorescence. These limitations have hindered crosstalk-free dual-channel experiments needed to establish relationships between multiple neural populations. The

channelrhodopsin from *Chloromonas oogama* (CoChR), recently discovered from large-scale transcriptome sequencing, possesses high blue-light sensitivity, but slow channel kinetics. This kinetics compromised the temporal precision of light-activation and limited the firing frequency in both one-photon and two-photon photostimulation applications. We sought to optimize the channel kinetics of CoChR such that it could enable high temporal precision in one-photon optogenetics. We rationally designed and engineered a kinetic-optimized CoChR variant that was three times faster than native CoChR while maintaining photosensitivity. Additionally, our CoChR variant exhibited comparable photosensitivity and channel kinetics as other recently-developed opsins. When expressed in cultured hippocampal pyramidal neurons, our CoChR variant improved high-frequency spiking fidelity under one-photon illumination. Our CoChR variant's blue-shifted excitation spectrum enabled simultaneous high fidelity cyan photostimulation and red calcium imaging with negligible photocurrent crosstalk from the imaging orange illumination. The faster kinetics of our CoChR variant extended the frequency range for stimulating spikes in cultured neurons with high fidelity, while the lack of photocurrent crosstalk minimized undesirable neuron activation by imaging light. Both properties of our CoChR variant would support one-photon dual-channel optogenetic applications when combined with red-shifted calcium indicators or voltage indicators. We also anticipate that the large photocurrent amplitude and moderate channel kinetics of our CoChR variant could have additional applications in two-photon optogenetics that integrates photocurrent over millisecond duration scans.

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Poster

499. Development and Application of Optogenetic Tools

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH U01 NS118288

Title: A set of conditional GtACR2 reporter mouse lines with improved functionality for optogenetic inhibition

Authors: *Z.-L. CAI^{1,3}, H. CHEN^{1,3}, Y. WANG^{1,3}, M. XUE^{1,2,3};

¹Neurosci., ²Mol. Human Genet., Baylor Col. of Med., Houston, TX; ³The Cain Fndn. Labs., Jan and Dan Duncan Neurolog. Res. Inst. at Texas Children's Hosp., Houston, TX

Abstract: Light-gated chloride channels such as *GtACR1* and *GtACR2* are powerful optogenetic tools for suppressing neuronal activity due to their large photocurrents and fast kinetics. However, photoactivating chloride channels can also cause axonal depolarization and transmitter release because the high chloride concentration in the axons leads to inward currents. We previously screened somatodendritic motifs and generated a hybrid motif (Kv2.1C-linker-TlcnC)

that targets *GtACR2* expression to soma and dendrites to reduce the axonal excitatory effect. To facilitate the use of this optogenetic tool, we generated three Rosa26 knock-in mouse lines that conditionally express *GtACR2*-EYFP-Kv2.1C-linker-TlcnC in a Cre-, Flp-, or Cre- and Flp-dependent manner. We characterized *GtACR2* photocurrent and axonal excitation in cortical excitatory and inhibitory neurons, benchmarking with the state-of-art reporter line *GtACR1*-ts-Fred-Kv2.1C. We found that *GtACR2*-EYFP-Kv2.1C-linker-TlcnC mouse lines generated larger photocurrents and lower axonal excitation than *GtACR1*-ts-Fred-Kv2.1C in both excitatory and inhibitory neurons. We also optimized photostimulation protocols that sufficiently suppress action potentials without causing axonal excitation. Furthermore, *GtACR2*-EYFP-Kv2.1C-linker-TlcnC mouse lines can more effectively suppress neuronal activity than *GtACR1*-ts-Fred-Kv2.1C *in vivo*. Thus, we provide a set of powerful optogenetic inhibitory tools with improved functionality for neuroscience research.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.04

Topic: I.08. Methods to Modulate Neural Activity

Support: NS047384
NS122316

Title: Light-activated regulation of eIF4E by an opto4EBP for conditional and inducible protein synthesis inhibition in the brain

Authors: *J. M. ALAPIN, M. MOHAMED, M. M. OLIVEIRA, P. SHRESTHA, H. L. BOWLING, H. G. KHALED, E. KLANN;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The protein kinase mechanistic target of rapamycin (mTOR) is one of the primary triggers for initiating cap-dependent translation via phosphorylation of eIF4E-binding proteins (4E-BPs) and p70 S6 kinase 1 (S6K1). mTORC1 signaling is known to be required for multiple forms of protein synthesis-dependent synaptic plasticity and various forms of long-term memory, including associative threat memory. To demonstrate that eIF4E-dependent translation is required for associative threat memory consolidation, we previously utilized the pharmacological inhibitor 4EGI-1 (Hoeffler et al. PNAS 108: 3383-3388). Pharmacological inhibitors such as 4EGI-1 offer temporal control, but they lack cell type-specificity. More recently we used a eIF4E knockdown (4Ekd) mouse line (4Ekd) to demonstrate that eIF4E-dependent translation in excitatory neurons in the lateral amygdala (Shrestha et al. Nat. Neurosci. 23: 281-292) and in somatostatin-expressing inhibitory neurons in the centrolateral amygdala (Shrestha et al. Nature 586: 407-411) is required for associative threat memory. Although the 4Ekd mice permit

conditional and inducible protein synthesis inhibition (ciPSI) in vivo, these mice rely on a Tet-Off system to knockdown eIF4E. Therefore, the 4Ekd mice lack precise on-off control for investigating the temporal window for eIF4E-dependent translation in memory consolidation. We have now designed a novel optogenetic tool (Opto4E-BP) for light-dependent regulation of eIF4E to probe memory consolidation in vivo with high spatiotemporal resolution. We have shown that light-activation of Opto4E-BP effectively decreases protein synthesis in HEK cells and cultured neurons. Moreover, light-activation of Opto4E-BP in acute amygdala slices following in vivo viral expression in excitatory neurons resulted in decreased protein synthesis. We are currently utilizing this Opto4E-BP tool to determine the temporal windows and cell types in the amygdala that require eIF4E-dependent protein synthesis for associative threat memory consolidation in vivo. This work was supported by NIH grants NS047384 and NS122316 (E.K.).

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Poster

499. Development and Application of Optogenetic Tools

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Title: Optogenetic tool for protein translation control through sequestration of specific mRNA

Authors: *H. JEONG¹, M. LEE², H. MOON³, H. PARK¹, Y. SHIN²;

¹Dept. of Electrical and Computer Engin., Univ. of Minnesota, Minneapolis, MN; ²Dept. of Mechanical Engin., Seoul Natl. Univ., Seoul, Korea, Republic of; ³Bioengineering, Univ. of California, Berkeley, CA

Abstract: Precise spatiotemporal regulation of mRNA localization and translation is critical in neuronal cells with highly asymmetric structures. External stimuli-queued dynamic assembly and disassembly between endogenous mRNAs and RNA binding proteins (RBPs) have been suggested to be an underlying mechanism to control local protein synthesis. To mimic the transient control of protein translation, we developed an optogenetic tool to control translation using liquid-liquid phase separation induced by blue light exposure. Optogenetic control of protein translation through sequestration of mRNA has advantages in achieving high spatial and

temporal resolution. However, previous approaches required a multi-component system, which resulted in a substantial burden on a cell and a low efficiency arising from the uncontrolled ratio between clustering components. Here, we developed 'optoMCP-FUS', a single-component optogenetic platform for mRNA sequestration that uses IDR-mediated phase-transition in living cells. We applied the optoMCP-FUS system to control the translation of endogenous β -actin mRNA in dissociated hippocampal neurons cultured from the Actb-MBS knock-in mice. We confirmed that most of the endogenous β -actin mRNA tagged with 24 repeats of MS2 binding sequence (MBS) were sequestered into the optoMCP-FUS droplets after blue light exposure. Considering neural activity-dependent localization of β -actin mRNA and its role in the stabilization of actin-based structures, we investigated whether optoMCP-FUS dependent sequestration of β -actin mRNA could influence the maintenance of structural long-term potentiation (sLTP). We induced sLTP by stimulating neurons with glycine-dependent chemical LTP protocol. Global translation inhibition by cycloheximide (CHX) completely blocked sustained spine enlargement consistent with previous reports. Expression of OptoMCP-FUS on wild-type neurons coupled with blue light illumination did not affect spine enlargement. However, light illumination on homozygous Actb-MBS knock-in neurons expressing OptoMCP-FUS impaired the maintenance of spine enlargement, suggesting that local translation of β -actin mRNA has a role in the maintenance of sLTP. We expect the OptoMCP-FUS system will be a valuable tool for studying the role of mRNA localization and local translation in living cells.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.06

Topic: I.08. Methods to Modulate Neural Activity

Title: A genetically encoded toolbox for observation and manipulation of spatiotemporal cAMP & cGMP dynamics

Authors: *J. RAI¹, H. LI², M. VALENCIA³, T. LUYBEN¹, F. BERGIN¹, M. ZHEN¹, K. OKAMOTO²;

¹Lunenfeld Tanenbaum Res. Inst., ²Lunenfeld Tanenbaum Res. Inst., Toronto, ON, Canada;

³Lunenfeld-Tanenbaum Res. Inst., Mount Sinai Hosp., Toronto, ON, Canada

Abstract: Cyclic adenosine/guanosine monophosphate (cAMP/cGMP) are ubiquitous second messengers in various intracellular signaling pathways that mediate sensory transduction, neuromodulation and further regulate higher-order brain network functions such as learning and memory. However, their spatiotemporal dynamics and related regulatory functions for neural circuit activity remain unclear due to limitations for observation and spatiotemporal specificity for acute perturbation in living neurons of the brain. Here, we have tested live-imaging and optogenetic approaches that enable direct observation and light-dependent manipulation of

endogenous cAMP and cGMP levels using genetically encoded fluorescent indicators and photoactivatable metabolic enzymes in living hippocampal neurons. For cAMP/cGMP observation, we prepared a set of cAMP and cGMP FRET/FLIM (Förster resonance energy transfer/fluorescence lifetime imaging microscopy) indicators utilizing CFP donor and YFP acceptor pair separated by either the cAMP-binding (Epac) or cGMP-binding (PKG) domain, with modified linker sequence. To make a compatible version with blue light activated photoactive enzymes such as PAC (photoactivatable adenylyl cyclase) or BlgC (blue light activated guanylyl cyclase), we also prepared another set of cAMP and cGMP FRET/FLIM indicators that use the YFP and RFP pair. After optimizing and validating the sensitivity of cAMP/cGMP indicators *in vitro*, we co-expressed these indicators with optogenetic enzymes which synthesize/hydrolyze cAMP/cGMP in hippocampal CA1 pyramidal neurons of organotypic cultured slices and have demonstrated that these probes can detect photoactivation of these optogenetic enzymes in living neurons by two-photon FRET/FLIM imaging. We also detect the endogenous cAMP/cGMP dynamics upon synaptic activation, indicating the ability of these tools to measure cAMP/cGMP levels in living neurons. Furthermore, we examined circularly permuted fluorescent protein-based cAMP/cGMP indicators and light-sensitive metabolic enzymes in the CA1 neurons *in vivo*. We will discuss the advantages and limitations of these approaches for *in vivo* neural network level applications in the brain of behaving mice.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.07

Topic: I.08. Methods to Modulate Neural Activity

Title: Mapsi: miniscope with all-optical patterned stimulation and imaging

Authors: J. ZHANG¹, *J. KIM¹, R. N. HUGHES¹, N. KIM¹, I. P. FALLON², K. BAKHURIN¹, F. ULLOA SEVERINO¹, H. H. YIN¹;

¹Dept. of Psychology and Neurosci., ²Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Recent advances in optical techniques for monitoring or modulating neural activity have led to numerous neuroscience findings. Particularly, all-optical approaches combining *in vivo* calcium imaging and optogenetics have enabled recording neuronal activity with cellular resolution as well as selective manipulation of neurons in the same animal. However, current tools often require stationary bench-top systems such as two-photon miniscopes which limit the portability of the system and animal behaviors. To address these limitations, we have developed a Miniscope with All-optical Patterned Stimulation and Imaging (MAPSI) by integrating a one-photon endoscope with a digital micromirror device. MAPSI enables simultaneous calcium imaging and photo-stimulation. Using this system, we were able to successfully image striatal

neurons from the direct or indirect pathway while simultaneously activating any neuron of choice within the field of view. MAPSI can identify neurons tuned to a particular behavior and mimic the activity pattern of neurons to recreate the behavior. MAPSI can also produce arbitrary spatiotemporal stimulation patterns generated by an experimenter. Thus, MAPSI will be a useful tool for all-optical investigation of neural circuit function in freely behaving animals.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.08

Topic: I.08. Methods to Modulate Neural Activity

Title: Open source frameworks for development and building of laser microscopes. Software and hardware.

Authors: ***M. NIKITCHENKO**¹, M. LORING², E. A. NAUMANN³;
¹Duke Univ., Durham, NC; ²Neurobio., Duke Univ. Hlth. Syst., Durham, NC; ³Neurobio., Sch. of Med., Durham, NC

Abstract: We present several open-source hardware and software frameworks which can be used for building and operating laser microscopes, and complex experimental equipment in general. All of them are built with a goal of being user-friendly, modular, and asynchronous. Using these frameworks, we designed and built 2 different two-photon microscopes with photostimulation modules, and a light-sheet microscope (SPIM) with two illumination paths, and are using them in our daily research of larvae zebrafish. These builds include: 3D models, purchasing system to allow for easy modification of assemblies and parts replacement, and multiple custom components with documentation and associated software. Most of the modules can be built in different ways, and the resulting microscopes can be either assembled from off-the-shelf components, or by making DIY parts (or ordering them from manufacturing vendors). To maximize the flexibility and easiness for combining different software modules (controlling hardware modules, running analysis software, etc.) and coordinating their activity in real time, we developed a new software platform, which we call MODULE CONDUCTOR. This platform allows users to combine software modules written in different languages, and running locally or remotely, using legible text configuration files. As a result, each assembly of shared modules is chosen by simply listing the modules of interest. Complementing existing modules with a plug-and-play add-on allows them to communicate between each other and the Module Conductor, either locally, or remotely (via the zmq protocol). On top of it, the Module Conductor includes a new paradigm-style language, which allows running complex coordinated tasks with the modules. E.g., to acquire a volumetric stack, it is only necessary to create a new sequence of steps for communication between modules and the Module conductor, which is written in text

format.

We hope that our work will provide a common platform for optical scientists developing new software, while remaining highly accessible for scientists who are new to the field of optical engineering, but would like to build highly-customizable, cutting-edge, equipment at an affordable price.

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Poster

499. Development and Application of Optogenetic Tools

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Topic: I.08. Methods to Modulate Neural Activity

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Title: Open-source hardware for optogenetic stimulation integrated with high-density CMOS microelectrode arrays

Authors: *K. VOITIUK¹, D. EHRLICH¹, T. SHARF¹, T. VAN DER MOLEN², J. SEVETSON¹, J. GENG¹, A. ROBBINS¹, C. PAZ FLORES¹, M. A. T. ELLIOTT¹, D. F. PARKS¹, S. TORRES MONTOYA¹, M. G. KEEFE³, T. J. NOWAKOWSKI³, D. HAUSSLER¹, K. S. KOSIK², S. R. SALAMA¹, M. TEODORESCU¹;

¹Univ. of California, Santa Cruz, CA; ²Univ. of California, Santa Barbara, CA; ³Univ. of California, San Francisco, CA

Abstract: We propose an open-source optogenetics platform for stimulating in vitro human 2D iPSC-derived neurons and 3D cortical organoid cultures on high-density CMOS multielectrode arrays. Using a multielectrode recording system, our hardware addition allows neurons expressing opsins to receive light stimulation protocols aligned to their neural activity data. The optogenetic stimulation can further be performed in response to the recorded neural activity. The platform uses off-the-shelf optoelectronic equipment and 3D printed components to be reproducible. Modularity allows users to select 385 nm - 625 nm LEDs for different optogenetic actuators. Our experiments use an optical fiber coupled to a blue 475nm LED to stimulate cells expressing channelrhodopsin-2 via pAAV-Syn-ChR2(H134R). We measure open and closed-loop responses to different programmed stimulation protocols, varying pulse frequencies, timings, and amplitudes on longitudinal experiments spanning several days.

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Poster

499. Development and Application of Optogenetic Tools

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Topic: I.08. Methods to Modulate Neural Activity

Support: European Research Council (ERC) - DISCONN; no. 802371 to A.G.

Title: Optogenetic modulation of the mouse default mode network with a single tapered optical fiber

Authors: E. DE GUZMAN¹, A. GALBUSERA¹, B. SPAGNOLO², F. PISANO², M. PISANELLO², M. DE VITTORIO^{2,4}, T. FELLIN³, F. PISANELLO², A. GOZZI¹;

¹Functional Neuroimaging Lab., Inst. Italiano di Tecnologia, Rovereto, Italy; ²Ctr. for Biomolecular Nanotechnologies, Inst. Italiano di Tecnologia, Arnesano (Lecce), Italy; ³Optical Approaches to Brain Function, Inst. Italiano di Tecnologia, Genova, Italy; ⁴Dept. di Ingegneria dell'Innovazione, Univ. del Salento, Lecce, Italy

Abstract: The default mode network (DMN) is a functional network of the human brain widely studied with fMRI due to its association with higher cognitive processes and frequent dysregulation in human brain disorders. Evolutionarily relevant precursors of the DMN have been described in primates and rodents, offering opportunities to unveil the core constituents and neurobiological underpinnings of DMN (dys)function. However, the widely distributed topography of this network and its peculiar antero-posterior organization have so far prevented reliable manipulation of its constitutive elements via optogenetics. Tapered optical fibers (TFs) offer homogeneous illumination of large cortical volumes while minimizing risk of tissue heating and spurious hemodynamic responses as assessed with fMRI. Here, we describe reliable network-scale manipulation of the rodent DMN via single TF optogenetic stimulation of the mouse medial prefrontal cortex (PFC), an evolutionary conserved hub-region. We show that a single TF implanted at a 15° angle resulted in bilateral light delivery to the PFC in slice preparations, with negligible tissue damage. In keeping with this, in vivo optogenetic-fMRI studies revealed that stimulation of pyramidal cells with a single TF in the PFC resulted in exquisitely bilateral stimulation of key DMN afferents of the mouse PFC, with the largest response in the thalamus, a region recently recognized as an important component of the DMN. Notably, the bilateral response achieved with a single low-invasive TF was comparable to that obtained with a canonical dual flat fiber configuration, and distinct from the unilateral response obtained with a single flat fiber implant. Corroborating the specificity of the mapped effects, studies in opsin free mice did not reveal any heat or visually induced fMRI responses at irradiance up to 100mW/mm². Similarly, blood pressure recordings showed that stimulation was

uncoupled from peripheral cardiovascular changes. Proof-of-concept experiments performed using this technology showed that rhythmic stimulation of the PFC resulted in differential engagement of cortical and subcortical DMN substrates, demonstrating the possibility of using this platform to produce and test network-level perturbations of high translational relevance.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.11

Title: WITHDRAWN

Poster

499. Development and Application of Optogenetic Tools

Location: SDCC Halls B-H

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Topic: I.08. Methods to Modulate Neural Activity

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Title: Understanding the spatial precision of optogenetic stimulation: a computational study

Authors: *D. BERLING, L. BARONI, J. ANTOLÍK;
Fac. of Mathematics and Physics, Charles Univ., Prague, Czech Republic

Abstract: Optogenetic stimulation is a powerful tool for studying the brain with a clinical application in neural prostheses. Crucial for the success of optogenetics-based brain-interfaces is the spatial resolution at which they can control neural activity. Major effort is being invested into improving this aspect through dense arrays of light elements and subcellularly precise expression of light-gated ion channels. However, there is a lack of quantitative understanding of how light source properties, neuron morphology, and the expression of light-gated ion channels constrain the spatial precision of stimulation. This knowledge is a key for guiding the development of stimulation devices, optogenetic constructs, and for designing and interpreting experiments utilizing optogenetic stimulation. Expanding upon existing computational work, we address these

questions by simulating optogenetic stimulation of channelrhodopsin-2 expressing pyramidal neurons (layer 2 and 5). The simulated scenario replicates illumination with an optical fiber placed on top of the cortex. According to our simulations, the neurons are stimulated in a highly anisotropic manner along the cortical space which strongly depends on the extent and spatial heterogeneity of their morphology. Somatic confinement of expression of channelrhodopsin improves the spatial precision and reduces anisotropy. We find that expression level and illumination power have to be tuned depending on the neuron type to achieve optimal spatial precision and that increasing the stimulation duration sharpens the spatial activation profile. Finally, we show that small fiber diameter and divergence yield optimal spatial precision. Fine-tuning one of these two parameters only improves precision if the other one is already small. In conclusion, our simulations identify important parameters of the optogenetic setup that are likely to impact the spatial precision at which the targeted neural populations can be externally controlled.

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Poster

499. Development and Application of Optogenetic Tools

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Title: All-optical whole-brain imaging and control of larval zebrafish neuronal activity

Authors: L. TURRINI^{1,2}, P. RICCI¹, M. SORELLI^{1,3}, G. DE VITO^{1,4}, M. MARCHETTI⁶, F. VANZI^{1,5}, L. SILVESTRI^{1,3}, *F. S. PAVONE^{1,2,3};

¹LENS, European Lab. for Non-Linear Spectroscopy, Sesto Fiorentino, Italy; ²Natl. Inst. of Optics (INO), Natl. Res. Council (CNR), Sesto Fiorentino, Italy; ³Dept. of Physics and Astronomy, Univ. of Florence, Sesto Fiorentino, Italy; ⁴Dept. of Neuroscience, Psychology, Drug Res. and Child Hlth., Univ. of Florence, Firenze, Italy; ⁵Dept. of Biol., Univ. of Florence, Sesto Fiorentino, Italy; ⁶L4T, Light4Tech, Sesto Fiorentino, Italy

Abstract: One of the most ambitious goals in neurosciences is the disentangling of the causal relationships among functional activity of neuronal populations on a whole-brain scale. This target, prohibitive just a couple of decades ago, is getting within reach in the last few years. Indeed, the tremendous technological improvement of both optical methods and genetically-

encoded indicators and actuators, coupled with the tiny and transparent larval zebrafish as an animal model, led to the possibility of recording and perturbing the activity of large neuronal populations, spanning an entire vertebrate brain. Here, we present the development of a custom optical system consisting in the integration of a two-photon light-sheet microscope, optimized for high-speed volumetric imaging, with a module devised for 3D random-access two-photon excitation applicable to optogenetic actuators. We show that this system allows us to simultaneously perform fast whole-brain functional imaging and targeted optogenetic stimulation. In particular, the photostimulation unit employs four acousto-optic deflectors (AODs) driven by an electronic control system optimized for homogeneous energy delivery across the stimulation volume (up to $100 \times 100 \times 100 \mu\text{m}^3$). This 3D light-addressing module allows tailored efficient optogenetic activation of selected populations of neurons during light-sheet whole-brain recording of neuronal evoked responses at high spatio-temporal resolution. Employing a double-transgenic zebrafish line, pan-neuronally expressing both the green fluorescent calcium indicator GCaMP6s and the red-shifted light-gated cation channel ReaChR, we adopted a crosstalk-free non-invasive all-optical approach to identify neurons functionally connected to the stimulated regions. In this way, we laid the foundations for the brainwide reconstruction of larval zebrafish functional connectivity.

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Poster

499. Development and Application of Optogenetic Tools

Location: SDCC Halls B-H

Time: Tuesday, November 15, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 499.14

Topic: I.08. Methods to Modulate Neural Activity

Support: CRSNG

Title: Development of structured optogenetic stimulation in behaving animals for functional mapping of the mouse cortex.

Authors: *V. CHOUINARD¹, I. DJEROUROU¹, V. DAIGNEAULT¹, N. CORTES¹, L. LAPLANTE¹, M. PTITO¹, F. LESAGE², C. F. CASANOVA¹, M. VANNI¹;
¹Univ. de Montréal, Montréal, QC, Canada; ²École Polytechnique Montréal, Montréal, QC, Canada

Abstract: All-Optical imaging is a robust tool for exploring the functional networks of the cortex. Light-sensitive proteins such as genetically encoded indicators and ion-conducting proteins can be targeted to cellular sub-compartments to record and manipulate neurons simultaneously in awake or anesthetized animals. However, large-scale functional mapping and optical control that comprises multiple cortical areas in a behaving animal create many new challenges. Our goal is to integrate a multi-modal system of spatially structured

photostimulation, widescale imaging and behaviour in awake head-fixed mice. We first created a protocol of functional imaging using calcium indicators and spatially targeted optogenetics using the digital micromirror device (DMD). The DMD uses mirrors to shape a light beam of blue light into a large surface with large power (9x9mm, 10mW/mm², 470nm). Such innovative technology opens new doors in optogenetics as brain areas often have complex shapes and are interconnected with remote areas. We virally expressed the calcium indicator jrGECO1a and the opsin ChR2 in excitatory neurons using CaMK2 promoter. Calcium imaging allowed us to do cortical mapping like retinotopic maps and measure the optogenetics response amplitude (~20% increase in fluorescence). In the second set of experiments, we injected mice with a virus expressing ChR2 in inhibitory neurons (AAV2/9 - Dlx-ChR2.mCherry) to develop a protocol of reversible inactivation of the mouse visual cortex during a visually guided task. We first validated that inhibitory neurons were correctly driven by photostimulation through electrophysiological recordings in a first subset of mice. Another subset of mice was then trained to discriminate orientation angles until good performance was achieved. Amongst the mice that performed the task, we showed that reversible inactivation of the primary visual cortex impaired the visual discrimination of orientation. In the end, we expect to effectively develop a system combining fast and precise optogenetic stimulation, functional mapping, and accurate measures of mouse perception.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.15

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH K99 NS116122
AP Giannini Fellowship
Stanford Dean's Award Fellowship
LSRF
NIH
HHMI

Title: Brainwide neural circuitry for controlling orofacial movements

Authors: *T. A. MACHADO¹, I. V. KAUVAR¹, Y. CHEN¹, J. KOCHALKA², S. BRADBURY², K. DEISSEROTH³;

¹Bioengineering, Equal Contribution, ²Bioengineering, Stanford Univ., Stanford, CA;

³Bioengineering and Psychiatry, Stanford Univ. and HHMI, Stanford, CA

Abstract: Even seemingly simple movements require context-dependent processing of sensory signals and motor commands. How does the joint activity of neural circuitry distributed across the brain subserve motor behavior? We first studied these behaviors in head-restrained mice using a new widefield optical imaging method, Cortical Observation by Synchronous Multifocal Optical Sampling (COSMOS) (Kauvar*, Machado*, et al., Neuron, 2020), to record with true simultaneity from over a thousand neuronal sources spread across the entirety of curved mouse dorsal neocortex at ~30 Hz; analysis suggests that individual COSMOS sources represent mixtures of signals from 1-15 neurons. Using a three-spout, memory-guided, lick-to-target task, we found that distributed neural activity throughout cortex encodes targeted licking actions, with no apparent spatial structure ($p < 0.05$, permutation test for spatial clustering of sources with similar tunings; 4 mice). We also found that across cortex, unaveraged (but not trial-averaged) activity correlations showed local structure (at distances < 1 mm, unaveraged correlations were consistently lower than trial-averaged correlations; $p = 0.0001$, paired t test; 4 mice). Analysis of population dynamics revealed similar encodings of history-guided motor plans in areas across cortex. But how are these widespread representations transformed into motor output? To address this question, we measured neural activity in medullary circuits while kinematically similar licks were made towards three spouts in different behavioral contexts. During this behavior, we optogenetically inhibited cortex (using VGAT-ChR2 mice), and using 3D kinematic tracking, we revealed that cortical silencing influenced licking movements in a context-specific manner (i.e. it selectively affected long-distance licks to the middle spout, but not closer licks to the side spouts; $n=3$ mice with at least 5 sessions per mouse). We subsequently obtained simultaneous recordings from the cortex and the medulla to analyze the effects of this perturbation along the output pathway between cortex and the medulla. We found units modulated by cortical inhibition in both cortex-and the medulla. These data are suitable for alignment to the Allen Atlas Common Coordinate Framework, where we co-register information about neuronal connectivity (from rabies tracing and hydrogel-based brain clearing) and neuronal cell typology (using 1000-gene STARmap for in situ sequencing of the brainstem transcriptome). Combining these diverse data streams into a coherent map may advance our understanding of how distributed representations and descending commands drive movements.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.16

Topic: I.08. Methods to Modulate Neural Activity

Title: Data-driven identification and perturbation of multi-regional and cell-type-specific neural population dynamics in reward history computation

Authors: *Y. JO, E. L. SYLWESTRAK, S. VESUNA, S. QUIRIN, A. DRINNENBERG, T. X. LIU, J. SHI, D. K. KIM, C. RAMAKRISHNAN, R. CHEN, K. V. SHENOY, D. SUSSILLO, K. DEISSEROTH;
Stanford Univ., Stanford, CA

Abstract: Neural computations for storing and processing history of reward and punishment are essential for the survival of animals. The timescale of such computations tends to be substantially longer than that of intrinsic membrane time constants of single neurons or even of typical single trials or tasks, suggesting the need for specific mechanisms generating persistent activity of relevant neural populations. Here we measured multi-regional and cell-type-specific neural population activity in awake mice performing reward history-guided decision-making tasks, using Neuropixels extracellular electrophysiology and two-photon Ca^{2+} imaging. With these datasets in hand, we conducted data-driven dynamical systems modeling using LFADS (latent factor analysis via dynamical systems) to reverse-engineer the population dynamical structures. We identified relevant persistent activity in multiple brain regions including retrosplenial cortex (RSP; $N=6$ mice), anterior lateral motor cortex (ALM; $N=8$), and medial habenula (MHb; $N=9$). Within MHb, genetic dissection of cell types revealed cell-type-specific line attractor dynamics underlying reward history integration. Learned dynamical systems models were utilized to make predictions on temporal evolution of activity and behavior, which in turn guided perturbation experiments. One-photon optogenetic inhibition of the reward history-integrating populations resulted in degraded behavioral performance ($p=0.025$, $N=5$), consistent with the hypothesized importance of the underlying circuitry and computation. Two-photon holographic optogenetic perturbation could be designed based on the identified dynamical structures to be orthogonal or parallel to the line attractor ($N=3$). Taken together, our integrated approach using a tight experiment-theory loop presents a framework by which the large-scale neurophysiology experiments can be efficiently guided by quantitative data-driven models for multi-regional and cell-type-specific investigation of neural population dynamics underlying fundamental behaviorally-relevant computations.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.17

Topic: I.08. Methods to Modulate Neural Activity

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5199990614277)

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Title: Transient response of trigeminal ganglion to halorhodopsin mediated modulation on trigeminal neuralgia rat model.

Authors: *E. KC¹, J. ISLAM¹, K. T. HYUN², Y. Y. PARK², Y. S. PARK^{1,2};

¹Neurosci., Chungbuk Natl. Univ., Cheongju, Korea, Republic of; ²Neurosurg., Chungbuk Natl. Univ. Hospital, Cheongju, Korea, Republic of

Abstract: Alterations in the expression of several molecules in trigeminal ganglion (TG) neurons are likely to cause orofacial sensory dysfunctions associated with trigeminal nerve injury or orofacial inflammation. TG is the principal site of aberration in trigeminal neuralgia (TN), and thus an important locus for modulating afferent input. We have shown that suppressing TG neuronal activity would decrease brainstem trigeminal nucleus caudalis (TNC) action, and have proposed this inhibition they mediate could contribute to the pain attenuating effect following trigeminal nerve injury. In this study, we aim to determine the halorhodopsin mediated TG inhibition and its effect on trigeminal pain circuitry. We have generated TN by infraorbital nerve constriction in female Sprague Dawley rats, with naive and sham rats serving as controls. We have injected each group of rats with TG-directed microinjections of adeno-associated virus containing either the optogenetic or null vector. We have carried out in vivo optogenetic experiments in TG with simultaneous electrophysiological recordings from the ventral posteromedial nucleus (VPM) of the thalamus. We also examine the impact on pain behavioral responses. We find evidence of yellow laser-driven inhibition on TG mediate improved pain behavioral responses. We also find the recordings in TN rats demonstrated a decrease in burst firing activity during optogenetic inhibition of TG by yellow laser, indicative of thalamic manipulation and GABA disinhibition. To blue laser stimulation, meanwhile, we observe sustained hypersensitivity and enhanced tonic firing. Our findings provide functional evidence that TG inhibition could coordinate trigeminal pain signal transmission in a TN animal model. We propose that halorhodopsin mediated TG inhibition precisely interrupt the trigeminal pain circuitry and could result in analgesia. Together, these findings identify TG neurons as prospective target for trigeminal neuralgia therapeutic intervention.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.18

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH
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Title: Cardiogenic control of affective behavioral states

Authors: *R. CHEN¹, B. HSUEH¹, D. D. TANG¹, M. RAFFIIE², Y. JO³, Y. KIM¹, M. INOUE⁴, C. RAMAKRISHNAN², L. TAN⁵, M. M. ZEINEH⁶, M. GOUBRAN⁷, K. DEISSEROTH⁸;

¹Stanford Univ., Palo Alto, CA; ²Stanford Univ., Stanford, CA; ³Applied Physics, Stanford Univ., Palo Alto, CA; ⁴Bioengineering, Stanford Univ., Menlo Park, CA; ⁵Bioengineering, Stanford Univ., Palo Alto, CA; ⁶Radiology, Stanford Univ., Stanford, CA; ⁷Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada; ⁸Stanford, Stanford, CA

Abstract: Physiological theories of emotion have suggested that bodily signals (e.g., changes in heart rate) might influence emotional states such as fear and anxiety. However, this hypothesis-widely debated for more than a century- has remained experimentally intractable. To alter cardiac rhythms, nonspecific interventions such as drugs or painful shocks would be required. Even with high-resolution tools like optogenetics, optical control of heart rate in freely moving animals would not have been possible (until recently), since existing opsins were not sensitive enough to control a large organ like the heart with enough precision and power. To determine how altered cardiac rhythms might affect emotional or affective states, we developed a noninvasive optogenetic pacemaker utilizing 1) ChRmine under control of the mouse cardiac troponin T promoter delivered via AAV9 for enhanced tropism in cardiac tissue (AAV9-mTNT::ChRmine-2A-oScarlet) and 2) wearable micro-LED optics. Using the ultrapotent opsin ChRmine enabled precise control of cardiac rhythms up to 1000 beats per minute in freely-moving mice within a safe range of illumination power similar to conventional optogenetics. We tested the behavioral impact of truly precise and specific induced cardiac changes on behavior and affective state. We found that this primary and direct tachyarrhythmia indeed potently enhanced anxiety-like behavior in mice (n=16), demonstrating causal impact of the body to brain axis. We observed behavioral changes only in risky contexts, suggesting that both exteroceptive and interoceptive processes were synergistically required to modulate behavioral state changes. To identify potential brain regions involved in cardiac interoception, we performed a whole brain activity screen in in double transgenic TRAP2: Ai14 mice, and identified posterior insular cortex as a potential mediator of bottom-up cardiac signal processing (n=8). We then showed that simultaneous optogenetic inhibition of posterior insular cortex with iC++ during optical cardiac pacing was sufficient to reverse the induced behaviors, implicating this region as a necessary site for mediating cardiogenic anxiety-like and apprehensive behavior (n=8). Our findings offer insights into the specific mechanisms by which such bodily signals can causally influence complex behaviors, and reveal that body and brain must be considered together. We also present robust and generalizable tools for non-invasive, temporally precise control of precision-targeted cells, tissues, and organs throughout the body.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.19

Topic: I.08. Methods to Modulate Neural Activity

Support: NIMH Grant ZIAMH002958
NIMH Intramural Research Training Award (IRTA) Fellowship Program

Title: Behavioral detectability of optogenetic stimulation of inferior temporal cortex varies with the visibility and size of concurrently viewed objects

Authors: *R. LAFER-SOUSA¹, K. WANG¹, R. AZADI¹, E. LOPEZ¹, S. BOHN², A. AFRAZ¹;
¹NIMH, NIH, Bethesda, MD; ²Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: We have previously demonstrated that macaque monkeys can behaviorally detect a subtle optogenetic impulse delivered to their inferior temporal (IT) cortex. We have also shown that the ability to detect the cortical stimulation impulse varies depending on some characteristics of the images viewed at the time of stimulation. This observation raises an intriguing question about the phenomenological nature of the perceptual event induced by stimulation: Does stimulation of the same neural population induce a consistent perceptual event, independent of the concurrently fixated image, that is more or less difficult to detect due to figure-ground effects? Or does stimulation induce a variable perceptual event depending on the concurrent visual input? To tease apart these two interpretations, we systematically tested how diminishing the visibility of the visual input affected detection of the cortical event. In the first experiment visibility was diminished by reducing the contrast, saturation, and spatial frequency of the objects viewed during stimulation. In the second experiment visibility was diminished by reducing object size. If cortical stimulation evokes a consistent perceptual event, it should be similarly if not more easily detected when the onscreen images are less visible. Two macaque monkeys were implanted with LED arrays over a region of their central IT cortex transduced with the depolarizing opsin C1V1. In each trial, following fixation an image was displayed on the screen for 1s. In half of the trials, randomly selected, an LED was turned on for 200ms halfway through image presentation, and the animal was rewarded for correctly identifying whether the trial did or did not contain cortical stimulation. The image set for the first experiment consisted of 5 objects that degraded in contrast, saturation, and spatial frequency to near uniform gray in 4 steps. The image set for the second experiment consisted of 5 objects that were reduced in size in 4 steps, from 8 to 1 degree(s) of visual angle. A “no image” condition was also included in both experiments. Attenuating the visibility of the objects by diminishing their contrast, spatial frequency, and saturation significantly decreased detection performance

(ANOVA: M1 $p = 0.004$, M2 $p < 0.001$), as did reducing object size (ANOVA: M1 $p = 0.01$, M2 $p < 0.001$). These results show that identical stimulation impulses delivered to the same neural population induce variable perceptual events depending on the visibility of the objects viewed at the time of brain stimulation. The findings carry significant implications for the design and interpretation of perturbation studies, and for the development of visual prosthetics.

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Poster

499. Development and Application of Optogenetic Tools

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Support: American Heart Association postdoctoral fellowship
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NIH R01 NS119395

Title: Investigating the frequency response of sensorimotor cortex in non-human primates using optogenetics

Authors: ***B. M. SMITH**¹, **A. YAZDAN-SHAHMORAD**²;

¹Bioengineering, ²Bioengineering and Electrical Engin., Univ. of Washington, Seattle, WA

Abstract: Optogenetics is a powerful tool that enables artifact free recordings and millisecond-level control of neuronal activity within specific groups of neurons. This technique is ideal for relating brain function to behavior in animals with great behavioral capabilities such as non-human primates (NHPs). We have developed a large-scale optogenetic interface in NHPs that enables large-scale ($>1\text{cm}^2$) stimulation and recording from sensorimotor cortex. This interface in combination with the potentials of optogenetics creates an unprecedented opportunity for us to study the frequency response of sensorimotor cortex at both the local and network scale. Two adult male rhesus monkeys were used in this study. We used convection enhanced delivery to express AAV-CamKIIa-C1V1-EYFP across primary somatosensory (S1) and motor (M1) cortices. Semi-transparent micro-electrocorticography (μECoG) arrays were implanted on top of the expressing areas to provide network recording from these cortical areas during optical stimulation. To investigate the frequency response of the network, we optically stimulated one location in either M1 or S1 with one-second pulse trains. We varied the pulse width (0.5, 1, 5 ms) and frequency (10, 20, 30, 35, 40, 50, 70, 100, 150 Hz) of these trains in an interleaved fashion while recording from the μECoG arrays covering both M1 and S1. Each parameter was repeated 60 times. These recordings provided light evoked neural responses from areas of sensorimotor cortex that were both close to the site of stimulation and areas that were far from the site of stimulation. We were able to induce clear oscillations at the site of stimulation when

stimulating at frequencies in the beta (e.g. 10, 20 and 30 Hz), gamma (35, 40, 50 Hz), and high gamma (70 Hz) ranges. At higher stimulation frequencies (100 and 150 Hz) however, reliable evoked activity was only observed at the beginning of the 1-second stimulus train. This frequency-dependent response of the neural activity is likely due to the C1V1 channel off-kinetics, however other factors such as the underlying neural network could play a role. We will further investigate this phenomena by evaluating the frequency response of the above stimulation parameters within the sensorimotor cortex. Specifically, we will evaluate the propagation of neural activity from the site of stimulation to the distant areas in response to various stimulation parameters.

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Poster

499. Development and Application of Optogenetic Tools

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH U01 NS116377
Schmitt Program on Integrative Neuroscience
Del Monte Neuroscience Institute

Title: Intersectional optogenetics for excitation and inhibition of cortico-cortical projections in the mouse and marmoset brain

Authors: ***L. SHAW**¹, **K. PADMANABHAN**¹, **J. F. MITCHELL**², **K. H. WANG**¹;
¹Univ. of Rochester Med. Ctr., Rochester, NY; ²Univ. of Rochester, Rochester, NY

Abstract: Modulation of neural circuits based on projection patterns is critical to causal understanding of brain functions. Intersectional viral labeling methods have enabled targeting specific populations of projection neurons for optogenetic manipulation in mice. These methods have been less explored in non-human primates and generally thought to be less efficient. Here we tested intersectional adeno-associated virus (AAV) delivery of the excitatory opsin channelrhodopsin 2 (ChR2) and inhibitory red-shifted opsin Jaws to cortico-cortical projection neurons in marmoset monkeys. We began testing the intersectional expression of ChR2 in marmosets by utilizing the callosal connections between premotor cortices. AAVrg-hSyn-Cre was injected into one hemisphere for retrograde delivery of the Cre recombinase, and AAV9-CAG-FLEX-ChR2 was injected into the contralateral site for local Cre-dependent expression of ChR2. This AAV9 intersection yielded highly efficient but leaky expression of ChR2. To explore vectors that may yield higher specificity, we tested AAV8-CAG-FLEX-ChR2 in marmoset callosal connections and found reduced efficiency but higher specificity compared to AAV9. Furthermore, we tested intersectional expression of both ChR2 and Jaws in the ipsilateral connections between marmoset dorsal premotor cortex and posterior parietal cortex. By co-

injecting AAV8-CAG-FLEX-ChR2 and AAV8-CAG-FLEX-Jaws into premotor cortex and AAVrg-hSyn-Cre into parietal cortex, we found that the intersectional expression of ChR2 and Jaws was specific and moderately efficient, with a high degree of opsin co-expression at the premotor injection site. Finally, we verified by electrophysiology in mice that the same vectors enabled optical excitation and inhibition of cortical neurons expressing Jaws and ChR2. We also found that ChR2-evoked neuronal spiking can be effectively suppressed by Jaws excitation at the cell body, but not at the afferent terminals. Ongoing electrophysiology experiments aim to validate the excitation and inhibition of cortical projections from premotor to parietal cortex in marmosets, which may facilitate future functional investigation of cortical feedback pathways in action control.

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Poster

499. Development and Application of Optogenetic Tools

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Program #/Poster #: 499.22

Topic: I.08. Methods to Modulate Neural Activity

Title: In vitro seizure suppression by patterned optogenetic stimulation while allowing information processing in hippocampus

Authors: *M. ABEDIN¹, Y. BERDICHEVSKY²;

¹Bioengineering, ²Bioengineering, Electrical and Computer Engin., Lehigh Univ., Bethlehem, PA

Abstract: Most prevalent refractory form of epilepsy is mesial temporal lobe epilepsy which involves seizures arising from hippocampus. An alternative option to surgical resection for treating patients with intractable epilepsy is to suppress seizures with electrical stimulation. Current stimulation protocols suppress seizures by delivering electrical pulses at high frequency, which effectively prevents activity in stimulated area. We are interested in developing an effective method for suppressing seizures in such a way that activity in stimulated region is not completely shut off during the intervention. This will allow stimulated area to participate in information processing and exchange of information with other brain regions, and may be a more gentle method of suppressing seizures than existing protocols. During ictal activity, neurons in a network fire at high rate. Ictal episode terminates when neurons run out of neurotransmitter. Therefore, if we keep the synapses in the epileptic network depressed (short term depression) by delivering asynchronous stimulation, we may be able to suppress seizures without shutting off all neural activity. We tested this hypothesis by delivering spatially patterned optical pulses to rat (post-natal day 7/8) organotypic hippocampal slice cultures expressing channel rhodopsin (ChR2), a blue light activated cation channel in excitatory neurons under CaMKII promoter. Slices also expressed jRGECO1a, a red fluorescent protein sensitive to intracellular calcium

under synapsin promoter to enable us to monitor ictal-like activity. We developed a protocol to deliver patterned optogenetic stimulation to the whole slice. Recordings were 60 minutes long in total (20 mins each for spontaneous-with stimulation-spontaneous recording). Mightex's Polygon Pattern Illuminator for delivering stimulation, a CCD camera and a 4X objective were used to record fluorescent changes at 20 frames per second. Videos were then analyzed to extract raw mean grey value using ImageJ and fluorescent change, ΔF over baseline, F was calculated in MATLAB using asymmetric least square mean smoothing method. We found that 92% of slices were seizure free during stimulation with a $\Delta F/F$ of 1.7 ± 0.63 % (mean \pm SD) at steady state but were seizing spontaneously when there was no stimulation ($n= 12$ slices, 3 animals). This low level of intracellular calcium illustrates that activity is not shut off entirely which allows the room for information processing. Our findings provide an alternative, gentle yet effective means of suppressing seizures. This method may improve the quality of life for epileptic patients by reducing side effects of neural stimulation.

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