Nanosymposium

013. Neuron Signaling, Neuroendocrine, and Physiology

Location: SDCC 24

Time: Saturday, November 12, 2022, 1:00 PM - 3:00 PM

Presentation Number: 013.01

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Photoperiod impacts nucleus accumbens dopamine signaling dynamics

Authors: *A. N. JAMESON, J. K. SIEMANN, K. G. MOORE, J. R. MELCHIOR, E. CALIPARI, C. A. GRUETER, D. G. MCMAHON, B. A. GRUETER;
Vanderbilt Univ., Nashville, TN

Abstract: Light signals have long been recognized as having a powerful influence on the structure and function of specific brain systems. The light-entrained circadian clock and circadian photoperiod are associated with affective and reward-related behaviors, but the underlying neurobiological mechanisms are largely unknown. The mesolimbic dopamine pathway is one of the most well-studied pathways within the brain's reward circuit. Within this pathway, dopamine input to the nucleus accumbens (NAc) plays a critical role in maintaining efficiency and flexibility coordinating goal-directed, motivated behaviors. Here, we provide novel findings of how day length, or photoperiod, affects dopaminergic-signaling dynamics in the NAc. We assess reward circuit dopamine content and signaling dynamics using mass spectrometry and fast-scan cyclic voltammetry (FSCV) in mice maintained in different photoperiods. To determine behavioral consequences of DA signaling we investigate cocaine-induced behaviors. We find differential NAc DA content and that summer-like photoperiod enhances dopamine release and uptake in the NAc of female mice, but not in males. Further, we demonstrate that the dopamine transporters are a primary locus of action for sex-specific photoperiod modulation of dopamine signaling dynamics. Lastly, we demonstrate that photoperiod-driven basal differences in dopamine signaling dynamics manifest in observable differences in dopamine-dependent behaviors. Taken together, this work uncovers a potential neural circuit basis for sex-linked seasonality of neuropsychiatric disorders. By revealing novel mechanisms regarding circadian light input and neural plasticity, the findings presented here can change conceptualization of the impact on brain and behavior of a pervasive stimulus - environmental light cycles. This work creates an integrated picture of a novel circuit mechanism that has potential to stimulate a basis for discovery of environmental influence on typical and atypical neural function.

Disclosures: A.N. Jameson: None. **J.K. Siemann:** None. **K.G. Moore:** None. **J.R. Melchior:** None. **E. Calipari:** None. **C.A. Grueter:** None. **D.G. McMahon:** None. **B.A. Grueter:** None.

Time: Saturday, November 12, 2022, 1:00 PM - 3:00 PM

Presentation Number: 013.02

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01 R01DK129548
                ADA 1-17-PDF-138

Title: The sexual dimorphic role of SK current in serotonin neurons regulates feeding behavior during chronic isolation

Authors: B. FENG, J. HARMS, P. GAO, T. SMILEY, **Y. HE**;
Pennington Biomed. Res. Ctr., Baton Rouge, LA

Abstract: Dysfunctions of serotonin neurons are implicated in multiple psychological disorders, including feeding disorder, anxiety, depression, and mania behavior. However, whether and how altered serotonin neural activity may affect these complex behaviors differently in different genders remain unclear. We found that 5-Hydroxytryptamine (5-HT) neurons in the dorsal Raphe nucleus (5-HT^{DRN}) displayed basal neuronal firing activity in males V.S. females. Male 5-HT^{DRN} neurons have lower spontaneous action potential firing frequency compared to female 5-HT^{DRN} neurons. This was associated with higher expression of a small conductance Ca2+-activated K+-3 channel (SK3, encoded by the Kcnn3 gene) in male mice compared to female mice. When male mice were isolated from group-housed to single-house conditions, male 5-HT^{DRN} neurons reduced neuronal firing activity with elevated SK3 currents while 5-HT^{DRN} neuronal firing activity in female mice did not significantly change. Male mice also showed slow recovery of daily food intake compared to female mice when isolated from group-housed to single-housed. To examine the physiological role of SK3 in serotonin neurons, we generated a conditional knockout mouse model, in which SK3 was deleted selectively in mature serotonin neurons (Kcnn3f/f/Tph2-CreER). We found that serotonin neurons in these mutant mice showed diminished SK3-mediated outward potassium currents, decreased after-hyperpolarization, increased firing frequency, and elevated resting membrane potential. More importantly, both male and female mice with SK3 deleted from 5-HT^{DRN} neurons failed to show a reduction in daily food intake after isolation. In summary, we demonstrated that SK3 is required to maintain the regular firing activity of serotonin neurons, and we further suggest that enhanced SK3 functions in serotonin neurons will be involved in the reduction of daily food intake during the onset of chronic isolation.

Disclosures: B. Feng: None. **J. Harms:** None. **P. Gao:** None. **T. Smiley:** None. **Y. He:** None.

Nanosymposium

013. Neuron Signaling, Neuroendocrine, and Physiology

Location: SDCC 24

Time: Saturday, November 12, 2022, 1:00 PM - 3:00 PM

Presentation Number: 013.03

Topic: B.04. Synaptic Transmission

Title: Multilayered control of the KaiR1D autoreceptor distribution and gating properties

Authors: *W.-C. HSIEH, T. HAN, R. VICIDOMINI, P. NGUYEN, Z. LI, M. SERPE;
Natl. Inst. of Health, HHS, Bethesda, MD

Abstract: At excitatory synapses, autoreceptors provide a feedback mechanism that modulate neurotransmitter release and ensure stable neuronal network activities. Disruptions to this feedback have been linked to various neuronal disorders. Autoreceptors localize on presynaptic membranes and respond to neurotransmitter released by the cell on which they sit. Detection of these low abundant modulators has been challenging; so far, the presynaptic distribution has been inferred from functional and genetics studies. At *Drosophila* NMJ, an autoreceptor containing the KaiR1D glutamate receptor subunit controls the glutamate release; in the absence of KaiR1D, basal neurotransmission is reduced to half of the normal levels. We and others previously showed that KaiR1D requires at least two auxiliary proteins, Neto-α and Sol1, to fulfill its *in vivo* functions. Here, we focus on the roles of Neto-α in modulating KaiR1D properties and subcellular distribution. Neto-α limits KaiR1D *in vivo* activities: Basal neurotransmission is reduced by ~50% in *neto-α^{null}* animals and neuronal overexpression of KaiR1D cannot rescue this defect. Using fast perfusion on outside-out patches of HEK cells transfected with KaiR1D, we measured the gating properties of KaiR1D alone or in complexes with Neto-α. We found that Neto-α modulates the gating properties of KaiR1D channels, decreasing the desensitization and deactivation rates. Neto proteins have conserved extracellular domains, including two CUB domains and a LDL motif, and variable intracellular domains (CTD). CUB1 is required for modulation of KaiR1D gating properties as well as *in vivo* autoreceptor activities. The CTD participates in the modulation of KaiR1D gating properties but is dispensable *in vivo*. This difference may be reconciled by the presence of Sol1 *in vivo* but not in reconstituted systems. To search for a role for Neto-α in the subcellular distribution of KaiR1D, we examined KaiR1D localization in primary rat hippocampal neurons. KaiR1D localizes to dendrites with or without Neto-α. However, KaiR1D alone cannot effectively enter into the axon even when overexpressed. Neto-α or ΔCUB1, but not ΔCTD, promote KaiR1D axonal localization. All Neto variants distributed at both neurites and formed puncta largely colocalizing with KaiR1D. Finally, Neto-α and KaiR1D colocalize in the proximity of the active zones marked by the presynaptic scaffold Bassoon, suggesting that Neto-α may stabilize KaiR1D at the site of autoreceptor function. Our data indicate that multiple layers of modulation ensure proper autoreceptor activities and reveal potential targets for pharmacological interventions.

Disclosures: W. Hsieh: None. **T. Han:** None. **R. Vicidomini:** None. **P. Nguyen:** None. **Z. Li:** None. **M. Serpe:** None.

Nanosymposium

013. Neuron Signaling, Neuroendocrine, and Physiology

Location: SDCC 24

Time: Saturday, November 12, 2022, 1:00 PM - 3:00 PM

Presentation Number: 013.04

Topic: B.04. Synaptic Transmission

Title: Synaptic modulation of thalamocortical circuits by endogenous and exogenous opioids in mice

Authors: *E. ARIAS HERVERT, W. BIRDSONG;
Pharmacol., Univ. of Michigan, Ann Arbor, MI

Abstract: The goal of the present study was to elucidate how opioid drugs modulate excitatory and inhibitory synaptic transmission between the mediodorsal thalamus and the anterior cingulate cortex, two interconnected brain regions involved in pain and reward processing. Opioids are one of the most prescribed drugs in the United States for their efficacy to ameliorate pain, but little is known about how opioid drugs and their receptors modulate neuronal circuits in the central nervous system to elicit pain relief. Endogenous opioids and exogenous opioid drugs like morphine can elicit pain relief through actions in the ACC. Morphine primarily activates the mu opioid receptor expressed in MD terminals, while endogenous opioids like enkephalin (Enk) and endorphin can activate both mu and delta opioid receptors expressed on MD terminals and cells within the ACC, respectively. Based on our previous work describing cellular expression of mu and delta opioid receptors, our hypothesis is that Enk attenuates inhibitory transmission more than excitatory transmission in the ACC, causing a relative increase in the activity of ACC layer V pyramidal neurons ultimately leading to pain relief. To test this hypothesis, we used optogenetic manipulations to selectively excite thalamic, cortical or claustral inputs to ACC, and measured changes induced by opioid drugs using brain slice electrophysiology. We compared the effects of Enk and selective mu and delta opioid receptor agonists (and antagonists) on excitatory and inhibitory synaptic currents in L5 ACC pyramidal neurons and measured the relative change in excitation-inhibition balance in response to different opioid drugs. We also compared the effect of Enk versus mu and delta opioid receptor agonists on the membrane potential in current clamp configuration and found that mu and delta opioid receptor mediate opposite neuronal responses: mu agonists depressed the postsynaptic potential, while delta agonists facilitate it. Finally, to understand how the ACC excitability and opioid sensitivity may be altered in animals undergoing pain, we used the complete Freund's adjuvant (CFA)-induced inflammatory pain model. We injected CFA in the hind paw of naïve mice to induce chronic pain and five days later we euthanized these animals and examined synaptic transmission to determine how the responses to opioid drugs were affected by chronic pain. We hope that these results will contribute to the identification of novel pharmacological targets and the development of more selective opioid-based therapies for chronic pain management with lower risk of unwanted effects.

Disclosures: E. Arias Hervert: None. **W. Birdsong:** None.

Nanosymposium

013. Neuron Signaling, Neuroendocrine, and Physiology

Location: SDCC 24

Time: Saturday, November 12, 2022, 1:00 PM - 3:00 PM

Presentation Number: 013.05

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Adrenomedullary stress response dynamics in the living anestethized rat

Authors: *J. R. LOPEZ RUIZ[1], R. W. HOLZ[2], E. L. STUENKEL[1];
[1]Mol. & Integrative Physiol., [2]Pharmacol., Univ. of Michigan, Ann Arbor, MI

Abstract: The stress response has a major role in survival and prepares the body to deal with
different challenges, however if prolonged, often drives adverse health consequences. A key
pathway of the stress response is through the adrenal medulla. Adrenal medullary cells are
architecturally clustered and electrically coupled, however the in vivo dynamics of these cells
remain unknown. Therefore, the goal of this project is to dynamically and precisely monitor the
electrophysiological responses from the adrenal medulla in the living animals during normal and
under stress conditions to topographically dissect the chromaffin cells population and their
interactions. To achieve this, a high-density silicon probe was slowly inserted into the
immobilized left adrenal gland while recording, then the system was challenged first by
electrically stimulating the splanchnic nerve with different intensities, frequencies, and train
durations, and thereafter by inducing hypoglycemia or hypoxia to assess the evoked stress
response. Under basal conditions, spontaneous single as well as compound action potentials with
distinct firing rates that ranged from 0.5 to 4.5 Hz were recorded in the anesthetized subjects.
Spontaneous activity was partially blocked by locally applying TTX to the splanchnic nerve or
completely abolished by severing the nerve. The recordings revealed submillisecond interactions
that most likely reflect the activity from highly synchronized neighboring chromaffin cells firing
in a specific order. This synchronization was also occasionally observed between units separated
by several hundreds of micrometers. Electrical stimulation to the splanchnic nerve evoked the
same spatio-temporal firing patterns that were recorded during basal conditions, with latencies
from 5 to 30 ms after the stimulus and distinct thresholds. The recorded units were able to follow
up frequencies of up to 40Hz. To assess the physiological response to a stress condition,
hypoglycemia was induced by insulin shock with three incremental doses, producing a drop in
the glycemic levels that correlated to a gradual increase in the firing rate in a subset of
chromaffin cells. Hypoxia by respiratory arrest was induced through an anesthesia overdose,
resulting in a generalized increase in the firing rate of the recorded units at the moment breathing
stopped, reaching a maximum several seconds after and then gradually dropping to a complete
silence upon the animal's demise. For the first time we were able to document the internal

dynamics of the intact adrenal medulla, showing the extend of the local circuitry that drives the adreno-medullary stress response in the living animal.

Disclosures: J.R. Lopez Ruiz: None. **R.W. Holz:** None. **E.L. Stuenkel:** None.

Nanosymposium

013. Neuron Signaling, Neuroendocrine, and Physiology

Location: SDCC 24

Time: Saturday, November 12, 2022, 1:00 PM - 3:00 PM

Presentation Number: 013.06

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R00HD099307

Title: Microbiome composition predicts DNA methylation of NR3C1

Authors: *C. R. LEWIS[1], K. BONHAM[2], S. H. MCCANN[2], S. C. L. DEONI[3], V. KLEPAC-CERAJ[2];
[1]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [2]Dept. of Biol. Sci., Wellesley Col., Wellesley, MA; [3]Warren Alpert Med. Sch., Brown Univ., Providence, RI

Abstract: Background: The gut-microbiota plays a role in regulation of emotions, behavior, and higher cognitive functions through the 'microbiome-gut-brain axis'. Much research demonstrates microbiome composition influences hypothalamic-pituitary-adrenal (HPA) axis function. Microorganisms that live in the gut can influence host brain through various mechanisms and mediators including cytokines, short chain fatty acids, hormones, and neurotransmitters. A lesser studied mechanism between microbiome composition and host behavior, is through epigenetic pathways. For example, folate-mediated, one-carbon metabolism refers to a complex network of pathways resulting in a supply of methyl groups for DNA methyltransferase (DNMT) to regulate DNA methylation and 13% of gastrointestinal reference genomes contain all genes required for complete *de novo* folate synthesis. Therefore, host microbiome composition and relative abundance of folate-producing strains may influence bioavailable folate and epigenetic machinery, especially DNA methylation patterns. Understanding these relationships in early life is especially important as the microbiome and epigenome are more responsive to the environment during this period. We tested the hypotheses that relative abundance of the phylum containing the highest percent of folate producing organisms (Proteobacteria 68%) would predict DNA methylation levels of *NR3C1*, the glucocorticoid receptor gene in infants and children. **Methods:** Study sample included healthy infants and children (age range 2m – 15 yrs; M = 4.8 yrs, SD = 3.5; N = 146, Female = 65). Nucleic acids extracted from fecal samples were used for metagenomic sequencing; metagenomic data were analyzed using bioBakery workflows. DNA extracted from buccal cells was used for DNA methylation analyses on the Illumina EPIC array. PCA was used to generate a methylation summary score for *NR3C1*. **Results:** Multiple regression analysis with age, sex, and sequencing depth used as covariates revealed a significant global test $F(4, 131) = 37.38$, $p <$

2.2e-16) and that relative abundance of Proteobacteria predicts DNA methylation of *NR3C1* (p = 0.000199). **Conclusions:** These results suggest that one pathway by which microbiome composition may influence HPA physiology is through bioavailable folate and epigenetic regulation. Future analyses will assess more HPA genes.

Nanosymposium

013. Neuron Signaling, Neuroendocrine, and Physiology

Location: SDCC 24

Time: Saturday, November 12, 2022, 1:00 PM - 3:00 PM

Presentation Number: 013.07

Topic: F.02. Neuroendocrine Processes and Behavior

Title: Hypothalamic Sh2b1 protects against obesity and metabolic syndrome by enhancing BDNF signal transduction

Authors: *Y. LI, D. OLSON, L. RUI;
Univ. of Michigan, Ann Arbor, ANN ARBOR, MI

Abstract: Sh2b1 is an SH2 domain-containing adaptor protein mediating cell signal transduction in response to leptin, insulin, brain-derived neurotrophic factor (BDNF), and additional ligands. Sh2b1 directly binds to BDNF receptor TrkB and enhances BDNF signaling in cell cultures. Global deletion of *Sh2b1* results in obesity, type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD). In humans, *SH2B1* deletion or mutations are linked to obesity and metabolic syndrome, indicating that Sh2b1's metabolic function has conserved across mammals. Given that leptin, insulin, and BDNF act on hypothalamic neurons to regulate metabolism, we conditionally deleted *Sh2b1* specifically in ventromedial (VMH, Sh2b1$^{\Delta VMH}$) and paraventricular (PVH, Sh2b1$^{\Delta PVH}$) hypothalamic neurons using the Cre/loxp system. Sh2b1$^{\Delta VMH}$ mice were prone to high fat diet-induced obesity and metabolic disorders. In contrast, Sh2b1$^{\Delta PVH}$ male and female mice spontaneously developed obesity, insulin resistance, glucose intolerance, and NAFLD on a chow diet. Sh2b1$^{\Delta PVH}$ mice consumed more food relative to wild-type littermates, while their energy expenditures were lower. We bilaterally microinjected AAV-CAG-BDNF vectors into the PVH. PVH-specific overexpression of BDNF reduced body weight and metabolic disorders to a higher degree in wild-type mice relative to Sh2b1$^{\Delta PVH}$ mice, indicating that Sh2b1 deficiency in the PVH impairs hypothalamic BDNF/TrkB signaling and actions. Taken together, these results unveil a novel hypothalamic BDNF/TrkB/Sh2b1 signaling cascade that governs body weight and metabolic homeostasis.

Nanosymposium

013. Neuron Signaling, Neuroendocrine, and Physiology

Location: SDCC 24

Time: Saturday, November 12, 2022, 1:00 PM - 3:00 PM

Presentation Number: 013.08

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: The pandemic stay-at-home orders linked to dysregulated cortisol in children

Authors: *K. MCDONALD[1], L. GABBARD-DURNAM[1], K. BEAUDRY[2], M. DE LISIO[2], L. B. RAINE[1], T. MORRIS[1], Y. BERNARD-WILLIS[3,1], J. N. H. WATROUS[1], T. CLINE[1], S. WHITFIELD-GABRIELI[1], A. F. KRAMER[1,4], C. H. HILLMAN[1];
[1]Northeastern Univ., Boston, MA; [2]Univ. of Ottawa, Ottawa, ON, Canada; [3]Brigham & Women's Hosp., Boston, MA; [4]Univ. of Illinois, Champaign-Urbana, IL

Abstract: The COVID-19 pandemic globally altered daily life in March 2020 and impacted the lives of children, as their normal routines were disrupted by community lockdowns, online learning, limited in-person social contact, more screen time, and less physical activity. Considerable research has been conducted on the symptoms and physical health impact of COVID-19 infection, but far less research has investigated the psychological impact of the pandemic, especially in children. The purpose of this study was to investigate neurophysiological outcomes associated with the stay-at-home orders on preadolescent children by leveraging an ongoing randomized controlled trial that included the collection of salivary cortisol samples before, during, and following the orders. Preadolescent children (n=94) provided salivary cortisol on three testing days. Cortisol was collected prior to any procedures and averaged across the three testing days. For this analysis, participants were divided into three groups relative to the onset of the stay-at-home orders: Before Orders (n=40), During Orders (n=25), and Following Orders (n=29). Results suggest that cortisol was lower in children in the During Orders group (M=.06 µg/dL) compared to children in the Before (M=.12 µg/dL) or Following (M=.17 µg/dL) Orders groups, suggesting that the chronic stress associated with staying at home potentially dysregulated the hypothalamic-pituitary-adrenal (HPA) axis that regulates cortisol in children, resulting in hypocortisolism (p<.001). Further, the number of days of the stay-at-home orders experienced prior to the initial testing day related to salivary cortisol, such that the early days of the orders showed the most intense dysregulation (p<.001). In previous research, hypocortisolism has been linked to fatigue, depression, sleep disturbances, stress sensitivity, and negative health outcomes. These findings suggest that in a sample of preadolescent children, the pandemic stay-at-home orders was considered a chronic stressor that resulted in dysregulation of the HPA axis. This dysregulation may influence the mental health of children. These findings emphasize the need for social and mental support for children following the pandemic stay-at-home orders, as mental health concerns may be the newest pandemic stemming from COVID-19.

Disclosures: K. McDonald: None. **L. Gabbard-Durnam:** None. **K. Beaudry:** None. **M. De Lisio:** None. **L.B. Raine:** None. **T. Morris:** None. **Y. Bernard-Willis:** None. **J.N.H. Watrous:**

None. **T. Cline:** None. **S. Whitfield-Gabrieli:** None. **A.F. Kramer:** None. **C.H. Hillman:** None.

Nanosymposium

014. Other Dementias, Proteinopathies, and Pathologies

Location: SDCC 11

Time: Saturday, November 12, 2022, 1:00 PM - 3:30 PM

Presentation Number: 014.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Frontotemporal dementia associated behavioural and transcriptomic abnormalities in 'humanized' progranulin-deficient mice: a novel model for progranulin-associated frontotemporal dementia

Authors: *B. LIFE[1,3], T. L. PETKAU[1,3], G. N. F. CRUZ[2], E. I. NAVARRO-DELGADO[2], N. SHEN[2], K. KORTHAUER[2], B. R. LEAVITT[1,3];
[1]Med. Genet., [2]Statistics, Univ. of British Columbia, Vancouver, BC, Canada; [3]Ctr. for Mol. Med. and Therapeut., Vancouver, BC, Canada

Abstract: Frontotemporal dementia (FTD) is an early onset dementia characterized by neuropathology and behavioural changes. A major genetic cause of FTD is loss of function of a single copy of the gene progranulin (*GRN*). Mouse models of progranulin deficiency have provided some insight into progranulin neurobiology, but currently available models incompletely recapitulate important features of FTD. We asked if genetic expression of human *GRN* in mice would better model FTD-like phenotypes. To this end, we have developed a novel mouse model that expresses a single, targeted copy of human *GRN* in the absence of mouse progranulin. As the *GRN* transgene is expressed from the X-linked *Hprt* locus, only male animals were studied to avoid issues with X-inactivation. We performed a parallel, longitudinal characterization of wild type, humanized progranulin-deficient, and heterozygous mouse progranulin-null mice over 18 months. FTD-associated behaviour was probed at multiple timepoints, neuropathology was assessed at 12 and 18 months of age, and the cortical transcriptome was evaluated at 18 months of age. Our analysis yielded several progranulin-dependent physiological and behavioural phenotypes in both models of progranulin deficiency, including increased marble burying, open field hyperactivity, and thalamic microgliosis. RNAseq analysis of cortical tissue revealed an overlapping profile of transcriptomic dysfunction with 302 genes commonly dysregulated in both progranulin deficient strains relative to wild type mice. Further transcriptomic analysis uncovered potential functional differences between mouse and human progranulin in the regulation of lipid metabolic gene expression, suggesting that humanized progranulin-deficient mice may offer new insights into certain aspects of progranulin neurobiology. In sum, we have identified several consistent phenotypes in two independent mouse models of progranulin deficiency that are expected to be useful endpoints in the development of therapies for progranulin-deficient FTD. Furthermore, the presence of the human

progranulin gene in the humanized progranulin-deficient mice will expedite the development of clinically translatable gene therapy strategies.

Nanosymposium

014. Other Dementias, Proteinopathies, and Pathologies

Location: SDCC 11

Time: Saturday, November 12, 2022, 1:00 PM - 3:30 PM

Presentation Number: 014.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A C-terminally truncated TDP-43 splice isoform exhibits neuronal specific cytoplasmic aggregation and contributes to TDP-43 pathology in ALS

Authors: *M. SHENOUDA[1], S. XIAO[2], L. MACNAIR[1], A. LAU[2], J. ROBERTSON[1];
[1]Lab. Med. & Pathobiology, Univ. of Toronto, Toronto, ON, Canada; [2]Tanz Ctr. for Res. in Neurodegenerative Dis., Toronto, ON, Canada

Abstract: Neuronal mislocalization of the normally nuclear TDP-43 to the cytoplasm, aggregation and ubiquitination is the most common disease pathology in Amyotrophic Lateral Sclerosis (ALS). TDP-43 pathology is characterized by the presence of low molecular weight TDP-43 species generated through proteolytic cleavage and/or abnormal RNA processing events. In addition to TDP-43 C-terminal fragments, it has become evident that N-terminal fragments (NTFs) generated through alternative splicing in exon 6 also contribute to the pathophysiology of ALS. Three such NFTs have been previously reported each sharing the same C-terminal unique sequence of 18 amino acids containing a putative nuclear export sequence. Herein, we identify an additional NFT of TDP-43 in human spinal cord tissue. This variant, termed TDP43C-spl, is generated through use of non-canonical splice sites in exon 6, skipping 1020 bp and encoding a 272 aa protein lacking the C-terminus with the first 256 aa identical to full-length TDP-43 and the same 18 amino acid C-terminal unique sequence. Ectopic expression studies in cells revealed that TDP43C-spl is localized to the nucleus in microglial and astrocytic cell lines, while forming ubiquitinated aggregates in neuronal cell lines. Generation of an antibody raised to the unique 18 amino acid sequence showed elevated levels of NFTs in ALS spinal cord tissues, and co-labeled TDP-43 pathology in ALS tissue samples. The retention of this 18 amino acid sequence among several TDP-43 NTFs suggests an important functional relevance. Our studies of TDP43C-spl

suggest this may be related to the selective neuronal vulnerability to TDP-43 pathology and cell-subtype differences in nuclear export.

Disclosures: M. Shenouda: None. S. Xiao: None. L. Macnair: None. A. Lau: None. J. Robertson: None.

Nanosymposium

014. Other Dementias, Proteinopathies, and Pathologies

Location: SDCC 11

Time: Saturday, November 12, 2022, 1:00 PM - 3:30 PM

Presentation Number: 014.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A Novel Source of Protein Aggregates Derived from Non-mitochondrial BaxΔ2 in Alzheimer's Disease

Authors: *Q. YAO[1], A. MASCARENHAS DOS SANTOS[1], J.-F. POMBERT[1], S. TASAKI[2], J. XIANG[1];
[1]Dept. of Biol., Illinois Inst. of technology, chicago, IL; [2]Rush Alzheimer's Dis. Ctr., Rush Univ., Chicago, IL

Abstract: Bax is a well-known mitochondria-targeted pro-death tumor suppressor. We previously discovered a Bax variant (BaxΔ2) that cannot target mitochondria but aggregates in the cytosol and triggers caspase 8-driven cell death. Unlike Bax, BaxΔ2-positive cells were detected at a low rate (1-4%) in most human normal organ tissues, including the brain, but rarely in cancer. As protein aggregates are a hallmark of Alzheimer's disease (AD), we wondered if such BaxΔ2 aggregates would present in AD brains. Here, we analyzed BaxΔ2 expression in AD using tissue immunostaining and RNAseq database of ROSMAP. We found that a strikingly high number of BaxΔ2-positive neurons were mainly located in the AD-susceptible brain regions: up to 30% in the hippocampus and 20% in the frontal and temporal lobes, but primarily negative in AD occipital and parietal lobes and control brains. Consistently, RNAseq database analysis indicated an upregulation of BaxΔ2 transcripts in the AD brains compared with the controls. Further analysis of BaxΔ2 protein intracellular distribution showed that BaxΔ2 aggregates could coexist with T-tau or P-tau in the same cell but with no colocalization. Interestingly, many BaxΔ2 aggregates colocalized well with stress granules, a large protein-RNA complex involved in AD pathogenesis. Although the underlying mechanism remains to be explored, these data suggest that off-target mitochondrial proteins, like BaxΔ2, could serve as a new source of protein aggregates which may interfere with neuronal stress signaling regulation and contribute to AD pathogenesis.

Disclosures: Q. Yao: None. A. Mascarenhas dos Santos: None. J. Pombert: None. S. Tasaki: None. J. Xiang: None.

Nanosymposium

014. Other Dementias, Proteinopathies, and Pathologies

Title: Detection of MAP2K3 Immunoreactivity in the Human Cerebral Cortex and Primary Human Microglia, and loss in Frontotemporal Lobar Degeneration with TDP-43 Proteinopathy

Authors: *A. BAHRAMI[1], C. SWOPE[2], I. A. AYALA[3], M. E. FLANAGAN[3], R. TAEFI[4], E. J. ROGALSKI[5], M.-M. MESULAM[6], T. GEFEN[7], C. GEULA[8];
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Abstract: Mitogen Activated Protein Kinase Kinase 3 (MAP2K3) is a dual specificity serine/threonine cell-signaling kinase activated by stress. The MAP2K3 pathway has been implicated in processes such as embryonic development, cell proliferation, β-Amyloid (Aβ) deposition associated with Alzheimer's Disease (AD), and learning and memory. Evidence from the mouse brain indicates high MAP2K3 expression in microglia, which is associated with production of inflammatory proteins. Consistent with the role of MAP2K3 in learning and memory, different variants of this kinase distinguish cognitive SuperAgers, individuals 80 years or older with memory performance equal to or better than individuals 20-30 years their junior, when compared with age-matched cognitively normal peers. Previous studies have investigated MAP2K3 protein and downstream target abundance in mouse brain tissue. However, detection and measurement of MAP2K3 protein in the human brain has not been described. The purpose of this study was 1) to detect MAP2K3 protein in the human cerebral cortex as well as in cultured primary human microglia, and 2) to investigate potential changes in its level in cases diagnosed with behavioral variant frontotemporal dementia due to TDP-43 proteinopathy of frontotemporal lobar degeneration (FTLD-TDP). An antibody against human MAP2K3 (Proteintech, 1/500) was used for Western blot analysis of levels in the middle frontal gyrus of FTLD-TDP participants (N=5) and age-matched cognitively normal participants (N=5), and in primary human microglia isolated and cultured from postmortem frontal cortices of cognitively normal participants. We successfully detected and measured MAP2K3 in all brain specimens and in microglia. Cultured microglia contained high levels of this protein. On average, microglia contained more than five-fold concentration of MAP2K3 than tissue homogenates from normal human frontal cortex. The participants with FTLD-TDP displayed substantial loss of MAP2K3 (average of 83%) when compared with controls (p=0.02). These results demonstrate that the MAP2K3 protein can be

detected and measured in the human brain, and that similar to the mouse brain, it is present at high levels in human microglia. The substantial decrease in MAP2K3 in FTLD-TDP is consistent with the proposed role of this protein in cognitive processes.

Nanosymposium

014. Other Dementias, Proteinopathies, and Pathologies

Location: SDCC 11

Time: Saturday, November 12, 2022, 1:00 PM - 3:30 PM

Presentation Number: 014.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A novel white matter injury model with dementia

Authors: F. CHEN[1,3], Z. WENG[1,3], C. CAO[2], I. BHUIYAN[1], D. SUN[1,3], Q. WANG[2,3], R. K. LEAK[4], *G. CAO[1,3];
[1]Neurol., [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]Grecc, VA Pittsburgh Healthcare Syst., Pittsburgh, PA; [4]Pharmacol., Duquesne Univ., Pittsburgh, PA

Abstract: Background: Vascular cognitive impairment and dementia (VCID) is the second leading cause of dementia, but there is no animal model that can faithfully replicate all pathological changes of VCID. Here, we developed a new VCID model with long-term cognitive impairment. **Methods:** the right murine common carotid arteries (CCA) was ligated temporarily with a 31-gauge needle to stop the blood flow and the left CCA with a 33-gauge needle. Needles were removed immediately after ligation. Blood flow was monitored by Perivascular Flow Module and two-dimensional laser speckle. White matter (WM) injury was analyzed by immunohistochemical staining and diffusion tensor imaging (DTI). Sensorimotor and cognitive impairments were assessed weekly for 8 weeks, and then at 24 weeks. **Results:** Removal of the needle led to ~23% blood flow recovery in the left CCA and ~52% recovery in the right CCA. The needle model caused hypoperfusion in both hemispheres, but more severe hypoperfusion in the left hemisphere, with similar hypoperfusion extents at 2, 4, 6 and 8 weeks after surgery. Immunohistochemical staining indicated that brain injury mainly occurred in the external capsule, corpus callosum, internal capsule, and striatum in the left hemisphere. DTI showed reductions of fractional anisotropy in the above regions. Needle mice showed gradual increases in the latency to fall in rotarod testing, starting 7d after surgery, and this loss of sensorimotor function increased thereafter. Similarly, gradually reduced sensorimotor function was observed in adhesive tape removal and the corner and cylinder tests. Needle mice spent longer locating the submerged platform and less time in the goal quadrant in the Morris water maze at the 6th week

after surgery. The new object recognition test and the passive avoidance test further confirmed impaired cognitive function in needle mice. Importantly, WM injury and cognitive impairments in needle mice lasted for up to 24 weeks after surgery. **Conclusion:** A novel and versatile VCID model was established. Compared with other WM injury models, the needle model is easy to master, low in cost, causes lower mortality, and better replicates the pathophysiology of long-term cerebral hypoperfusion. Furthermore, asymmetric brain injuries allow for special behavioral assessments and fiber tract-tracing. Finally, no metal items are implanted into the brain, allowing for live DTI imaging to monitor WM injury. Supported by: NIH grant RF1NS117509 (to GC) and VA Merit Review grants BX003923 (to G C).

Disclosures: F. Chen: None. **Z. Weng:** None. **C. Cao:** None. **I. Bhuiyan:** None. **D. Sun:** None. **Q. Wang:** None. **R.K. Leak:** None. **G. Cao:** A. Employment/Salary (full or part-time):; GRECC, VA Pittsburgh Healthcare System, Pittsburgh PA 15240.

Nanosymposium

014. Other Dementias, Proteinopathies, and Pathologies

Location: SDCC 11

Time: Saturday, November 12, 2022, 1:00 PM - 3:30 PM

Presentation Number: 014.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS R56NS117465

Title: Distinct inclusions of α-synuclein and tau in temporal cortex of Dementia with Lewy Bodies

Authors: D. L. FISCHER[1], L. S. STOYKA[1], E. D. ROBERSON[1], N. M. KANAAN[2], T. G. BEACH[3], G. E. SERRANO[4], ***L. A. VOLPICELLI-DALEY**[1];
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Abstract: A strong association of variations in *MAPT* 17q.21.31 locus H1 haplotype occurs with PD. Recombinant α-synuclein and tau proteins interact *in vitro*. Reducing tau is protective in models of Alzheimer's disease, tauopathies and some α-synuclein transgenic mice. Here, we examined the relationship between tau and α-synuclein using the α-synuclein pre-formed fibril mouse model in tau heterozygous and tau knock-out mice. We show using proximity ligation assays in primary neurons, and expansion microscopy in cortex from wild type mice, that tau and α-synuclein colocalize and interact at excitatory presynaptic terminals. However, tau heterozygous and tau knock-out mice do not show a reduction in fibril-induced α-synuclein inclusion formation in the substantia nigra pars compacta, amygdala, hippocampus or cortex. Further, reduction of tau did not rescue loss of dopamine neurons induced by α-syn inclusion formation. Thus, reduction of endogenous tau does not appear to prevent the templated formation of α-synuclein inclusions or subsequent neurodegeneration. We further analyzed the potential interactions between α-synuclein and tau by performing dual label immunofluorescence in

temporal cortex tissue from human individuals with Dementia with Lewy Bodies. The cortex shows abundant pathologic tau and α-synuclein inclusions. Notably, the tau and α-synuclein aggregates were almost completely non-colocalized. Rarely, neurofibrillary tau appears to wrap around Lewy neurites or Lewy bodies. Thus, the data suggest that pathologic α-synuclein and pathologic tau do not co-assemble in the same inclusions, at least in DLB temporal cortex. Instead, the aggregates of α-synuclein and tau may contribute to DLB phenotypes by affecting independent neurons and disrupting circuitry.

Disclosures: D.L. Fischer: None. **L.S. Stoyka:** None. **E.D. Roberson:** None. **N.M. Kanaan:** None. **T.G. Beach:** None. **G.E. Serrano:** None. **L.A. Volpicelli-Daley:** None.

Nanosymposium

014. Other Dementias, Proteinopathies, and Pathologies

Location: SDCC 11

Time: Saturday, November 12, 2022, 1:00 PM - 3:30 PM

Presentation Number: 014.07

**Topic:** C.02. Alzheimer's Disease and Other Dementias

Title: Contribution of GLT1 and NMDARs to excitotoxicity through the interplay of FOXO1 and TXNIP leading to neurodegeneration in YAC128 model of Huntington's disease

Authors: S. JAMAL[1], X. WEE[1], S. KHO[1,3], E. OTUGEN MOUSSU[1], S. CLAUDINE[1,3], S. PUANG[2], *****J. C. SNG**[2];
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Abstract: Dysfunction of glutamatergic corticostriatal synapse via excitotoxicity is thought to drive pathogenesis of striatum atrophy in Huntington's Disease (HD). Here, we hypothesized that reduced cortical GLT1 contributes to reduced synaptic glutamate clearance and NMDAR composition in the striatum is skewed towards GluN2B and its pro-death extrasynaptic signaling. The study was performed in the early stage of HD (3 months old, ES) and in the late stage of HD (7 month old, LS). We found that striatal GluN2B/GluN2A ratio was upregulated at ES and continued till LS of HD. This, in turn, resulted in GluN2B to be reduced and these reduction were localized at synaptic locations, revealed by immunohistochemistry. At LS of HD, the percentage of Glu2B at extrasynaptic site is significantly increased in HD (HD=93%; WT= 87%, $p<0.005$, n=3 per genotype). This increase exacerbated FOXO1, a transcriptional factor downstream of extrasynaptic GluN2B, and its localisation to the nuclei. Localisation of FOXO in nuclei in LS was significantly increased in HD (HD=38%; WT= 20%, $p<0.05$, n=3 per genotype) and at LS, TXNIP protein level was increased in HD (HD=59%; WT= 39%, $p<0.005$, n=3 per genotype). The interaction of FOXO1 with TXNIP was investigated and we found that they were

both localized in the nuclear compartments of the striatal neurons, as shown in the immunohistochemistry images. Further investigations with chromatin immunoprecipitation in LS HD mice found that FOXO1 associated with TXNIP 1.3 fold (p<0.05) more in HD compared to age-matched WT mice. This interaction of FOXO with TXNIP promoter regulated its expression and may lead to caspase activation and striatal degeneration that is observed in HD.
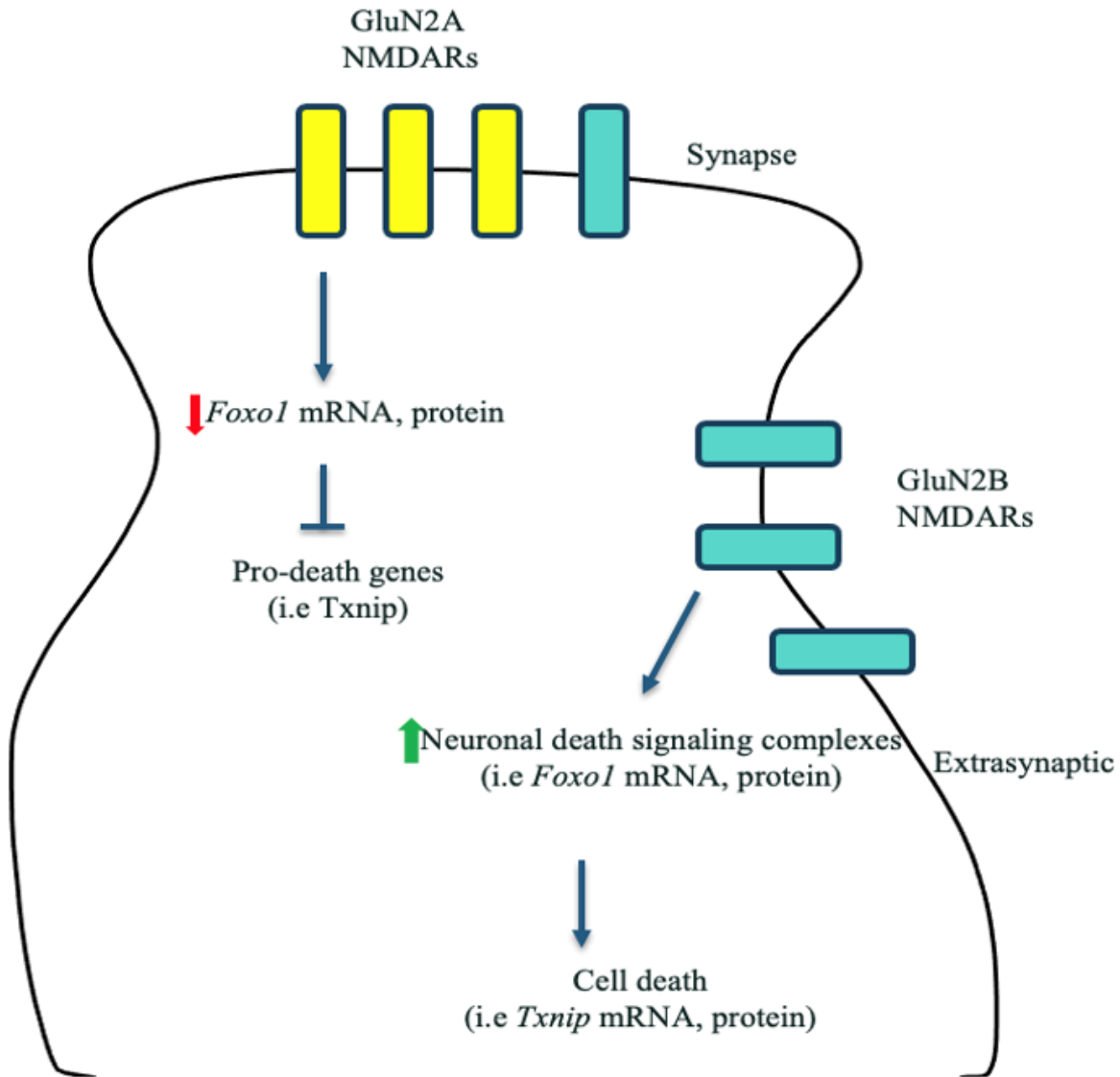
Nanosymposium

014. Other Dementias, Proteinopathies, and Pathologies

Location: SDCC 11

Time: Saturday, November 12, 2022, 1:00 PM - 3:30 PM

Presentation Number: 014.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: What childhood dementia tell us about Alzheimer's disease - new insights into CLN5 function

Authors: *R. STEINFELD[1], A. LUEBBEN[3], D. BENDER[1], S. BECKER[4], L. CROWTHER[1], C. BELLOTTI[5], A. STÄUBLE[2], R. KRÄTZNER[3];
[2]Neurol., [1]Univ. of Zurich, Zurich, Switzerland; [3]Univ. of Goettingen, Goettingen, Germany; [4]Max Planck Inst. for Multidisciplinary Sci., Goettingen, Germany; [5]Neurol., Kinderspital Zurich, Univ. of Zurich, Zurich, Switzerland

Abstract: The Neuronal Ceroid Lipofuscinoses (NCL) are the most common neurodegenerative disorders of childhood and presently 13 different genetic NCL variants are known. CLN5, one of them, is caused by mutations in the *CLN5* gene and is associated with variable types of neurodegenerative disorders ranging from late infantile epileptic encephalopathy to adult-onset dementia. In fact, the Cln5 variant Asn320Ser segregates with multiplex Alzheimer's disease families. However, Cln5 shows no significant homology to other proteins by primary amino acid sequence, and the molecular functions of Cln5 have not been previously demonstrated. We crystallized the glycosylated human Cln5 protein, determined its protein structure and revealed unexpected thioesterase activity of Cln5. We demonstrated complete loss of enzymatic activity for mutations affecting the catalytic H166 and C280 as well as for the two patient mutations Y258D and D279N and discovered that Cln5-deficient neuronal progenitor cells showed reduced thioesterase activity. These findings represent an important step toward understanding the neurodegenerative disease mechanisms associated with the CLN5 variant of NCL disease and will stimulate further investigations to elucidate the mechanism of neuronal loss in the state of unbalanced protein lipidation.

Disclosures: R. Steinfeld: None. **A. Luebben:** None. **D. Bender:** None. **S. Becker:** None. **L. Crowther:** None. **C. Bellotti:** None. **A. Stäuble:** None. **R. Krätzner:** None.

Nanosymposium

014. Other Dementias, Proteinopathies, and Pathologies

Location: SDCC 11

Time: Saturday, November 12, 2022, 1:00 PM - 3:30 PM

Presentation Number: 014.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Toward a better model: TDP-43 acetylation and neurodegeneration

Authors: *J. NECARSULMER, S. NAFEES, J. SIMON, S. MOY, T. COHEN;
Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Society for Neuroscience 2022 Annual Meeting Julie Necarsulmer**Poster title:** Toward a better model: TDP-43 acetylation and neurodegeneration**Abstract:**Frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) are two neurodegenerative disorders on a spectrum of disease related to the nucleic acid binding protein TAR DNA-binding Protein of 43 kDa (TDP-43). Most pathology specimens FTLD- and ALS-affected individuals possess the characteristic signature of aggregated and hyperphosphorylated TDP-43 protein within affected neurons. This classical TDP-43 pathology is often observed in other neurodegenerative disorders, such as Alzheimer's Disease and Hippocampal Sclerosis, which suggests a common mechanism linking TDP-43 dysfunction and neurodegeneration. How TDP-43 becomes dysfunctional and aggregated, and how this imparts neurotoxicity, remains poorly understood. Our lab identified an important post-translational modification, namely TDP-43 acetylation at a key lysine residue (Ac-K145), as a driver of TDP-43 pathology. With the goal of better modeling sporadic illness and enabling discovery of pathogenic mechanisms, we used CRISPR/Cas9 technology to insert a K145Q acetylation-mimic mutation in the endogenous mouse *Tardbp* locus (TDP-43^{K145Q}) to generate a novel model of TDP-43 proteinopathy in sporadic FTLD-ALS. TDP-43^{K145Q} mice develop signs of neurodegeneration, particularly FTLD, such as learning difficulties and social deficits. CNS tissue of homozygous TDP-43^{K145Q} mice display multiple neuropathologic signatures of TDP-43 pathology, particularly in the hippocampus and neocortex. These include TDP-43 mislocalization, TDP-43 hyperphosphorylation, and splicing defects. In summary, we have leveraged our knowledge of disease-associated posttranslational modifications to create a novel endogenous model of TDP-43 proteinopathy that recapitulates key protein and RNA dyshomeostasis signatures found in TDP-43-related neurodegeneration. We hope this model will allow the field to better study TDP-43 proteinopathy in sporadic disease and therefore enable discovery of pathogenic mechanisms and therapeutic interventions.

Disclosures: J. Necarsulmer: None. **S. Nafees:** None. **J. Simon:** None. **S. Moy:** None. **T. Cohen:** None.

Nanosymposium

014. Other Dementias, Proteinopathies, and Pathologies

Location: SDCC 11

Time: Saturday, November 12, 2022, 1:00 PM - 3:30 PM

Presentation Number: 014.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NS085770

Title: Strain-dependent Disease Progression in a Conditional Wild-type Human TDP-43 Transgenic Mouse Model

Authors: A. CHIODINI[1], I. A. AYALA[2], K. R. SADLEIR[3], R. J. VASSAR[4], H. DONG[2], M.-M. MESULAM[5], *C. GEULA[6];
[1]Mesulam Ctr. for Cognitive Neurol. and Alzheimer's Dis., [3]Neurol., [2]Northwestern Univ., Chicago, IL; [4]Dept. of Cell and Mol. Biol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [5]Norrthwestern Univ., Mesulam Ctr. For Cognitive Neurol. and Alzheim, Chicago, IL; [6]Northwestern Univ. Med. Sch., Chicago, IL

Abstract: Strain-Dependent Disease Progression in a Conditional Wild-Type Human TDP-43 Transgenic Mouse ModelAmber Chiodini, Ivan Ayala, Katherine R. Sadleir, Robert Vassar, Hongxin Dong, M.-Marsel Mesulam, and Changiz Geula Frontotemporal lobar degeneration (FTLD) is among the most prevalent dementias of early onset. Pathologically, FTLD presents with tauopathy or TAR DNA-binding protein 43 (TDP-43) proteinopathy. A biallelic mouse model of FTLD was produced on a mix FVB/129SVE background overexpressing wild-type human TDP-43 (hTDP-43) employing tetracycline transactivator (tTA). tTA activates hTDP-43 which was placed downstream of the tetracycline response element (TRE). We have backcrossed the tTA and hTDP-43 transgenic mice to the C57BL/6 background and found significant strain-dependent differences in disease progression. TDP-43 expression was turned on at 21 days postmortem by taking animals off doxycycline in diet. Bigenic animals on the mixed FVB/129SVE background displayed rapid progression of pathology and neurodegeneration. Punctate, cortical intraneuronal TDP-43 inclusions were detected after 5 days of expression. Inclusions attained a peak in density between 14-20 days of expression. Thereafter, they decreased in density, such that few were observed after 8 weeks of expression, and at 24 weeks, none could be identified. Neuronal degeneration and cortical thickness displayed the opposite pattern, with decreased neuronal density and cortical atrophy peaking at 24 weeks of expression. A subpopulation of cortical neurons contained intense ubiquitin immunoreactivity, which showed the same pattern of appearance and disappearance as TDP-43 inclusions. In contrast, bigenic animals on the C57BL/6 background displayed a considerably slower progression of disease and milder pathology. Accumulation of human TDP-43 immunoreactivity in cortical neurons slowly increased and was at a peak at 20 months of expression (the oldest age tested). Few and very small TDP-43 immunoreactive inclusion-like structures were visible in cortical neurons at 10 months of expression and thereafter. Ubiquitin immunoreactivity in cortical neurons was considerably less pronounced when compared with that in the mixed FVB/129SVE bigenic animals. These observations point to significant strain-dependent differences in disease progression and pathology in a conditional TDP-43 transgenic model. Thus, attention to strain of animals is crucial in studies using transgenic mice overexpressing proteins involved in neurodegenerative disorders.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.01

Topic: C.06. Neuromuscular Diseases

Support: NIH-NINDS R01NS118177
NIH-NINDS R21NS120126
Neurodegenerative Disease Research, Inc

Title: Transgenic expression of a mutant spastin variant linked to Hereditary Spastic Paraplegia elicits presymptomatic degeneration of corticospinal motor neurons.

Authors: M. PRIEGO[1], V. OCCIEPPO[1], E. PIERMARINI[2], A. ALTADONNA[3], J. DZIECHCIOWSKA[4], H. ZAKY[1], H. MARCHADI[5], P. W. BAAS[2], *G. MORFINI[1];
[1]Anat. and Cell Biol., Univ. of Illinois at Chicago, Chicago, IL; [2]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [3]Drexel Univ. Col. of Medicin, Philadelphia, PA; [5]Anat. and Cell Biol., [4]Univ. of Illinois, Chicago, IL

Abstract: The hereditary spastic paraplegias (HSP) comprise a heterogenous group of heritable neurodegenerative diseases characterized by progressive weakness and spasticity of lower limbs. *SPG4*-HSP, the commonest HSP variant, is caused by mutations in *SPAST*, a gene that encodes the microtubule-severing protein spastin. *SPG4*-HSP symptoms are associated with progressive degeneration of corticospinal motor neurons. Located in the cerebral cortex, the cell bodies of these neurons extend unusually large axons into various levels of the spinal cord. To better understand the etiology of *SPG4*-HSP, we recently generated a mouse model that expresses human spastin bearing a pathological HSP-linked mutation (transgenic hspastinC448Y mice). Starting at age P90 and older, transgenic hspastinC448Y+/- mice display mild gait deficiencies reminiscent of HSP and histological evidence of axonal degeneration in the spinal cord.To better evaluate and characterize degeneration of corticospinal neurons in transgenic hspastinC448Y+/- mice, we performed genetic crosses with two different YFP-based reporter mouse models. Because each of these reporter mice has attributes and shortcomings regarding specificity for corticospinal motor neurons, we also performed immunostaining analyses for cell-type-specific markers. Interestingly, we observed dystrophic corticospinal axons and a loss of corticospinal neuron somata long before the presentation of locomotor symptoms, manifested as gait deficits. These observations have implications relevant to the pre-symptomatic wiring of corticospinal axonal tracts, cellular changes associated with the onset of symptoms, and the timetable of potential therapeutic interventions in human patients.

Disclosures: M. Priego: None. **V. Occieppo:** None. **E. Piermarini:** None. **A. Altadonna:** None. **J. Dziechciowska:** None. **H. Zaky:** None. **H. Marchadi:** None. **P.W. Baas:** None. **G. Morfini:** None.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.02

Topic: C.06. Neuromuscular Diseases

Support: NIH R37 NS054154
                 NIH R24 NS098523
                 NIH R01 NS113583

Title: The role of ATF4 target-gene expression on neuropathy phenotype in mouse models of Charcot-Marie-Tooth disease type 2D

Authors: *T. J. HINES, A. L. D. TADENEV, R. W. BURGESS;
The Jackson Lab., Bar Harbor, ME

Abstract: Charcot-Marie-Tooth disease (CMT) is a rare, genetically heterogeneous neuromuscular disorder with causative mutations found in over 100 different genes. The tRNA synthetases (aaRSes) are the largest gene family associated with CMT. The integrated stress response (ISR) is activated through GCN2 in motor neurons of mouse models of aaRS-associated CMT, including multiple models of CMT type 2D (CMT2D - caused by dominant mutations in GARS1) and one model of dominant intermediate CMT type C (diCMTC - caused by dominant mutations in YARS1). Knockout of the ISR activting kinase, GCN2, in GARS1 mutant mice significantly improves the CMT2D phenotype, indicating ISR activation contributes to the disease phenotype. The ISR precipitates two major cellular consequences - shutdown of cap-dependent translation, and selective translation of the transcription factor ATF4 leading to expression of stress response genes. It is unclear which of these two events contribute to the neuropathy phenotype.
Gars/CMT2D mice are being crossed to motor neuron-specific ATF4 knockouts to determine if ATF4 target-gene expression is necessary for the neuropathy phenotype. Conversely, motor neuron-specific transgenic ATF4-overexpressing mice are being assessed to determine if ATF4 target-gene expression is sufficient to cause the phenotype. Neuromuscular performance, nerve histology, ATF4 target-gene expression, and translation levels will be assessed in these mice. Initial litters are just arriving from these crosses. Pilot data from the these mice will be presented showing neuromuscular performance and levels of ATF4 and its targets in motor neurons. The results of this project will further refine our understanding of the pathomechanism underlying aaRS-associated CMT, potentially uncovering additional therapeutic targets.

Disclosures: T.J. Hines: None. **A.L.D. Tadenev:** None. **R.W. Burgess:** None.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.03

Topic: C.06. Neuromuscular Diseases

Support: R01NS090335

Title: Neuromuscular junction and muscle pathology provides evidence for differential motor unit vulnerability in spinal and bulbar muscular atrophy

Authors: *E. MOLOTSKY[1], Y. LIU[1], A. P. LIEBERMAN[3], D. E. MERRY[2];
[2]Dept Biochem & Molec Biol, [1]Thomas Jefferson Univ., Philadelphia, PA; [3]Dept Pathology, Univ. of Michigan, Ann Arbor, MI

Abstract: Spinal and bulbar muscular atrophy (SBMA; Kennedy's Disease) is an X-linked neuromuscular neurodegenerative disease for which there is no cure. Disease is caused by a polyglutamine-encoding CAG repeat expansion in exon 1 of the androgen receptor (*Ar*) gene. SBMA is characterized by the loss of lower motor neurons from the spinal cord and brainstem motor nuclei, and by a selective decrease in fast-muscle power and fast-twitch muscle fibers. Motor units, defined as the lower motor neuron and the muscle fibers it innervates, are classified on a spectrum from slow-twitch to fast-twitch based on the contractile properties of the muscular cytoskeleton. However, the relationship between neuromuscular junction (NMJ) pathology and increased fast-twitch motor unit vulnerability seen in SBMA has yet to be explored. In this study, we aimed to evaluate NMJ pathology in relation to motor unit subtype in order to further understand the intersection of neuronal and myofibrillar pathology in SBMA. We used a cross-model comparison of two mouse models of SBMA to evaluate NMJ pathology, glycolytic-to-oxidative muscle fiber-type switching, and cytoskeletal alterations in pre- and postsynaptic termini of mouse hindlimb muscles tibialis anterior (TA), gastrocnemius, and soleus. We observed significantly increased NMJ and myofiber pathology in fast-twitch, glycolytic motor units of the TA and gastrocnemius compared to slow-twitch, oxidative motor units of the soleus, as seen by decreased pre- and post-synaptic membrane area, decreased pre- and post-synaptic membrane colocalization, increased acetylcholine receptor compactness, decreased endplate area and complexity, and deficits in neurofilament heavy chain. These studies provide significant evidence for correlated neuronal and muscular dysregulation that contribute to NMJ pathology, which is more severe in fast-twitch motor units. We propose a model in which the dynamic communicative relationship between the motor neuron and muscle, along with the developmental subtype of the muscle, promotes motor unit subtype specific vulnerability, metabolic alterations, and NMJ pathology. These data show specific fast-twitch motor unit vulnerability in SBMA and highlight the value of quantitative evaluation of the NMJ due to the dynamic nature of the NMJ and the combined muscular and neuronal contributions to disease.

Disclosures: E. Molotsky: None. **Y. Liu:** None. **A.P. Lieberman:** None. **D.E. Merry:** None.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.05

Topic: C.06. Neuromuscular Diseases

Title: Deciphering the mechanisms underlying the neuroprotective effect of glycolysis in ALS

Authors: *S. LOGANATHAN[1], H. BALL[1], M. MACIAS[1], N. MORTIMORE[1], B. WILSON[1], G. BIRCHAK[1], E. MANZO[1], D. C. ZARNESCU[1,2];
[1]Dept. of Mol. and Cell. Biol., Univ. of Arizona, TUCSON, AZ; [2]Dept. of Cell. and Mol. Physiol., Penn State Col. of Med., Hershey, PA

Abstract: ALS is a progressive neurodegenerative disease with no known cure. TAR DNA-binding protein (TDP-43) is a pathological marker of ALS and aggregation of TDP-43 is observed in 97% of ALS cases. Our lab has generated a *Drosophila* model of ALS via overexpression of human TDP-43 in motor-neurons which recapitulates key disease phenotypes. Glycolysis upregulation by an increase in Phosphofructokinase (PFK) has been shown to have a neuroprotective effect in *Drosophila* models of ALS based on TDP-43 proteinopathy. In this work we aim to decipher the mechanisms underlying the restorative effect of PFK overexpression (OE) in ALS. Evidence from other groups reveals that PFK can form clusters at the synapses of *C. elegans* where it is required for synaptic vesicle cycling, and it associates with other glycolytic enzymes to form Glycolytic bodies (G-bodies) in yeast under stress. In addition, pyruvate, the end product of glycolysis, was found to be increased in multiple ALS models. Interestingly, pyruvate has been shown to have strong Reactive Oxygen Species (ROS) scavenging capacity, protecting mitochondrial membrane potential, and regulating cell metabolism. These findings led us to hypothesize that 1) PFK localization and/or G-body assembly are altered at ALS synapses 2) PFK OE mitigates TDP-43 dependent SV cycling deficit at the larval NMJ in *Drosophila* and/or 3) PFK OE mitigates oxidative stress through the antioxidant activity of pyruvate. To test these hypotheses, we used confocal and expansion microscopy, and found that PFK forms puncta within *Drosophila* NMJs, in the context of TDP-43[G298S] OE. Furthermore, PFK clustering is observed in Drosophila primary motor neurons with TDP-43 proteinopathy. To identify protein partners of PFK in these puncta, we immunoprecipitated the puncta using anti-GFP nanobodies. Using Mass Spectrometry, we have preliminarily identified TDP-43-dependent changes in PFK complexes, suggesting a role for the interaction of synaptic proteins with PFK. Using FM1-43, a membrane probe, we found that PFK OE rescues TDP-43 induced SV cycling deficits. Together, these results suggest that PFK forms puncta at the synapse and mitigates TDP-43 induced SV cycling deficits

Disclosures: S. Loganathan: None. **H. Ball:** None. **M. Macias:** None. **N. Mortimore:** None. **B. Wilson:** None. **G. Birchak:** None. **E. Manzo:** None. **D.C. Zarnescu:** None.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.06

Topic: C.06. Neuromuscular Diseases

Title: The non-protein amino acid BMAA misincorporates into protein and exacerbates disease phenotypes in a multi-hit mouse model of ALS

Authors: *F. J. ARNOLD[1], M. BURNS[1], Y.-C. CHIU[2], A. LA SPADA[1], C. L. BENNETT[1];
[1]UC Irvine, Irvine, CA; [2]NC State, Raleigh, NC

Abstract: Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease characterized by the progressive loss of upper and lower motor neurons in the brain and spinal cord. Only about 10% of ALS cases are linked to a known, inherited genetic mutation, while the remaining ~ 90% are "sporadic," caused by complex, ill-defined interactions between genetic and environmental risk factors. To develop disease-modifying therapies for sALS patients, we must characterize the key risk factors that cause disease. BMAA is a non-protein amino acid produced by cyanobacteria found throughout the world. On the island of Guam, ingestion of high doses of BMAA by the indigenous Chamorro people remains the leading cause of an ALS/parkinsonism dementia complex (ALS/PDC). Studies have thus far ruled out a genetic basis for disease and the rate of ALS/PDC in the Chamorro population dramatically decreased in conjunction with westernization of their diet, further correlating BMAA exposure with disease. The key mechanism by which environmental BMAA exerts toxicity is through aberrant accumulation into proteins (due to tRNA mischarging), causing protein misfolding and cell death. The goal of the present study was to determine whether chronic exposure to a physiologically-relevant dose of BMAA leads to motor dysfunction or neuropathology in a mammalian model system and to further investigate whether genetic predisposition to motor neuron disease (TDP-43 mutation) synergistically exacerbates its effect. Cohorts of wildtype or transgenic TDP-43^{Q331K} mice were treated with a control diet or a matched diet containing 300ppm BMAA for 16 months. We found that BMAA exposure exacerbated motor dysfunction in transgenic mice, corresponding with reduced motor neuron area. Additionally, we used highly sensitive mass spectrometry analysis of BMAA protein incorporation to determine the extent to which free and protein-incorporated BMAA accumulates in the mammalian central nervous system (CNS) and peripheral tissue, finding significant accumulation of free and protein-incorporated BMAA in liver, and low levels of free BMAA in the brain. This corresponded with dysregulation of the unfolded protein response (UPR) in mouse liver, but not brain. Altogether, these findings indicate that while chronic exposure to BMAA is not acutely toxic, as suggested by prior studies, it may significantly contribute to 'multi-hit' sALS, warranting consideration as an environmental risk factor for disease.

Disclosures: F.J. Arnold: None. **M. Burns:** None. **Y. Chiu:** None. **A. La Spada:** None. **C.L. Bennett:** None.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

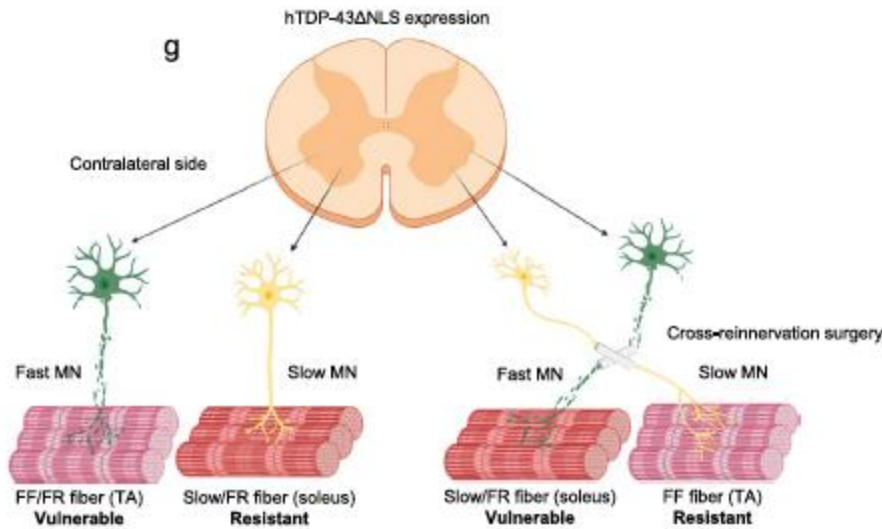**Presentation Number:** 015.07

Topic: C.06. Neuromuscular Diseases

Title: Slow motor neurons resist pathological TDP-43 and mediate motor recovery in the rNLS8 model of amyotrophic lateral sclerosis

Authors: *S. HUR[1,2], V. M. LEE[3];
[1]Neurosci. department, Genentech Inc., South San Francisco, CA; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Perelman Sch. of Medicine,, Univ. of Pennsylvania, Philadelophia, PA

Abstract: In the intermediate stages of amyotrophic lateral sclerosis (ALS), surviving motor neurons (MNs) that show intrinsic resistance to TDP-43 proteinopathy can partially compensate for the loss of their more disease-susceptible counterparts. Elucidating the mechanisms of this compensation may reveal approaches for attenuating motor impairment in ALS patients. In the rNLS8 mouse model of ALS-like pathology driven by doxycycline-regulated neuronal expression of human TDP-43 lacking a nuclear localization signal (hTDP-43ΔNLS), slow MNs are more resistant to disease than fast-fatigable (FF) MNs and can mediate recovery following transgene suppression. In the present study, we used a viral tracing strategy to show that these disease-resistant slow MNs sprout to reinnervate motor endplates of adjacent muscle fibers vacated by degenerated FF MNs. Moreover, we found that neuromuscular junctions within fast-twitch skeletal muscle (tibialis anterior, TA) reinnervated by SK3-positive slow MNs acquire resistance to axonal dieback when challenged with a second course of hTDP-43ΔNLS pathology. The selective resistance of reinnervated neuromuscular junctions was specifically induced by the unique pattern of reinnervation following TDP-43-induced neurodegeneration, as recovery from unilateral sciatic nerve crush did not produce motor units resistant to subsequent hTDP-43ΔNLS. Using cross-reinnervation and self-reinnervation surgery in which motor axons are disconnected from their target muscle and reconnected to a new muscle, we show that FF MNs remain hTDP-43ΔNLS-susceptible and slow MNs remain resistant, regardless of which muscle fibers they control. Collectively, these findings demonstrate that MN identity dictates the susceptibility of neuromuscular junctions to TDP-43 pathology and slow MNs can drive recovery of motor systems due to their remarkable resilience to TDP-43-driven degeneration. This study highlights a potential pathway for regaining motor function with ALS pathology in the advent of therapies that halt the underlying neurodegenerative process.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.08

Topic: C.06. Neuromuscular Diseases

Title: Uncovering shared and circuit specific mRNA targets of TDP-43 proteinopathy in Drosophila models of Amyotrophic lateral sclerosis (ALS) and Frontotemporal Dementia (FTD)

Authors: *D. ZARNESCU[1], R. GODFREY[1], R. BJORK[1], E. ALSOP[2], H. RUVALCABA[1], K. VAN KEUREN JENSEN[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) encompass a spectrum of neurodegenerative diseases affecting upper and lower motor neurons and cells in the anterior cingulate cortex (ACC) and frontoinsula (FI), with age dependent progressive degeneration in other brain regions. The classification of ALS and FTD as related diseases is based on clinical, pathological, and genetic overlap among patients. It has been hypothesized that overlapping cellular and molecular pathways link neurodegeneration in these diseases, however the precise mechanisms underlying degeneration in different neuronal circuits remain poorly understood. Given its presence in ubiquitin-positive aggregates among greater than 95% of ALS and nearly half of FTD cases (FTLD-TDP), the trans active response (TAR) DNA-Binding protein 43 (TDP-43) provides a common denominator for identifying the

molecular mechanisms linking disease across the ALS/FTD spectrum. We have recently developed a Drosophila model of FTD based on overexpression of human TDP-43 in the mushroom bodies (MBs), a higher-order associative circuit that overlaps in function and gene expression with neurons in the human hippocampus and frontal cortex, making it an appropriate brain structure to model human cognitive disease. Using this approach we identified age dependent TDP-43 mislocalization, axonal degeneration as well as working memory and sleep deficits. Here, using *Drosophila* models of TDP-43 proteinopathy based on expression of human TDP-43 either in motor neurons or MBs we identify shared as well as circuit specific mRNA targets of TDP-43. Genetic interaction approaches in conjunction with ALS and FTD relevant behavioral assays show that dally like protein, (Dlp), a glypican regulating wingless/wnt signaling is a TDP-43 target in vivo, in both the motor and MB circuits. We are currently evaluating other candidate mRNA targets for their ability to mediate TDP-43 dependent toxicity in both ALS and FTD models and validating them in patient tissues.

Disclosures: D. Zarnescu: None. **R. Godfrey:** None. **R. Bjork:** None. **E. Alsop:** None. **H. Ruvalcaba:** None. **K. Van Keuren Jensen:** None.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.09

Topic: C.06. Neuromuscular Diseases

Support: Ulla-Carin Lindquist's Foundation
        The Swedish research council
        Wallenberg Foundation

Title: Updates on *in vivo* seeding studies: SOD1 prions transmit aggregation and fatal ALS-like disease

Authors: *E. EKHTIARI BIDHENDI[1], P. M. ANDERSEN[2], S. L. MARKLUND[3];
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Abstract: Evolving evidencesuggest the presence of propagating prion-like species in several types ofneurodegenerative diseases associated with misfolding of host-encodednon-mutated proteins. Among these are AD, PD, the tauopathies, HD and amyotrophiclateral sclerosis (ALS). A hallmark of prions is the presence of differentconformational aggregate strains with different biological activities. Anothercharacteristic of prions is that subsequent prion passage within the same hostcan lead to a shorter incubation period. In ALS, using binary epitope mapping(BEM) we identified two structurally different strains of mutant humansuperoxide dismutase 1 (hSOD1) aggregates (named A and B) in the CNS oftransgenic (Tg) mice models expressing full-length hSOD1 variants. When seededinto spinal cords of adult hSOD1G85R Tg mice, these strains

transmitexponentially propagating hSOD1 aggregation selectively in the motor systemwith concomitant development of muscle wasting and paresis. To furtherinvestigate prion-like properties of hSOD1 strains in ALS, and explore thespreading characteristic of the disease in vivo, stereotaxic inoculation ofhSOD1 aggregate strains into spinal cords of pre-symptomatic Tg-hSOD1D90A micewere performed. This mutation is essentially wt-SOD1 like. Normally,Tg-hSODD90A only develop MND phenotype when the transgene is homozygous. Micehemizygous for the hSOD1D90A transgene insertion do not spontaneously developALS pathology and have a normal murine lifespan (>700d). First & second passagestudies were performed to investigate further prion-like properties of hSOD1.Inoculations of strain A or B seeds into the lumbar spinal cord of 100-day-oldhemizygous hSOD1D90A mice induced progressive hSOD1 aggregations and prematurefatal ALS-like disease after ≈250 and ≈350 days, respectively. BEM analysis ofaggregates in the terminal stage lead to a surprise discovery: Inoculation ofstrain A into hemizygous hSOD1D90A mice gave rise to a new strain named C,which has the C-terminal end apparently recruited to the aggregate core. Secondpassage inoculations were then performed in hemizygous hSOD1D90A mice, usingspinal cord homogenates with strain C aggregates as seeds. The novel prionstrain was much more efficient, and transmitted progressive hSOD1 aggregationand ALS-like disease which was fatal 100 days after inoculation. We providefurther evidence of the similarities between hSOD1 and the prion protein. Ourdata suggest that mutations in SOD1 are inducing aggregation and ALS pathologyvia a prion mechanism.

Disclosures: E. Ekhtiari Bidhendi: None. **P.M. Andersen:** None. **S.L. Marklund:** None.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.10

Topic: C.06. Neuromuscular Diseases

Title: Extracellular Vesicles induce neurodegeneration in vivo in C9-ALS

Authors: *M. CICARDI, D. TROTTI;
Thomas Jefferson Univ., Thomas Jefferson university, Philadelphia, PA

Abstract: Amyotrophic Lateral Sclerosis (ALS) and Fronto-Temporal Dementia (FTD) are two neurodegenerative diseases that belong to the same disease spectrum. ALS-FTD's most common genetic cause is a G_4C_2 repetition in the intron 1 of the C9orf72 gene. This mutation results in three main toxic events: C9orf72 protein reduction, RNA foci formation, and aberrant repeat-associated non-AUG translation (RAN-T). The products of RAN-T are five dipeptide repeat proteins (DPRs: polyGA, polyGP, polyGR, polyPA, and polyPR). These proteins can be

transmitted from one cell to another, possibly causing disease progression.To monitor *in vivo* spreading of DPRs, we employed a mouse model which expresses one single DPR (poly(GR)) tagged with GFP upon Cre recombination. We focally injected an AAV-mCherry-Cre in the ventral horn of the spinal cord of these mice. Interestingly, we detect some DPR signals even in distant areas from the injection point, suggesting *in vivo* poly(GR) spreading. One of the means of protein propagation is extracellular vesicles,, double membrane organelles released by cells through different pathways whose content varies according to cells' patho/physiological conditions. We already know that neurons *in vitro* shed poly(GR)$^+$ EVs. We thus checked whether in vivo neurons could also spread poly(GR) through the EVs and whether these could be detected in biofluids. We employed a mouse that expressed CD63-GFP, and we injected lentiviral particle expressing poly(GR) in the motor cortex. After 14 days,, we detected CD63-GFP particles containing poly(GR) in areas surrounding the injection site indicating EVs as one of the means of poly(GR) spreading. We then analyzed circulating EVs in the plasma, and we found that a small but detectable percentage of them were poly(GR)$^+$. Last, to check whether poly(GR)$^+$ EVs were able to cause a neuronal loss *in vivo*, we intraspinal inject poly(GR)$^+$ EVs. We observed that after 14 days, poly(GR)$^+$ EVs could cause significant neuronal loss paralleled by astrogliosis and microgliosis. In conclusion, with this work,, we determined that poly(GR) is distributed in the CNS through EVs and that poly(GR) transmission can cause neuronal loss.

Disclosures: M. Cicardi: None. **D. Trotti:** None.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.11

Topic: C.06. Neuromuscular Diseases

Title: Tnpo1 hinders the neurotoxicity of the glycine-arginine dipeptides linked to c9orf72-als/ftd

Authors: *D. TROTTI, M. CICARDI;
Thomas Jefferson Univ., Thomas Jefferson Univ., Philadelphia, PA

Abstract: The most common cause of amyotrophic lateral sclerosis and frontotemporal dementia is the presence of a G_4C_2 expansion in the C9orf72 gene. This expansion is aberrantly translated by non-AUG-dependent translation into dipeptide repeat proteins (DPRs), which accumulate into inclusions and are, to a different degree, toxic to neurons. The arginine-rich DPRs are known to be highly aggregation-prone and to interact with multiple other proteins aberrantly. Building on preliminary results showing the interaction between the dipeptide poly-glycine-arginine (polyGR) and TNPO1, we sought to explore the effect of this interaction in disease relevant-

models. We observed in neurons *in-vitro* and mice that polyGR co-precipitated with TNPO1. We found by FRAP assay that TNPO1 could not disaggregate polyGR inclusions nor change their liquid-like properties. We thus hypothesized that this aberrant interaction could hinder TNPO1 function and that restoring TNPO1 levels could benefit neurons bearing polyGR. Overexpression of TNPO1 in neurons did not change their normal viability *in-vitro*. We then co-expressed polyGR and TNPO1 and found that TNPO1 relieved neuronal toxicity caused by poly(GR) and restored TDP-43 levels in the nucleus. This work dissects the role of TNPO1 in counteracting poly(GR) toxicity in neurons and opens a new therapeutic avenue centered on the modulation of TNPO1 in C9orf72-linked ALS/FTD.

Disclosures: D. Trotti: None. **M. Cicardi:** None.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.12

Topic: C.06. Neuromuscular Diseases

Title: Antisense, but not sense, repeat expanded RNAs activate PKR/eIF2α-dependent integrated stress response in C9orf72 FTD/ALS

Authors: *J. PARAMESWARAN[1], N. ZHANG[1], E. BRAEMS[3], K. TILAHUN[1], D. C. PANT[1], G. CHILUKURI[1], S. ASRESS[2], A. BANERJEE[1], E. DAVIS[1], G. J. BASSELL[1], L. BOSCH[4], J. JIANG[1];
[1]Dept. of Cell Biol., [2]Dept. of Neurol., Emory Univ., Atlanta, GA; [4]Dept. of Neurosciences, [3]Univ. of Leuven, Leuven, Belgium

Abstract: GGGGCC (G_4C_2) hexanucleotide repeat expansion in the C9orf72 gene is the most common genetic cause of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). C9orf72 repeats are bidirectionally transcribed into sense G_4C_2 and antisense CCCCGG (C_4G_2) RNAs and are proposed to confer gain of toxicity by sequestering key RNA binding proteins into RNA foci and/or by production of toxic dipeptide repeat (DPR) proteins via non-canonical repeat-associated non-AUG-dependent (RAN) translation from all reading frames. Several lines of studies, by expressing either G_4C_2 repeats or individual codon-optimized, ATG-driven DPR proteins, support that gain of toxicity from the repeat expanded RNAs plays a central role in disease pathogenesis. However, the underlying toxic species is debated, and whether antisense C_4G_2 expanded RNAs contribute to C9FTD/ALS and thus are targets of intervention is less clear. Several studies show antisense RNA foci are as abundant as sense RNA foci in multiple brain region, raising a possibility that antisense C_4G_2 repeat expanded RNAs also contribute to diseases. To test this hypothesis, we generated antisense constructs containing 2 or

75 C_4G_2 repeats using recursive directional ligation and checked for neuronal toxicity. We expressed these constructs in primary cortical neurons together with an mApple construct and performed an automated longitudinal microscopy to track the survival of hundreds of neurons as indicated by the mApple fluorescence over several days. We find that neurons expressing $(C_4G_2)75$ die much faster than those expressing control 2 repeats, suggesting that C9rof72 antisense C_4G_2 expanded repeats are neurotoxic. Mechanistically, our study shows that C9orf72 (C_4G_2) antisense repeat expanded RNAs trigger the activation of the PKR/eIF2α-dependent integrated stress response independent of dipeptide repeat proteins that are produced through RAN translation in HEK293T cells and primary neurons. Such activation further leads to global translation inhibition and stress granule formation. We also observed the increased phosphorylation of PKR/eIF2α in the frontal cortex of C9orf72 FTD/ALS patients (n=6) compared to age matched controls (n=6) by immunocytochemistry and immunoblotting analysis respectively. Finally, we find that only antisense (C_4G_2), but not sense (G_4C_2), repeat expanded RNAs can activate the PKR/eIF2α pathway. Together, these findings provide a mechanism by which antisense repeat expanded RNAs elicit neuronal toxicity in FTD/ALS caused by C9orf72 repeat expansions.

Nanosymposium

015. ALS, CMT, and Degenerative Diseases

Location: SDCC 33

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 015.13

Topic: C.06. Neuromuscular Diseases

Title: Glucose hypometabolism drives dipeptide repeat protein accumulation in C9orf72-ALS/FTD in-vitro and in-vivo models modifying disease phenotypes.

Authors: *A. T. NELSON, M. CICARDI, A. R. HAEUSLER, D. TROTTI;
Neurosci., Thomas Jefferson Univ., Philadelphia, PA

Abstract: The *C9orf72* (G4C2)n repeat expansion is one of the most common genetic causes of ALS/FTD. It leads to the production of dipeptide repeat proteins (DPRs) in neurons by an aberrant, non-canonical mechanism known as repeat-associated non-AUG (RAN) translation. These DPRs are toxic in various model systems and are thought to be a primary driver of neurodegeneration in *C9orf72*-linked ALS/FTD. Recently, various forms of cellular stress have been shown to increase RAN translation in neurons, specifically via activation of the integrated stress response (ISR) pathway. The core event in this pathway is the phosphorylation of eIF2α by one of four kinases: PERK, PKR, GCN2, or HRI, each of which is activated by different forms

of stress, leading to increased DPR production. One potential source of stress in neurons is altered metabolic homeostasis. Indeed, a growing list of metabolic imbalances is observed in ALS patients and model systems, including increased metabolic rate, glucose hypometabolism, and mitochondrial dysfunction. Notably, glucose hypometabolism is explicitly seen in carriers of the *C9orf72* repeat expansion prior to symptom onset and could potentially serve as an early trigger of RAN translation and DPR production. However, whether this is the case remains unexplored. To test this hypothesis, we transduced either primary or human iPS-derived neurons with a lentiviral vector that encodes a GFP-tagged (G_4C_2) repeat expansion, then applied a glucose hypometabolism paradigm - which sharply reduces the metabolic rate of the cells - and monitored its effect on the accumulation of DPR aggregates monitored via the reporter GFP tag. We observed that glucose hypometabolism indeed enhances the accumulation of DPRs in parallel with activation of the ISR. Furthermore, DPR accumulation can be entirely blocked by co-treating the cells with a pharmacological inhibitor of the GCN2 kinase, which is known to respond to nutrient and metabolic imbalances. These data suggest that glucose hypometabolism enhances RAN translation, and that it does so precisely via activation of the GCN2 arm of the ISR. We also show that glucose hypometabolism negatively impacts the survival of C9orf72 patient-derived iPS neurons and worsens behavioral phenotypes in a BAC transgenic mouse model of C9orf72 ALS. These studies highlight new potential therapeutic targets and strengthen conclusions in the field about the mechanism and molecular triggers of C9orf72-linked RAN translation.

Disclosures: A.T. Nelson: None. **M. Cicardi:** None. **A.R. Haeusler:** None. **D. Trotti:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.01

Topic: D.06. Vision

Support: Wellcome 223144

Title: Visuomotor association orthogonalizes visual cortical population codes

Authors: *S. W. FAILOR, M. CARANDINI, K. D. HARRIS;
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Abstract: When an animal sees a stimulus, a pattern of activity is triggered across many neurons in its visual cortex. Together, these neurons' firing rates define a representation of the stimulus in a high-dimensional vector space. Recordings from individual neurons in primary visual cortex (V1) have shown that their encoding of visual stimuli can change when animals associate these stimuli with motor actions. However, it remains to be established how this representational plasticity is coordinated across neurons and how it affects population coding.
One suggested purpose for the plasticity of cortical representations is to improve the precision of

stimulus encoding in the face of noise. However, V1 stimulus representations are extremely precise even in naive animals [Stringer 2021], so representational plasticity is not required to improve coding precision. This does not mean it is useless: a neural population could represent stimuli in multiple formats, some better suited for downstream circuits to associate them with appropriate behaviors. Indeed, representations vary in their generalizability, captured by the angle between two population activity vectors. Identical representations will generalize, whereas orthogonal representations will not at all. Similarly, stimuli that evoke similar representations in the brain are likely to evoke similar behaviors, while stimuli that evoke orthogonal representations could evoke opposing behaviors.

We used two-photon calcium imaging to investigate how the tuning of populations of V1 neurons changes after mice learn to associate opposing actions to two visual stimuli. At a single-cell level, the results appear complex: neuronal tuning curves evolve according to a lawful but complicated dependence on their prior stimulus preference and tuning strength. At the population level, however, a simple principle emerges: visuomotor association transforms response vectors by a nonlinear function, whose convexity is largest for motor-associated stimuli. This transformation sparsens the population representations and makes them more orthogonal. The degree of sparsening varied consistently across the population on a trial-by-trial basis, suggesting it emerges from rapid circuit dynamics (e.g. local inhibition). Sparsening did not improve the precision of representations in V1, in fact, stimuli could be decoded perfectly with only a few neurons in all experiments. However, a feedforward-inhibition model demonstrated that orthogonalization could amplify and further differentiate motor-associated stimulus responses in downstream regions, supporting its role in generating stimulus-specific behaviors.

Disclosures: S.W. Failor: None. **M. Carandini:** None. **K.D. Harris:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.02

Topic: D.06. Vision

Title: Specialized basal dendrites with co-tuned inputs drive cortical neurons

Authors: *L. F. ROSSI[1], A. LIU[2], L. MARIOTTI[5], C. REDDY[2], N. GHANI[3], K. D. HARRIS[4], M. CARANDINI[2];
[1]Wolfson Inst. for Biomed. Res., [2]Inst. of Ophthalmology, [3]Sainsbury Wellcome Ctr., [4]Inst. of Neurol., Univ. Col. London, London, United Kingdom; [5]Inst. of Neurosci., Consiglio Nazionale delle Ricerche, Padova, Italy

Abstract: A key question in neuroscience concerns the functional role of dendrites: are all dendrites the same, or are individual dendrites specialized to integrate dedicated input? We addressed this question in the primary visual cortex (V1), where the aggregate orientation preference of synaptic spines on pyramidal cells matches those of the neuron, but individual synaptic inputs can exhibit diverse preferences. It is not clear whether these synaptic inputs are organized, and whether different dendrites play distinct roles in conferring selectivity. Thus, we sparsely expressed the glutamate sensor iGluSnFR3 to measure the visual selectivity of synaptic inputs to different basal dendrites in mouse V1, relating these dendrites to the preferred orientation of the neuron after careful measurement of the local map of retinotopy. The results revealed a marked asymmetry in the distribution of synaptic inputs: the inputs onto dendrites oriented parallel to the preferred orientation were mostly selective for the same orientation, whereas those on dendrites orthogonal to the preferred orientation exhibited a variety of preferences. We then speculated that the co-tuned inputs on parallel dendrites could be strengthened by plasticity to provide greater drive. To test this hypothesis, we reconstructed the dendritic arbor of neurons sparsely labeled with a red structural marker, and characterized their tuning with somatic imaging of GCaMP6. Spine density was similar across dendrites, but spines were indeed larger on parallel than on orthogonal dendrites. Finally, in the same mice, we tested causally the impact of synaptic inputs onto different basal dendrites, by using two-photon optical micro-dissection. Pruning the dendrites parallel to the preferred orientation dramatically reduced the responses of V1 neurons to gratings, significantly decreased orientation selectivity and occasionally shifted preferred orientation; by contrast, pruning the orthogonal dendrites had much weaker effects. These results reveal a new form of specialization in the basal dendrites of pyramidal neurons in the cortex: the dendrites parallel to the neuron's preferred orientation receive co-tuned, stronger synaptic inputs that provide a dominant drive. This specialization opens new questions about the role of the orthogonal dendrites. These dendrites receive a mixture of inputs, which likely reflects the variety of stimulus preferences of nearby neurons. They do not affect the responses to gratings, but perhaps they carry contextual signals that shape the responses to more complex natural stimuli.

Disclosures: L.F. Rossi: None. **A. Liu:** None. **L. Mariotti:** None. **C. Reddy:** None. **N. Ghani:** None. **K.D. Harris:** None. **M. Carandini:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.03

Topic: H.04. Executive Functions

Title: Global synchronization of spontaneous activity across layer 5 pyramidal neurons during anesthesia-induced loss of consciousness

Authors: A. BHARIOKE[1], M. MUNZ[1], A. BRIGNALL[2], G. KOSCHE[1], M. EIZINGER[2], D. HILLIER[1], K.-K. CONZELMANN[3], E. MACÉ[4], B. ROSKA[2];
[1]Botond Roska Group, [2]Inst. of Mol. and Clin. Ophthalmology Basel, Basel, Switzerland; [3]Max von Pettenkofer-Institute, Virology, Med. Fac. and Gene Center, Ludwig Maximilians Univ., Munich, Germany; [4]Max Planck Inst. of Neurobio., Planegg, Germany

Abstract: Identifying a circuit mechanism underlying the loss of consciousness induced by general anesthesia is a long-standing question in neuroscience. Activity within cortex drives conscious perception and, hence, loss of consciousness is thought to result from the disconnection of cortex. Here, we identified an aperiodic temporal synchronization of spontaneous neuronal activity during general anesthesia, specific to layer 5 pyramidal neurons, a major output of cortex. This synchronous activity was maintained across different anesthetics with diverse molecular modes of action, despite having different event frequencies and amplitudes. Additionally, during transitions to and from anesthesia, the change in synchrony within layer 5 coincided with the loss and recovery of consciousness. Further, this synchronous layer 5 activity extended globally across cortex. In contrast, neurons in each of the other cortical layers, targeted through mouse Cre lines, did not show a consistent change in synchrony across anesthetics. We demonstrated that the synchronous activity in layer 5 pyramidal neurons results in a decrease in information entropy, with the entire population acting as a single effective unit. Hence, our results show that, during general anesthesia, cortex shifts from a mode characterized by spatially asynchronous outputs transmitting high information, to a mode characterized by spatially synchronous outputs, transmitting low information. This reduction in information output disconnects cortex and, thereby, provides a possible mechanism for the loss of consciousness.

Disclosures: A. Bharioke: None. **M. Munz:** None. **A. Brignall:** None. **G. Kosche:** None. **M. Eizinger:** None. **D. Hillier:** None. **K. Conzelmann:** None. **E. Macé:** None. **B. Roska:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.04

Topic: D.06. Vision

Title: The neural code for face cells is not face specific

Authors: *K. VINKEN[1], **J. S. PRINCE**[2], **T. A. KONKLE**[2], **M. S. LIVINGSTONE**[1];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Psychology Dept, Harvard Univ., Cambridge, MA

Abstract: Face cells are neurons that generally respond more to faces than non-faces, leading to the wide-spread belief that face cells encode semantic or visual information that is mainly or even exclusively applicable to faces. Indeed, most previous face-cell studies have used only faces to characterize face-cell tuning, with no consideration of the smaller responses to non-faces. Here, we asked whether these non-face responses contain information about face-cell tuning that cannot be characterized using only faces. By analyzing responses to hundreds of images (N=1408) in macaque inferotemporal cortex (N=448 sites), we asked how accurately we can infer the defining property of a face cell, namely its face versus non-face selectivity, from only the non-preferred category of non-faces. We found that the response selectivity for only non-face images can predict a neural site's face versus non-face selectivity (R^2=0.69) better than the response selectivity for only face images (R^2=0.49). Thus, non-face responses contain information about face-cell tuning that cannot be characterized with only faces. The link between non-face responses and face versus non-face selectivity was not explained by color or simple shape features, but by complex image statistics encoded by higher classification-trained DNN layers, particularly when the DNN had been pre-trained on general object classification (ImageNet; R^2=0.54) rather than on face identity (R^2=0.41). Even in the most face-selective neural sites (face d'>1.5), we found some face images with a significantly lower response than some non-face images, suggesting that face cells do not merely encode the extent to which an object looks like a face. Overall, our work challenges the assumption that face cells owe their face selectivity to face-specific information, instead supporting the notion that category-selective neurons are best understood as tuning directions in a domain-general object space.

Disclosures: K. Vinken: None. **T.A. Konkle:** None. **M.S. Livingstone:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.05

Topic: D.06. Vision

Title: Whole-brain functional mapping of face patch neurons during rest

Authors: *D. ZALDIVAR, R. BHIK-GHANIE, K. W. KOYANO, F. Q. YE, D. C. GODLOVE, S. PARK, D. A. LEOPOLD;
SCNI, Lab. of Neuropsychology, NIMH, NIH, SCNI, Lab. of Neuropsychology, NIMH, NIH, Bethesda, MD

Abstract: Resting state fMRI (rs-fMRI) is a widely used method that measures the coordination of slow fluctuations (<0.1 Hz) across the brain in the fMRI response. Brain areas showing coherent activity are operationally defined to be functionally connected, with patterns that often recapitulate networks observed in task-based testing. While multiple mechanisms contribute to the fMRI activity correlation across the brain, direct neural projections are thought to be a central determinant of observed network structure. However, little is known about the functional connectivity of individual neurons with fMRI signals across the brain. Would the functional connectivity of individual neurons primarily reflect the known anatomical connections of the area in which they reside? To what extent would individual cells exhibit distinct patterns of correlation across the brain? We addressed these questions by concurrently measuring local single-unit activity and fMRI signals across the brain of five macaques. Specifically, the spiking fluctuations of individual neurons in two face patches were compared with brain-wide hemodynamic fluctuations to create maps of functional connectivity. We found neurons within each face patch population exhibited a high degree of overlap in their large-scale fMRI correlations, with the strongest correlations in visual areas V4, TEO, other face patches, and ventral premotor cortex. At each location, subpopulations of neurons had inverse time courses and were thus anticorrelated with these structures. Many neurons also exhibited a pronounced anticorrelation with thalamic structures and brainstem neuromodulatory centers. Corresponding maps derived from fMRI seeds and local field potentials from the same recording location showed marked differences in their structure, particularly regarding the inverse correlations. These findings demonstrate a restricted and largely shared pattern of functional connectivity among neurons in functionally defined face patches that partially reflects known anatomical connections. The direct linking of local circuitry to whole-brain functional architecture offers new avenues to study how diverse types of signals are integrated within functionally defined brain areas.

Disclosures: D. Zaldivar: None. **R. Bhik-Ghanie:** None. **K.W. Koyano:** None. **F.Q. Ye:** None. **D.C. Godlove:** None. **S. Park:** None. **D.A. Leopold:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.06

Topic: D.06. Vision

Title: Optogenetic inactivation of cortico-cortical feedback modulates gamma oscillations in marmoset primary visual cortex

Authors: *A. CLARK[1], A. ANGELUCCI[2];
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Abstract: Top-down cortico-cortical feedback (FB) is hypothesized to contribute to numerous cognitive and perceptual phenomena. There is also evidence that FB connections are involved in the generation and/or modulation of gamma oscillations, a network phenomenon hypothesized to be critical for facilitating inter-areal communication and neural coding, in primary visual cortex (V1). That is, the effect of stimulus size on V1 gamma power is greater in FB-recipient layers, and cooling of V2/V3 decreases the amplitude of V1 gamma. Using optogenetic inactivation of FB terminals, we previously demonstrated that FB from V2 to V1 play a critical role in regulating single-unit response amplitude and receptive field size in V1. Here, we have investigated the effects of selective inactivation of V2-to-V1 FB on V1 gamma oscillations. We used the inhibitory opsin ArchT to silence V2-to-V1 FB projections. Specifically, we drove selective expression of ArchT in V2 pyramidal cells in 2 marmosets. After allowing time for ArchT expression, we performed V1 recordings using 64 channel linear electrode arrays (LEA). To activate ArchT in V2 FB terminals and silence V2-V1 FB, we applied focal surface photostimulation to an approximately 1.5mm radius area around the LEA. We measured size tuning to full contrast drifting sinusoidal gratings of optimal orientation and spatiotemporal frequency with and without FB inactivation. Consistent with prior work (Clark et al 2021; Gieselmann & Thiele, 2022), V1 gamma power was size tuned, with some suppression at large stimulus diameters, and the peak frequency of the gamma oscillation decreased with increasing diameter. Also consistent with prior findings, we observed a selective decrease in the gain of multi-unit (MU) responses when stimuli were matched to the RF but an increase where the stimuli extended into the near surround, resulting in an increase in RF size during V2 FB inactivation. To look at the effects of FB inactivation on local field potential (LFP) gamma power, we examined the ratio of gamma power in baseline and FB-inactivation conditions for LEA channels that exhibited significant size tuning in the baseline condition. For each channel, we selected the stimulation intensity that elicited the largest change in MU RF size (critically, either a decrease or an increase). When FB was inactivated, we found a decrease in gamma power for stimuli that were either matched to the LFP RF size or extended into the near, but not the far, surround. We are currently examining laminar differences in these effects as well as changes in other frequency bands. Finally, we are examining a network model of visual cortex to identify putative circuit mechanisms.

Disclosures: A. Clark: None. **A. Angelucci:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.07

Topic: D.06. Vision

Title: Routing by avalanches: a theoretical framework for selective processing in the visual system

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Abstract: Neural information processing in dynamic, natural environments requires the brain to flexibly allocate limited computational resources to varying task demands. One example is selective attention in the visual system, which allows to preferentially route signals from behaviourally relevant stimuli to downstream visual areas, while suppressing irrelevant visual information. It is an open question which neural mechanisms realize selective routing, and how this process is controlled. Here we propose that spontaneous synchronization in recurrent networks is the key mechanism for selective processing. We study a hierarchical network consisting of recurrently coupled populations of spiking neurons which send activation to a common receiver/output population. The network is driven by two external signals of which one has to be routed to the output, while the other signal has to be ignored. Communication takes place via propagation of spike avalanches between sending and receiver populations. Routing is established by releasing inhibition from control populations which enhance avalanche generation and increase sizes of synchronous events carrying the information from the attended stimulus. Optimal routing configurations need to balance signal representation in the upstream areas and transmission to the downstream area and are derived analytically using a newly-developed theory for describing spike-pattern formation. Our framework provides a unifying account for selective information transfer through the visual hierarchy while reproducing a series of key experimental observations, such as typical rate modulations induced by attention in different visual areas, biased competition, and the emergence of gamma oscillations and inter-areal phase synchronization. In contrast to previously proposed routing schemes based on oscillatory dynamics such as Communication-through-Coherence, selective routing can be established quickly since it does not need an intricate control scheme organizing the phase relationship between different oscillatory units. In addition, it proposes a simple and biophysically plausible control mechanism in form of a population with a critical dynamics, which becomes engaged by attention and then boosts generation of synchronous events for enhancing signal transfer.

Disclosures: M. Schünemann: None. **U.A. Ernst:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.08

Topic: D.06. Vision

Title: Forming 3-dimensional multimodal object representations relies on integrative coding

Authors: *A. Y. LI[1], N. LADYKA-WOJCIK[1], H. QAZILBASH[1], A. GOLESTANI[1], D. B. WALTHER[1], C. B. MARTIN[2], M. D. BARENSE[1];
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Abstract: Combining information from multiple senses is essential to object recognition. Yet how the mind combines sensory input into coherent multimodal representations - the *multimodal binding problem* - is not well understood. Here, we applied multi-echo fMRI across a four-day paradigm, in which participants learned 3D multimodal objects that were created from well-characterized visual shape and sound features. Our novel paradigm decoupled the learned multimodal object representations from the baseline unimodal shape and sound feature representations, thus tracking the emergence of multimodal concepts as they were learned by healthy adults. Critically, we found that the whole object was different from the combined representation of its individual parts, with evidence of an integrative object code in anterior temporal lobe structures. Intriguingly, the perirhinal cortex - an anterior temporal lobe structure - was by default biased towards visual shape, but this initial shape bias was attenuated with learning. Pattern similarity analyses revealed that the perirhinal cortex orthogonalized combinations of visual shape and sound features with experience, transforming overlapping feature input into distinct multimodal object representations. These results reveal that sounds are integrated with existing shape representations in the perirhinal cortex, with visual content serving as the foundation by which new multimodal object concepts are constructed.

Disclosures: A.Y. Li: None. **N. Ladyka-Wojcik:** None. **H. Qazilbash:** None. **A. Golestani:** None. **D.B. Walther:** None. **C.B. Martin:** None. **M.D. Barense:** None.

Nanosymposium

Presentation Number: 016.09

Topic: D.06. Vision

Title: Experience drives the development of novel, reliable cortical sensory representations from endogenous networks

Authors: S. TRÄGENAP[1], D. E. WHITNEY[2], D. FITZPATRICK[2], *M. KASCHUBE[1];
[1]Frankfurt Inst. For Advanced Studies, Frankfurt am Main, Germany; [2]Max Planck Florida Inst., Jupiter, FL

Abstract: Cortical circuits embody remarkably reliable neural representations of sensory stimuli that are critical for perception and action. Building these network representations is a complex developmental process beginning prior to the onset of sensory experience with endogenous mechanisms generating an initial framework that is subsequently refined with experience. What we lack is a fundamental understanding of this nature-nurture transform: how endogenous networks respond to the onset of sensory experience, and the extent to which experience reorganizes these initial networks to generate a mature representation. Here we examine this question focusing on the representation of edge orientation in primary visual cortex of the ferret, a species that exhibits a well-defined modular network of orientation-selective responses. Using *in vivo* calcium imaging we find that the initial presentation of visual stimuli to endogenous networks generates robust modular patterns of activity, but these patterns lie on a high-dimensional manifold and are highly variable both across and within individual trials. With the onset of visual experience, the evoked manifold develops novel components, becomes low-dimensional, and the individual responses reliable. By using patterns of spontaneous activity to define endogenous network structure, we test whether the endogenous network could provide a stable framework for the emerging sensory representations. Inconsistent with this idea, we find that spontaneous patterns at eye opening are neither a good predictor of the initial nor the future visually evoked patterns. Instead, highly reliable visual responses emerge via an experience-dependent process that involves substantial reorganization of both evoked and spontaneous activity patterns to achieve a stable aligned network structure that is better predicted by the early evoked than by early spontaneous activity. Based on a computational network model whose predictions closely match the biology, we propose that visual experience drives the alignment of feedforward inputs and recurrent networks to achieve highly reliable sensory representations. Taken together, these results demonstrate that experience plays a critical role in transforming a nascent modular network with diverse and unreliable visual responses into a mature network with a distinctive modular structure and highly reliable visual responses and suggest that feedforward-recurrent alignment is a main factor in this process.

Disclosures: S. Trägenap: None. **D.E. Whitney:** None. **D. Fitzpatrick:** None. **M. Kaschube:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.10

Topic: D.06. Vision

Support: NIH Intramural NCT00001360

Title: How behaviorally-relevant object dimensions unfold over time during visual processing

Authors: *L. TEICHMANN[1], M. N. HEBART[2], C. I. BAKER[1];
[1]Lab. of Brain and Cognition, NIH, Bethesda, MD; [2]Vision and Computat. Cognition Group, Max Planck Inst. For Human Cognitive and Brain, Leipzig, Germany

Abstract: Our visual world consists of an immense number of unique objects and yet, we are easily able to identify, distinguish, interact, and reason about the things we see. Object processing encompasses more than being able to assign categories to things. For example, we are easily able to recognize a cat, but we also understand a variety of different properties that constitute that cat (e.g., living, moves, has ears). Similarly, we can discern properties even for objects we have never experienced before. In the current study, we examined how these rich object representations unfold in the human brain by testing how behaviorally-relevant object properties contribute to the neural signal.We recorded Magnetoencephalography (MEG) data while participants (n = 4) viewed 22,248 images from a large-scale image set (*THINGS, Hebart et al., 2019, PloS One*) sampled evenly from 1,854 object categories. Based on behavioral modelling results (*Hebart et al., 2020, Nature Human Behaviour*), we used 49 continuous and interpretable object dimensions to examine how object representations unfold in the neural signal over time. The dimensions are a behavioral measure of how strongly a given property is associated with an image class. For example, images of crayons score highly on the dimension "colorful" while images of zebras have lower scores on that dimension. Using these behavioral predictions, we trained a linear regression model to learn how the multivariate MEG response is related to these dimension weights. The results show at what timepoint each behavioral dimension is a good model to explain the variations in the MEG signal. We found that information about most dimensions was present in the multivariate MEG signal at some point in time. Dimensions describing low-level visual differences, such as the dimension "many small things", peak earlier than higher-level dimensions, such as "body-part related", showcasing that our method is able to differentiate between different stages of processing in the visual hierarchy. Using dynamic time warping and clustering methods, we compared dimension timecourses and extracted prototypical timeseries shapes. Collectively, our results show that continuous, multivariate predictors afford a high sensitivity to disentangle complex representations from MEG data. Our findings provide novel insights into how feature-rich, complex, and flexible object representations emerge and unfold in the human brain.

Disclosures: L. Teichmann: None. **M.N. Hebart:** None. **C.I. Baker:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.11

Topic: D.06. Vision

Support: Intramural Research Program (ZIAMH002909) of the National Institutes of Health—National Institute of Mental Health

Title: Real-world exploration of everyday objects alters their visual representation in the human brain

Authors: *S. G. WARDLE, B. RISPOLI, V. ROOPCHANSINGH, C. I. BAKER;
NIH, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, MD

Abstract: The neural basis of object recognition is typically studied using 2-D pictures of unfamiliar objects. In the human brain, regions of lateral occipital and ventral temporal cortex are known to respond more to pictures of intact objects than to pictures of scrambled objects. Although humans are skilled at making detailed inferences about an object from its photographic image, additional forms of object knowledge (e.g. multisensory perception, episodic memory, semantic associations) are obtained from real-world experience. It is unknown how real-world experience with a specific object affects the representation of its photographic image in the brain, particularly in areas of ventral temporal cortex implicated in object recognition. Accordingly, we conducted an fMRI study that involved real-world exploration of objects prior to scanning. Participants (N = 40) physically explored one of two sets of 12 ordinary household objects (e.g. clocks, mugs, bags) for 30 seconds each. Following real-world exploration, participants viewed photographs of both explored and unexplored objects in the MRI scanner (3.0T GE Discovery) in an event-related design using a whole-brain multiband, multiecho echo planar acquisition sequence. Each of the 24 individual objects were depicted twice in different contexts (indoor, outdoor) and at different viewing angles, for a total of 48 images. A post-scan memory test confirmed that participants correctly identified which objects they had explored from the photographs. Multivariate pattern analysis revealed that the identity of an object could be cross-decoded across image context in both lateral occipital and ventral temporal object-responsive cortex, regardless of whether an object has been physically explored or not (all $p < .0001$). However, real-world encounters with objects were associated with foci of increased activation in response to their 2-D image in the medial parietal lobe and posterior cingulate cortex. The effect of real-world experience was lateralized, with stronger activation in the left hemisphere in response to photographs of explored versus unexplored objects. Together, these results demonstrate that although the representation of objects in lateral occipital and ventral temporal object-responsive cortex is remarkably robust to both substantial image transformations and real-world experience, even brief encounters with objects alter their brain representation, particularly in medial parietal cortex. The richness of object representations beyond their photographic image has important implications for understanding object recognition in both the human brain and in computational models.

Disclosures: S.G. Wardle: None. **B. Rispoli:** None. **V. Roopchansingh:** None. **C.I. Baker:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.12

Topic: D.06. Vision

Title: Object space in human occipitotemporal cortex: category trumps shape

Authors: *E. YARGHOLI, H. OP DE BEECK;
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Abstract: Objects are represented in the occipitotemporal cortex (OTC). This large cortical area has a complex functional architecture, but it is unclear how different aspects of OTC's functional organization can be put together in one comprehensive model. Recently, Bao et al. (2020, *Nature*) proposed a characterization of object space in monkeys in which OTC is organized as a map with two main dimensions, stubby-spiky and animate-inanimate. However, the definition of their two dimensions might be confounded by selectivity for faces and bodies, because their face stimuli were mostly stubby and body stimuli were mostly spiky. To dissociate individual dimensions that might characterize object space, we prepared a novel set of stimuli including images of animates (face and body) and inanimates (natural and man-made) with a comparably wide range of aspect ratios in each of these four categories (52 stimulus images; 13 images for each category). We obtained fMRI (15 human subjects, 11 females, 19–46 years of age) and deep neural network (BigGAN) responses to stimuli and employed multivariate pattern analyses to examine the similarity of representational space between OTC, BigGAN, and models of animacy or aspect ratio. Results of representational dissimilarity analysis showed that object space in BigGAN and most category-selective regions in OTC was much better explained by the animacy rather than the aspect ratio model. In addition, no category-selective region showed sensitivity to the aspect ratio of stimuli from their selected category, an effect of aspect ratio was restricted to the right object-selective cortex. Brain decoding resulted in much higher accuracy for animate-inanimate classification across changes in aspect ratio than stubby-spiky classification across changes in animacy. We also observed transitions in the representational content along the anatomical posterior-to-anterior axis in OTC. Employing data-driven approaches, we clearly see clusters for face and body stimuli and separation of animate from inanimate stimuli in the representational space of OTC and BigGAN, but there is not any arrangement related to aspect ratio. In sum, aspect ratio remains an important shape dimension for representing object shape in general, but it does not function as a primary organizing principle for object representations in general, for which animacy and the face/body distinction are much more prominent candidates.

Disclosures: E. Yargholi: None. **H. Op de Beeck:** None.

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.13

Topic: D.06. Vision

Title: Deep neural networks and visuo-semantic models explain complementary components of human ventral-stream representational dynamics

Authors: K. M. JOZWIK[1], T. C. KIETZMANN[2], R. M. CICHY[3], N. KRIEGESKORTE[4], **M. MUR**[5];
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Abstract: The neural representation of objects dynamically unfolds over time across all stages of the human ventral visual stream. These representational dynamics are thought to reflect the cortical computations that support object recognition. Recent years have seen substantial progress in modeling such computations via deep neural networks (DNNs). However, the agreement between models and brain representational dynamics is far from perfect. This raises the question which representational features are left unaccounted for in the neural data.
We analyzed existing source-reconstructed MEG data acquired in human participants during object viewing (n = 15, mean age = 26 years, 10 females). Stimuli were 92 colored object images from a range of categories. MEG data consisted of representational dissimilarity matrix (RDM) movies for lower visual areas V1-3, intermediate visual areas V4t/LO, and higher visual areas IT/PHC. We used general linear modeling to explain variance in the RDM movies using DNNs (CORnet-Z and CORnet-R) and visuo-semantic models. Visuo-semantic models consisted of human-generated labels of object features and categories.
We found that DNN features explain variance over and above visuo-semantic features in lower and intermediate visual areas, especially during the early phase of the response (< 125 ms after stimulus onset; $p < .05$ corrected, signed-rank test; Fig. 1). In contrast, visuo-semantic features explain variance over and above DNN features in higher visual areas during a more prolonged time window (100 – 650 ms after stimulus onset; $p < .05$ corrected, signed-rank test; Fig. 1). Among the visuo-semantic features, object parts and basic categories drive the explanatory advantage over DNNs.
Our results suggest that current DNNs provide a promising image-computable model of ventral-stream computations but fail to fully capture the visuo-semantic features represented in higher human visual cortex. Future models of human ventral-stream representational dynamics may benefit from a stronger focus on visuo-semantic features during model development.
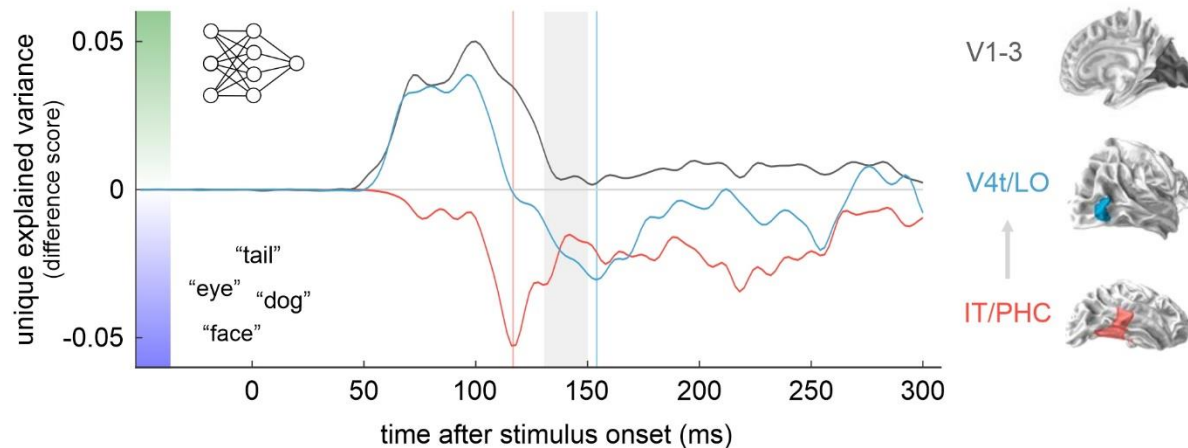
Figure 1. Deep neural networks (DNNs) and visuo-semantic models explain complementary components of human ventral-stream representational dynamics. During the early phase of the response (< 125 ms), DNNs outperform visuo-semantic models (positive deflection) in lower-level visual areas V1-V3 (grey line) and intermediate areas V4t/LO (blue line). This early time window is thought to be dominated by feedforward and local recurrent processing. In contrast, starting around 100 ms after stimulus onset, visuo-semantic models outperform DNNs in higher-level visual areas IT/PHC (red line, negative deflection). Stimuli were presented for 500 ms (Cichy et al. 2014). This result shows that DNNs fail to account for a significant component of variance in higher-level cortical dynamics, which is instead accounted for by visuo-semantic features, in particular object parts and basic categories. The peak of visuo-semantic model performance in higher-level areas (red vertical line) precedes the peak in intermediate areas (blue vertical line). This sequence of events aligns with the timing of feedback information flow from higher-level to intermediate areas (light grey rectangle and arrow; Kietzmann et al. 2019).

Nanosymposium

016. Functional Mapping and Properties of the Visual Cortex

Location: SDCC 7

Time: Saturday, November 12, 2022, 1:00 PM - 4:30 PM

Presentation Number: 016.14

Topic: D.06. Vision

Title: The time course of shape-invariant size processing and its relation to scene processing

Authors: *S. HAGEN, Y. ZHAO, M. V. PEELEN;
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Abstract: Real-world size is a behaviorally-relevant object property that can be decoded from neural activity, peaking around 190 ms after stimulus onset. Object size is also reflected in the organization of the visual cortex, where large objects activate scene-selective regions. It is currently unclear what drives these effects. One possibility is that they reflect visual feature

differences between large (typically rectilinear) and small (typically curvilinear) objects. Alternatively, or additionally, they may reflect functional associations of large versus small objects. For example, it has been proposed that large objects prime a sense of space. Here, using EEG, we aimed to dissociate the time course of object size from that of covarying shape properties and relate shape-invariant size responses to the temporal profile of scene processing. In Experiment 1, participants (N=33) viewed isolated objects that varied in real-world size (large, small), shape (rectilinear, curvilinear), and fixedness (fixed, transportable). We decoded each dimension (e.g., size) across the other dimensions (e.g., shape, fixedness). Across posterior electrodes, cross-decoding of shape (rectilinear vs curvilinear) peaked at around 190 ms after stimulus onset, in line with previous studies decoding object size without controlling for shape differences. By contrast, shape-invariant object size was significant only from 350 ms after stimulus onset, thus emerging relatively late during object processing. In Experiment 2, a new group of participants (N=32) viewed images of buildings (large and fixed objects), visually matched boxes (small and portable objects), scenes, and chairs. Conceptually replicating the first study, buildings and boxes could be distinguished from around 350 ms after stimulus onset. Interestingly, the building (vs box) classifier around 350 ms generalized to distinguish scenes from chairs earlier in time, at around 200 ms. Taken together, these results show that real-world object size can be decoded from EEG responses independently of visual feature differences (which are represented earlier). Furthermore, these large-object response patterns correspond to earlier scene-selective response patterns, in line with accounts that propose that large objects prime a sense of space.

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Nanosymposium

017. Fear and Aversive Learning and Memory: Mechanisms

Location: SDCC 23

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 017.01

Topic: G.01. Fear and Aversive Learning and Memory

Title: A cannabinoid sensitive amygdalo-accumbens circuit for learning proactive threat-coping responses.

Authors: *R. TRIANA-DEL RIO[1], A. YIN[1], C. FARB[1], M. HOU[1], K. HUBLEY[1], V. YARAGUDRI[3], E. BLESSING[4], E. KLANN[1], S. SONG[4], C. CONSTANTINOPLE[1], J. OLIVEIRA DA CRUZ[1], S. METHA[1], C. ALBERINI[1], P. SHRESTHA[5], E. ANDRADE[3], S. FLEM[2], R. SEARS[3], C. CAIN[3], J. LEDOUX[1];
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Abstract: Animals respond differently when recalling threatening memories, which is subject to conditioning. Conditioned proactive threat-coping behavior enhances the individual perception of control over a threatening environment, suppressing Pavlovian freezing or paralyzing responses. We used the rats' signaled active avoidance conditioning test (SigAA) to model proactive actions that suppress freezing. The cannabinoid receptor 1 (CB1r) and endocannabinoids (eCBs) modulate the circuit that reinforces the incentive properties of SigAA acquisition, representing a potential pharmacological target to facilitate this adaptive behavior. We hypothesized that SigAA responses are facilitated by cannabinoid-dependent plasticity in the nucleus accumbens (NAcc), stimulated by glutamatergic projections from the basal amygdala (BA). Initially, we found a positive correlation between the success in acquiring SigAA responses and eCB levels in the brain and serum of conditioned animals. Pharmacological approaches by systemic manipulation of CB1R, or locally by altering CB1R signaling in NAcc modulated SigAA responses. Using pharmacological, chemogenetic, and optogenetic inhibition, we showed that glutamatergic projections from BA to NAcc Core underlie the acquisition of SigAA responses and that those projections are cannabinoid-sensitive since activation of CB1r in NAcc Core while silencing the projections rescues the capability to acquire SigAA. To assess cellular and network mechanisms, chemogenetic and pharmacological approaches showed that parvalbumin-expressing cells in NAcc Core negatively modulate the acquisition of SigAA, and this cell population is sensitive to exogenous cannabinoids treatment.Additionally, a deep learning pipeline analysis of pose estimation allows for precisely assessing freezing responses during SigAA conditioning and circuit manipulation. For a neural mechanism substrate of our behavioral model, ongoing electrophysiological experiments in slices seek to explore CB1r-dependent synaptic plasticity in glutamatergic and GABAergic synapses in NAcc Core, stimulated by BA projections. Our initial and ongoing results show that in vivo endogenous and exogenous cannabinoids gate network dynamics of cell populations in NAcc that enable adaptive threat-coping. These data suggest the novel use of endocannabinoid-based drugs combined with proactive learning strategies might have a lasting effect on reducing anxiety disorder symptoms.

Disclosures: R. Triana-Del Rio: None. **A. Yin:** None. **C. Farb:** None. **M. Hou:** None. **K. Hubley:** None. **V. Yaragudri:** None. **E. Klann:** None. **C. Constantinople:** None. **J. Oliveira da Cruz:** None. **S. Metha:** None. **C. Alberini:** None. **P. Shrestha:** None. **E. Andrade:** None. **R. Sears:** None. **C. Cain:** None. **J. LeDoux:** None.

Nanosymposium

017. Fear and Aversive Learning and Memory: Mechanisms

Location: SDCC 23

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 017.03

Topic: G.01. Fear and Aversive Learning and Memory

Title: Affect updating of unwanted memories during human NREM sleep

Authors: *T. XIA[1], Z. YAO[1], X. GUO[2], J. LIU[3], D. CHEN[1], Q. LIU[2], X. HU[1];
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Abstract: Post-learning sleep contributes to memory consolidation. Yet, it remains contentious whether sleep affords opportunities to modify or update memory, particularly those unwanted memories that people prefer not to remember. For the first time, we examined whether we can update unwanted memories via pairing positive emotional words (vs. neutral words) with aversive memory cues during human non-rapid-eye-movement (NREM) sleep. We found that such pairing during NREM sleep reduced negative affect judgments toward paired aversive memory cues during the post-sleep tests. Cue-elicited EEG analyses showed that theta power differences between the positive words and the paired aversive memory cues predicted affect changes across sleep. Particularly, if the positive words elicited larger theta powers than the subsequent memory cues, participants judged the cues less negatively. The item-level analysis further showed that slow oscillation upstate, a state characterized by cortical excitability during NREM sleep, was conducive to effective affect updating: when the onset of the first positive words coincided with slow oscillation upstates, affect updating was more likely to happen. Our study revealed that the affect tones of unwanted memories can be updated via pairing with emotional stimuli during human deep sleep, with both theta power and slow oscillation upstates contributing to affect updating. These findings offer novel possibilities for modifying unwanted memories during sleep, without people being consciously confronted with such aversive memories.

Disclosures: T. Xia: None. **Z. Yao:** None. **X. Guo:** None. **J. Liu:** None. **D. Chen:** None. **Q. Liu:** None. **X. Hu:** None.

Nanosymposium

017. Fear and Aversive Learning and Memory: Mechanisms

Location: SDCC 23

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 017.04

Topic: G.01. Fear and Aversive Learning and Memory

Title: Fear-activated anterior basolateral amygdala neuronal ensembles: Memory or valence encoding?

Authors: *R. HAMMACK, L. HONAN, L. C. DAWS, G. M. TONEY;
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Abstract: Traumatic or fear-eliciting experiences can form long-lasting associative memories. Inappropriate recall of such memories can result in post-traumatic stress disorder (PTSD) and other negative valence disorders such as anxiety. Although there is a clear connection between fear memories and anxiety, circuit mechanisms coupling fear and anxiety behaviors are not well understood. Current theory holds that memory formation reflects structural and chemical changes among engram neurons and that recall of recently formed memories reflects reactivation of anatomically distributed ensembles of engram neurons. Over time, the engram circuit appears to reorganize such that memory storage migrates, partly or completely, to new neuronal ensembles. Despite engram migration, certain brain regions such as the basolateral amygdala (BLA) remain critical both for recent and remote memory recall. Here, we tested the hypothesis that ensemble neurons in the anterior BLA (aBLA), a brain region enriched with negative valence coding neurons, can drive both fear and anxiety-like behaviors. To investigate this, TRAP2 mice expressing a tamoxifen-inducible Cre-ER complex controlled by promoter/enhancer elements of the immediate early gene *c-fos* were exposed to contextual fear conditioning. Treatment with 4-hydroxytamoxifen immediately thereafter allowed for Cre-dependent expression of a fluorescent reporter (TdTomato) or an effector-reporter combination (ChR2+EYFP) in aBLA ensemble neurons - a process referred to as "TRAPing". First, we crossed TRAP2 and Ai14 mice for Cre-dependent tdTomato expression and used Fos immunostaining to quantify aBLA neurons re-activated during remote memory retrieval 3 weeks later. We found that remote memory recall re-activated more aBLA neurons in mice previously fear conditioned than control mice exposed to context alone (n=6/group). Next, we bilaterally injected AAV5-DIO-ChR2-EYFP into the aBLA and placed fiber optic cannulae directly above the BLA 2 weeks prior to context exposure or fear conditioning, thereby permitting remote photostimulation of TRAPed aBLA ensemble neurons. Photostimulation 3 weeks later did not alter freezing behavior during fear memory recall but increased freezing in a novel context and promoted anxiety-like behaviors in the open field and elevated pulse maze tests of anxiety (n=6-10/group, each paradigm tested in a separate cohort). Results indicate that aBLA ensemble neurons encode negative valence rather than fear specific memories per se. We posit that fear- and anxiety-related inputs converge on aBLA ensemble neurons to couple remote fear memories with anxiety-like behaviors.

Disclosures: R. Hammack: None. **L. Honan:** None. **L.C. Daws:** None. **G.M. Toney:** None.

Nanosymposium

017. Fear and Aversive Learning and Memory: Mechanisms

Location: SDCC 23

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 017.05

Topic: G.01. Fear and Aversive Learning and Memory

Title: A novel sex-specific role for degradation-independent lysine-63 polyubiquitination in the amygdala during fear memory formation

Authors: *K. FARRELL[1], M. MUSAUS[3], A. AUERBACH[3], S. V NAVABPOUR[2], W. K. RAY[3], R. F. HELM[3], T. J. JAROME[2];
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Abstract: The ubiquitin-proteasome system (UPS) controls the majority of protein degradation in cells through the protein ubiquitin. Multiple ubiquitin molecules can bind a target substrate and form polyubiquitin chains at 8 different linkage sites that have unique biological functions. The most abundant polyubiquitin modifications are linked at lysine-48 (K48) or lysine-63 (K63), the former of which is preferentially targeted for degradation by the proteasome, while the latter is thought to be independent of the degradation process. Over the last decade, numerous studies have demonstrated the necessity of UPS-mediated protein degradation for memory formation in the brain. However, the role of degradation-independent ubiquitin signaling, such as K63 polyubiquitination, during memory formation remains unknown. To investigate this, we used a K63-specific Tandem Ubiquitin Binding Entity (TUBE) and liquid chromatography mass spectrometry (LC/MS) to identify proteins targeted by K63 polyubiquitination in the amygdala of male and female rats following contextual fear conditioning (n = 5 per group per sex). Interestingly, we identified 13 proteins that gained and 9 proteins that lost K63 in females following fear conditioning, but only 1 protein was identified in males that lost K63, suggesting that changes in K63 polyubiquitination may be specific to females following fear conditioning. Western blot analysis revealed no change in levels of the top proteins identified by LC/MS (n = 5 per group, unpaired t-test, p < 0.05), which included a mitochondrial protein and proteasome subunit, indicating these proteins were not undergoing degradation. To manipulate K63 polyubiquitination in the amygdala, we used the CRISPR-dCas13b RNA-editing system to make site-specific modifications on the major ubiquitin coding gene, *Ubc*, at the nucleotide sequence coding for K63. Consistent with our proteomic analysis, we observed that CRISPR-dCas13-mediated reductions in K63 polyubiquitination in the amygdala impaired fear memory in females, but not males (n = 5-6 per group, unpaired t-test, p < 0.05). Furthermore, loss of K63 polyubiquitination in females reduced, but did not eliminate, learning-related increases in ATP levels and proteasome activity in the amygdala. This suggests that K63 polyubiquitination is involved in, but does not independently regulate, ATP synthesis and proteasome activity in the female amygdala during fear memory formation. Together, these findings provide the first evidence of a novel, sex-specific role for K63 polyubiquitination in the co-regulation of ATP synthesis and proteasome activity in the amygdala during fear memory formation.

Disclosures: K. Farrell: None. **M. Musaus:** None. **A. Auerbach:** None. **S. V Navabpour:** None. **W.K. Ray:** None. **R.F. Helm:** None. **T.J. Jarome:** None.

Nanosymposium

017. Fear and Aversive Learning and Memory: Mechanisms

Location: SDCC 23

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 017.06

Topic: G.01. Fear and Aversive Learning and Memory

Title: Neuron-specific H2B monoubiquitination is a master regulator of the transcriptome in the hippocampus during memory formation

Authors: *S. V NAVABPOUR[1], **K. FARRELL**[2], **A. AUERBACH**[3], **T. J. JAROME**[1];
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Abstract: It is well established that post-transcriptional modification of histone proteins, such as the transcription activator trimethylation of histone H3 lysine 4 (H3K4me3), are critically involved in the formation of long-term memory. Recently, we showed that monoubiquitination of histone H2B at lysine 120 (H2BubiK120) regulates H3K4me3 in the hippocampus during memory formation. However, the transcriptome controlled by H2BubiK120 and the cell-type specificity of this during memory consolidation remain unknown. Here, we report a vital role for histone H2B ubiquitination in active and repressive gene transcription during long-term memory formation. Using siRNA, the histone H2B ubiquitin ligase, *Rnf20*, was knocked down in the dorsal CA1 (dCa1) area of adult male Sprague Dawley rats' hippocampus (n=5/group) prior to training for contextual fear memory and their dCA1 was microdissected one hour later for whole genome RNA-sequencing. We found that training induced significant transcriptional changes in 31 genes (14 upregulated and 17 downregulated) after one hour. Remarkably, the loss of *Rnf20* completely abolished these changes in 30 of the 31 genes, suggesting a critical role for the H2BubiK120 epigenetic mark in active and repressive gene transcription during memory consolidation. We next studied the cell-type-specific role of H2BubiK120 in the hippocampus of adult male Sprague Dawley rats (n=7/group) during memory formation by using a modified version of CRISPR-dCas9 (SYN-driven) fused with a transcription suppressor (KRAB-MECP2) to manipulate *Rnf20* transcription only in neurons. Four weeks later, the rats were trained and tested for contextual fear memory. We found that the loss of *Rnf20* in neurons of the dCA1 impaired long-term memory consolidation. These findings suggest that neuronal H2B ubiquitination is essential for long-term memory formation. Taken together, our data suggest that monoubiquitination of histone H2B in neurons may be a master regulator of the transcriptome necessary for the memory consolidation process.

Disclosures: S. V Navabpour: None. **K. Farrell:** None. **A. Auerbach:** None. **T.J. Jarome:** None.

Nanosymposium

017. Fear and Aversive Learning and Memory: Mechanisms

Location: SDCC 23

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 017.07

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH01MH122387
             NIHF31MH124360

Title: Competition between contextual representations of threat and safety determines the success of extinction recall in humans

Authors: *A. HENNINGS[1,2], S. BIBB[3], J. A. LEWIS-PEACOCK[5], J. E. DUNSMOOR[4];
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Abstract: Following traumatic events, new memories of safety do not readily generalize to novel contexts, representing a barrier to the treatment of PTSD. Here, we sought to better understand the neural mechanisms by which the human brain promotes contextual generalization of associative memories. In a two-day fMRI experiment (N=30), we tagged and tracked the encoding and retrieval of neural representations corresponding to the episodic context in which associative memories were formed. On day 1, participants underwent aversive Pavlovian conditioning with auditory conditioned stimuli (CS). Two distinct tones were paired with a mild electric shock (CS+s), and one tone served as a safe control stimulus (CS-). Immediately following conditioning, participants underwent extinction, during which no shocks were delivered. Critically, only one of the CS+ tones was extinguished. During conditioning and extinction, participants were exposed to two distinct categories of visual cues (animals and tools), that served to differentiate and tag these distinct phases of associative learning. We have previously used this technique (Hennings 2020) to show that successful extinction recall is associated with reinstatement of the extinction mental context. Here, we predicted that we would be able to build on these results by showing that the competition between the conditioning and extinction mental contexts would meaningfully relate to both behavior and neural processing in canonical brain regions. The following day, participants underwent a fear renewal test, during which we used a multivariate pattern classifier to decode reinstatement of both the conditioning and extinction mental context tags. As predicted, the competition between the conditioning and extinction contextual representations was significantly correlated with explicit shock expectancy behavior, such that more decoded extinction context predicted safe responding. In addition, we found that this neural marker of contextual competition was significantly related to neural activity in canonical threat processing regions, including the ventromedial prefrontal cortex (vmPFC), amygdala, and anterior insula. These brain and behavior correlations with contextual competition were only observed for the ambiguous (extinguished) CS+, and were not observed for the unextinguished CS+. These results shed new light on how episodic contextual retrieval processes influence the outcome of associative memory competition, and will inform future clinical translation efforts seeking to enhance the contextual generalization of extinction learning.

Disclosures: A. Hennings: None. **S. Bibb:** None. **J.A. Lewis-Peacock:** None. **J.E. Dunsmoor:** None.

Nanosymposium

018. Neural Correlates of Decision Making in Mammalian Cortex

Location: SDCC 1

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 018.01

Topic: H.03. Decision Making

Title: Neural representation of latent cause in credit assignment

Authors: *Y. L. ZHANG[1], P. P. WITKOWSKI[1], S. A. PARK[2], E. D. BOORMAN[2];
[1]Univ. of California Davis, Davis, CA; [2]Ctr. for Mind and Brain, Univ. of California, Davis, Davis, CA

Abstract: Humans have a remarkable capacity to use an internal model of the environment to make inferences about unseen outcomes. How the brain assigns credit for outcomes to latent causes is poorly understood.We scanned hungry participants (N=28) while they tracked 2 probabilistic systems of stimulus-reward associations for 2 desserts, with each system comprising 2 stimuli of different visual categories but sharing the same reward probabilities. We hypothesize that the underlying latent cause is reinstated in the lateral orbitofrontal cortex (OFC). Behavioral results from a Bayesian learning model show that participants efficiently learned to track reward probabilities both from choice and the inferred stimulus in the same system (choices 1back t(27)>3.5 p<0.001). An univariate analysis of the DKL as a measure of belief confirmation of stimulus-outcome probabilities at feedback shows significant effects in the OFC, ventromedial prefrontal cortex and hippocampus (t(27)>4.3, p<0.0001 uncorrected). We used multivariate pattern analysis to test for a reinstatement of the causal choice at feedback (cross-validated across runs). Left lateral OFC and the hippocampus show a significant decoding accuracy relative to label-shuffled permutations, consistent with a role of OFC in choice reactivation during credit assignment (t(27)>3.745, p<0.001, uncorrected). To test if this reinstatement constitutes the identity representation of the stimulus, we trained a classifier on the stimuli in separate forced choice trials and decoded choice identity at feedback in free choice trials. Significant decoding accuracy was found in the bilateral OFC and the amygdala (t(27)>4.6, p<0.0001). We then tested for the reactivation of the inferred stimulus by training a classifier at forced choice for the paired but unpresented stimulus, and decoding at feedback in free choices. Left lateral OFC shows significant decoding accuracy (t(27)>3.7, p<0.0005) for the inferred stimulus that is informative for future decisions. These findings support a model whereby choices and inferred causes are reinstated at feedback time, coincident with prediction errors, to drive plasticity between co-active neural ensembles for the outcome and cause in the service of learning.

Disclosures: Y.L. Zhang: None. **P.P. Witkowski:** None. **S.A. Park:** None. **E.D. Boorman:** None.

Nanosymposium

018. Neural Correlates of Decision Making in Mammalian Cortex

Location: SDCC 1

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 018.02

Topic: H.03. Decision Making

Title: Value Compression and the Amplification of Choice Biases

Authors: *W. SHI[1], C. PADOA-SCHIOPPA[2];
[1]Yale Univ., Yale Univ., New Haven, CT; [2]Washington Univ. in St Louis, Washington Univ. in St Louis, Saint Louis, MO

Abstract: Behavioral variability and choice biases are ubiquitous in economic choices, and understanding the neural origins of these phenomena is a major goal for decision neuroscience. In previous work, we let monkeys choose between two juices offered in variable amounts. We interleaved two types of trials, with offers presented simultaneously (Task 1) or sequentially (Task 2). We found that choices were systematically less accurate in Task 2 compared to Task 1. Furthermore, examination of neural activity in the orbitofrontal cortex (OFC) revealed that the lower choice accuracy reflected reduced dynamic range in the activity of offer value cells (i.e., weaker offer value signals) (Shi et al., 2022). In the current study, we further explored the relationship between the activity of offer value cells, choice variability and biases in choices under sequential offers. First, assuming that the read-out of offer value cells does not depend on the choice task (sequential or simultaneous offers), we show that reduced activity ranges in Task 2 effectively induce a compression of the value space. We quantified the compression factor by calculating the average ratio between the activity ranges of N = 109 offer value cells in the two tasks. Second, using the choice pattern measured in Task 1 and the compression factor derived from neuronal measures, we simulated the choice pattern expected in Task 2 for each session. Simulated choices in Task 2 were significantly more variable than choices in Task 1, but not as variable as those measured experimentally in Task 2. This result confirmed that the increase of choice variability originated partly at the valuation stage (Shi et al., 2022). At the same time, this result suggested that additional factors contribute to the choice variability under sequential offers. Third, we conducted a series of analyses on choice hysteresis - a bias favoring, in any given trial, the same juice chosen in the previous trial (Padoa-Schioppa, 2013). Empirically, choice hysteresis was enhanced in Task 2 compared to Task 1. This result is somewhat counterintuitive because the time intervening between the end of the previous trial and the decision in the current trial was longer for Task 2 than for Task 1. However, simulation analyses demonstrated that value compression can explain enhanced choice hysteresis in Task 2. Taken together, our results indicate that changes in the activity of offer value cells can lead to changes in choice variability and choice biases. Specifically, value compression taking place under sequential offers induces higher choice variability and amplifies choice biases.

Nanosymposium

018. Neural Correlates of Decision Making in Mammalian Cortex

Location: SDCC 1

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 018.03

Topic: H.03. Decision Making

Title: Sex differences in flexible decision making and alcohol self-administration in mice

Authors: *S. L. THOMPSON[1], S. M. GROMAN[1,4], B. M. RAINFORD[1], S. PAK[1], J. R. TAYLOR[1,2,3];
[1]Psychiatry, [2]Neurosci., [3]Psychology, Yale Univ., New Haven, CT; [4]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Flexible decision-making is altered in individuals with psychiatric disorders, including alcohol use disorder. Excessive alcohol consumption has been linked to impaired flexible decision-making performance in reversal learning tasks. Most studies to date have exclusively examined decision making after alcohol exposure. How pre-existing differences in flexible decision making influence subsequent alcohol use is not known. Moreover, many rodent studies use deterministic schedules of reinforcement, forced alcohol exposure, or almost exclusively male animals. We have optimized a probabilistic reversal learning (PRL) task to assess dynamic decision-making strategies in mice. Here, we investigated the role of dynamic decision-making processes in alcohol self-administration in mice. Seventy-two adult male and female C57BL/6J mice were trained to acquire and reverse a three-choice discrimination problem to earn a probabilistically delivered 10% sweetened condensed milk reinforcer in the PRL task. Mice then were trained to self-administer 10% ethanol in 0.1% saccharin or 0.1% saccharin under a variable interval schedule for 24 days followed by contingency degradation to measure habit expression. Locomotor activity was then assessed and decision making reassessed in the PRL task. Finally, motivation for the sweetened condensed milk reinforcer was assessed using a progressive ratio schedule of reinforcement. We found that female mice performed better than male mice on aspects of the PRL task including better acquisition of, and adaptation to changes in, the reinforcement contingencies under select reinforcement schedules. In addition, female mice were more motivated for the sweetened condensed milk reinforcer under a progressive ratio schedule. Moreover, female mice self-administered more alcohol and reached higher blood ethanol levels compared to male mice. Female mice escalated intake of ethanol, but not saccharin, whereas male mice escalated their intake regardless of reinforcer. In male mice, alcohol caused lasting impairments in PRL performance, which were not explained by a loss of motivation or altered locomotor activity. The impact of alcohol in female mice was less clear, in part because behavior was disrupted in both ethanol- and saccharin-exposed groups. Overall, we

identified sex differences in flexible decision making and alcohol self-administration, in addition to within-subject impairments in flexible decision-making due to alcohol in male mice. These findings suggest sex differences in key reinforcement learning strategies that are altered by alcohol consumption and highlight the importance of inclusion of females.

Nanosymposium

018. Neural Correlates of Decision Making in Mammalian Cortex

Location: SDCC 1

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 018.04

Topic: H.03. Decision Making

Title: Primate-like perceptual decision making through deep recurrent reinforcement learning

Authors: *N. J. WISPINSKI[1], S. A. STONE[1], A. SINGHAL[1], P. M. PILARSKI[2], C. S. CHAPMAN[3];
[1]Psychology, [2]Med., [3]Fac. of Kinesiology, Sport, and Recreation, Univ. of Alberta, Edmonton, AB, Canada

Abstract: Many studies of perceptual decision making in animals use a random-dot-motion discrimination task, where an animal must guess in which of two directions dots are moving on a screen. In this task, difficulty is varied by changing the proportion of randomly moving dots. When making saccadic responses during this task, recordings from primate medial temporal (MT) cortex suggest this area encodes the direction and magnitude of momentary motion on the screen. Downstream, the lateral intraparietal area (LIP) is thought to accumulate this momentary motion evidence over time to a decision threshold, which determines what direction the animal responds with and when. These dynamics and resultant behavior are consistent with a general decision mechanism—evidence accumulation—which is thought to underlie many perceptual- and value-based decisions in humans, non-human primates, and other animals.
We trained an end-to-end deep recurrent neural network using reinforcement learning on a random-dot-motion discrimination task. Behavior and dynamics emerged in these agents that were consistent with an evidence accumulation mechanism, and similar to those observed in mammals. Trained artificial agents responded quicker and more accurately on easy trials, and varying discount rate between agents changed their balance between response speed and accuracy. The activation of the network's convolutional layer emerged to encode the direction and magnitude of motion, much like in MT. Further, the network's recurrent layer activations

emerged to accumulate momentary motion over time, similar to primate area LIP. Targeted stimulation of individual network units influenced speed and accuracy in patterns predicted by evidence accumulation models and primate microstimulation experiments. Further, using a decoder on the internal dynamics of the network showed that agents changed their mind on some trials before committing to a decision.

Finally, we extended the task for the artificial agent from a simulated saccadic response to a simulated reaching response. We compared this agent behavior to collected data from 14 humans performing a similar reaching task. In this continuous control task, both humans and artificial agents displayed more curved movement trajectories on hard trials, and changed their mind while moving to correct for initial errors.

These results support the idea that a general decision making mechanism found in biological agents emerges in artificial agents trained to maximize reward in the face of noisy, temporally-evolving information, suggesting the importance of neuroscientific tasks in artificial intelligence research.

Disclosures: N.J. Wispinski: None. **S.A. Stone:** None. **A. Singhal:** None. **P.M. Pilarski:** None. **C.S. Chapman:** None.

Nanosymposium

018. Neural Correlates of Decision Making in Mammalian Cortex

Location: SDCC 1

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 018.05

Topic: H.09. Spatial Navigation

Title: The neurobiology of flexible behaviour in virtually navigating monkeys

Authors: *R. A. GULLI, R. HASHIM, S. FUSI, D. SALZMAN;
Zuckerman Inst., Columbia Univ., New York City, NY

Abstract: Humans possess a remarkable ability to integrate vast amounts of information from the environment, internal states, context, and prior experiences to make adaptive choices in novel situations. Despite this, most studies of decision-making in animal models take a reductionist approach where animals repeatedly make choices in familiar, not novel, situations. To elucidate the neural basis of decision-making in new situations, behavioral paradigms must be designed that allow for large numbers of new situations to be created, while still retaining tight experimental control that allows for precise measurement of behavior in combination with electrophysiological recordings and neural perturbations (Jazayeri and Afraz, 2017; Krakauer et al. 2017).

Here we report the development of a new virtual reality experimental platform and behavioral task that *requires* subjects to use a spatial rule to make correct decisions, even when they are at locations they have never experienced before. Trained monkeys perform this task using a joystick to navigate across an open-field virtual environment, frequently making choices to collect colored objects. The rewarded color varied as a function of the subject's location in the

virtual environment. When monkeys first experience a virtual environment, choices are made only at a subset of locations in the environment. Subsequently, monkeys must make choices in completely novel locations within the environment. Correct choices at these novel locations cannot be based on memorized scenes or singular landmarks; rather, they rely on forming an "abstract" representation of space that lets them generalize from previous experiences in the virtual environment to infer their current location. Behavioral data indicate that monkeys are able such construct abstract cognitive maps of virtual environments, and use these cognitive maps to make decisions in new, never-before-encountered situations.

This new behavioral paradigm offers the opportunity to study decision-making in novel situations using electrophysiological approaches in behaving monkeys. We hypothesize that the ability to form correct decisions in novel situations will rest critically on the ability to form an abstract cognitive map of space during learning of new virtual environments (Ho et al. 2019). This type of map is hypothesized to be revealed by examining the geometry of neural representations, the pattern of firing in large neural ensembles across experimental conditions (Bernardi et al. 2020, Cell).

Disclosures: **R.A. Gulli:** None. **R. Hashim:** None. **S. Fusi:** None. **D. Salzman:** None.

Nanosymposium

018. Neural Correlates of Decision Making in Mammalian Cortex

Location: SDCC 1

Time: Saturday, November 12, 2022, 1:00 PM - 2:30 PM

Presentation Number: 018.07

Topic: D.06. Vision

Title: Role of orbitofrontal cortex in categorization of neutral valent visual objects: Evidence from effective EEG connectivity study

Authors: A. YN, S. SONI, M. PRIYA, S. KUMAR, P. TAYADE, S. KAUR, R. SHARMA, *S. MUTHUKRISHNAN;
Dept. of Physiol., All India Inst. of Med. Sci., New Delhi, India

Abstract: Most of our impressions of the world are based on sight and it is fundamentally object-centered. We make sense of the visual world by organizing objects into categories. Categorization is the basis of thinking and reasoning. Dynamic changes in the strength and directionality of information transfer between the brain regions involved in visual object categorization occur in the range of milliseconds which can be resolved better using high-density EEG based effective brain connectivity. The current study aimed to elucidate the causal interactions underlying the categorization of visual objects using effective EEG brain connectivity. Visual object recognition task (VORT) was designed using 100 standard images with neutral valence. 128 channel EEG data were acquired from healthy participants (n = 55) during baseline (eyes open) and 'categorization' of visual objects. Standardized low-resolution brain electromagnetic tomography (sLORETA), phase slope index (PSI), and BrainNet Viewer were used for the source analysis, effective brain connectivity analysis, and plotting of the

effective connectivity results respectively. Significant ($p < 0.05$) causal interactions were observed between orbitofrontal cortex (Brodmann area 11) and other brain regions (Brodmann area 5, 4, 27, 40) during the "visual object categorization" when compared to the baseline condition i.e. "eyes open". The current study results indicate that the orbitofrontal cortex (Brodmann area 11) implicated in abstract reasoning, long term memory and decision making could play a vital role in the categorization of visual objects. Data analysis using complex neural network analysis and neural network modelling could further refine our current understanding of visual object recognition in health and disease states.

Disclosures: A. Yn: None. **S. Soni:** None. **M. Priya:** None. **S. Kumar:** None. **P. Tayade:** None. **S. Kaur:** None. **R. Sharma:** None. **S. Muthukrishnan:** None.

Nanosymposium

019. Hippocampal Circuitry and Navigation

Location: SDCC 5

Time: Saturday, November 12, 2022, 1:00 PM - 3:15 PM

Presentation Number: 019.01

Topic: H.08. Learning and Memory

Title: Hippocampus learns metric spaces

Authors: *Z. LIAO, A. LOSONCZY;
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Abstract: The ability to map space, and use those maps to guide behavior, is central to our current understanding of the function of the hippocampus. Recent work has hypothesized a more general role for the hippocampus in mapping relationships independent of space. In this work, we directly pose the question of whether the hippocampus can learn representations of abstract metric spaces that cannot be embedded into 2D space. Using two-photon calcium imaging combined with simultaneous local field potential recording in a novel virtual reality learning task, we provide evidence that the hippocampus is able to learn metric spaces as cognitive maps. Conversely, we show that hippocampal representations are also constrained by rules governing metric spaces, notably the triangle inequality. Furthermore, we find that, once acquired, these representations are read out by hippocampal replay. Finally, we discuss a mathematical theory of how metric space learning can be implemented using biologically plausible primitives such as

STDP, and can be used to support abstraction as well as to compute relational queries about the "world" downstream.

Nanosymposium

019. Hippocampal Circuitry and Navigation

Location: SDCC 5

Time: Saturday, November 12, 2022, 1:00 PM – 3:15 PM

Presentation Number: 019.02

Topic: H.08. Learning and Memory

Title: Low rate hippocampal activity during delay periods encodes the recent past.

Authors: *M. ATHANASIADIS[1], S. MASSERINI[3], L. YUAN[4], D. FETTERHOFF[5,6], J. K. LEUTGEB[4,7], S. LEUTGEB[4,7], C. LEIBOLD[1,2];
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Abstract: Remembering what just happened is an essential component for the formation of long-term memories but also for establishing and maintaining working memory. The neural basis for working memory is debated and particularly the activity of hippocampal time cells, with the ability to switch on and off at specific timestamps during a waiting period is a controversial candidate (Pastalkova et al. 2008, Sabariego et al. 2019). Animals with lesions of the medial entorhinal cortex (mEC) show a behavioral deficit in working memory but time cell activity did not seem to be impaired (Sabariego et al., 2019). Here we further explored this delay activity in the hippocampus (CA1 & CA3) of control rats and mEC-lesioned rats trained on a spatial alternation task as well as a second data set from CA1 of Mongolian gerbils that were trained to run on two mazes in virtual reality (distinguished by left and rightward turns)(Fetterhoff et al., 2021) and had a 20s pause between trials during which they received a reward. We compared two versions of the task, one with familiar landmarks, one with recently changed landmarks. We used a linear neural network to examine whether the turn/choice direction in the preceding trials could be decoded using population vectors constructed from time bins of varying durations from 60ms to 1s. For bin sizes of 100ms, our classifier was able to predict the turn direction of the previous trial except for CA1 recordings from mEC-lesioned rats and for gerbils with unfamiliar landmarks establishing that CA1 delay activity correlates with both working memory

performance and familiarity of the environment. To directly identify the neuronal basis of the prediction scores of the classifier we visualized its decision boundary (DB) by applying adversarial attack techniques from machine learning (Goodfellow et al., 2015). From this set of boundary positions we then constructed most informative directions (MIDs) as clusters of orthogonal vectors to the boundary. For low signal strength and artificial surrogate data, we show that the method outperforms estimating the weight vector by bootstrapping. Our approach reveals that only few neurons (about 20%) and few time bins (~2%) contribute to the classification task with slightly but significantly increased firing rate (~2.5Hz) compared to uninformative time bins. Thus, the recent past is encoded by few temporally dispersed spikes in the CA1, independently of whether the animal is in a working memory task. Reduction of informative time bins in rats with mEC lesions suggest that this low-rate activity may underlie working memory.

Disclosures: M. Athanasiadis: None. **S. Masserini:** None. **L. Yuan:** None. **D. Fetterhoff:** None. **J.K. Leutgeb:** None. **S. Leutgeb:** None. **C. Leibold:** None.

Nanosymposium

019. Hippocampal Circuitry and Navigation

Location: SDCC 5

Time: Saturday, November 12, 2022, 1:00 PM – 3:15 PM

Presentation Number: 019.03

Topic: H.08. Learning and Memory

Title: The role of inhibition in shaping hippocampal memory-encoding sequences

Authors: *J. TAXIDIS[1,2], B. MADRUGA[1], M. Z. LIN[3], P. GOLSHANI[4];
[1]UCLA, Los Angeles, CA; [2]SickKids Res. Inst., Toronto, ON, Canada; [3]Stanford Univ., Stanford, CA; [4]Univ. of California Los Angeles, Los Angeles, CA

Abstract: Hippocampal spiking sequences encode external sensory cues and internally link them by tiling the gaps in time between them, tracking the passage of our experiences. We previously described pyramidal sequences composed of 'odor-cells' encoding specific olfactory cues, followed by 'time-cells' encoding time-points in the ensuing delay period after a cue (Taxidis et al., Neuron, 2020). But what is the role of inhibition by parvalbumin- (PV) or somatostatin-expressing (SST) interneurons in shaping such sensory and temporal representations? We employed *in vivo* high-frequency voltage imaging, using the ASAP3 voltage indicator on the CA1 of PV-Cre and SST-Cre mice. We recorded action potentials and subthreshold membrane dynamics from PV and SST cells while mice performed an olfactory delayed non-match-to-sample task (DNMS). Mice were imaged during untrained, passive exposure to DNMS trials as well as after training, during DNMS performance. We followed the same cells across multiple days, and some cells were imaged before and after DNMS training. We found that ~ 50% of PV and SST cells significantly increased their firing during the odor-cue presentation ('odor-cells').

Unlike pyramidal cells, most of these neurons responded to both odors similarly, yielding weak odor-selectivity. Only a small minority of cells had significant time-fields during the delay period. As in pyramidal cells, inhibitory odor-fields remained relatively stable across days with cells even retaining a stable odor-field before and after training to the DNMS task. Moreover, the number of odor-fields did not change across days or pre-vs-post-training and their activity did not get more tuned to a particular odor. Surprisingly, at the onset of the cue, many PV and SST cells exhibited a distinct delta-frequency hyperpolarization before their ensuing firing increase. This synchronous inhibition resulted in resetting their intracellular theta phase and aligning their subsequent spike timing, yielding synchronous theta-cycle inhibition across cells and across trials. Collectively, our findings reveal a distinct fine-timed, but not cue-specific, inhibition in CA1 during cue presentation but not during the delay period. This inhibition is independent of any association between the cue and a behavioral context, with a fixed number of cells driving it. We propose that its role is to dampen background activity and increase the signal-to-noise-ratio of the few pyramidal cells that form a task-encoding spiking sequence.

Disclosures: J. Taxidis: None. **B. Madruga:** None. **M.Z. Lin:** None. **P. Golshani:** None.

Nanosymposium

019. Hippocampal Circuitry and Navigation

Location: SDCC 5

Time: Saturday, November 12, 2022, 1:00 PM – 3:15 PM

Presentation Number: 019.04

Topic: H.08. Learning and Memory

Title: Experience-induced changes in gamma coherence of sharp-wave ripples, replay content, and CA1 network dynamics

Authors: *J. M. SMITH[1], B. E. PFEIFFER[2];
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Abstract: Hippocampal sharp-wave ripples (SWRs) and associated replay events are thought to facilitate different memory processes across behavioral states. SWR-encoded replay during awake behavior has been associated with retrieval and navigational planning, while sleep-based replay has been heavily implicated in memory consolidation. It is unclear how the brain uses the same events to facilitate different memory processes, and whether and how SWR-encoded replay changes to support these processes. In this study, we compared features of SWRs, replay content, and associated network activity within the CA1 region of the hippocampus (HIPP) before, during, and after a learning experience to identify differences across memory processes and behavioral states. To this end, we used *in vivo* electrophysiological recordings in 4 adult male Long-Evans rats across 2 behavioral sessions each (n=8). In each session, local field potential

(LFP) and single unit spike data from up to 213 neurons were recorded during a ~30 min performance on a Goal-Directed Spatial Navigation (GDSN) task, surrounded by ~1hr of pre- and post-experience rest in a remote location. SWR events were detected in each of the 3 behavioral epochs and spatial replay content was estimated using a Bayesian decoding algorithm. We found that many features of the SWR events themselves differed across behavioral epochs, as well as across wakefulness and putative SWS periods within rest epochs. Furthermore, gamma rhythms appeared to play unique roles in CA1 replay across experience. The involvement of slow (25-55 Hz), medium (65-95 Hz) and fast (100-140 Hz) gamma oscillations in SWRs differed across experience, and interestingly, phase locking of replay content to slow gamma was not present before experience, but emerged during behavior and persisted into post-experience rest. Together, these data suggest that various aspects of SWRs, replay content, and underlying CA1 network activity are influenced by experience and behavioral state. Furthermore, since the presence of slow and fast gamma in CA1 has been previously shown to reflect input from CA3 or the entorhinal cortex respectively, the differential involvement of gamma frequencies in SWRs across epochs suggests that the relevant circuitry and inputs to CA1 during replay may shift across experience.

Disclosures: J.M. Smith: None. **B.E. Pfeiffer:** None.

Nanosymposium

019. Hippocampal Circuitry and Navigation

Location: SDCC 5

Time: Saturday, November 12, 2022, 1:00 PM – 3:15 PM

Presentation Number: 019.05

Topic: H.08. Learning and Memory

Support: Samsung Science and Technology Foundation SSTF-BA1801-10

Title: Single cell and ensemble activity patterns correlated with retrieval of a contextual memory in the dorsal CA1 of mouse hippocampus

Authors: *H.-S. LEE, J.-H. HAN;
Korea Advanced Inst. Of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Retrieval of memory is associated with specific neuronal activity and proper behavior. In mice, the hippocampus dorsal CA1 (dCA1) activity is necessary for retrieving contextual memories, and neurons whose activation is essential for the retrieval have been identified. Despite recent advances in recording neural activity during behaviors, however, little is known about how the activity patterns of dCA1 neurons are correlated with memory performance. To address this question, using a recently developed calcium indicator (jGCaMP7f) and advanced calcium signal extraction method (CNMF_E) enabling improved detection of individual spikes, we optically imaged neuronal activity in the mouse dCA1. We found that there was an overall increase in neuronal response to the context after contextual fear conditioning (CFC) compared to simple re-exposure. Not all but only a portion of neurons increased their response by CFC, and

such increase correlated with memory strength during recall and context-specific memory retrieval. At the cell population level, correlated cell activity patterns correlated with context-specific retrieval of memory. These results suggest that increase in response of individual neurons and correlated cell activity patterns in dCA1 are critically involved in representing a contextual memory.

Disclosures: H. Lee: None. **J. Han:** None.

Nanosymposium

019. Hippocampal Circuitry and Navigation

Location: SDCC 5

Time: Saturday, November 12, 2022, 1:00 PM – 3:15 PM

Presentation Number: 019.06

Topic: H.08. Learning and Memory

Title: Cell-specific synaptic wiring within the hippocampal CA3 network

Authors: *J. F. WATSON, V. M. VARGAS-BARROSO, P. JONAS;
IST Austria, Klosterneuburg, Austria

Abstract: The hippocampus is crucial for learning and memory, and the interconnected pyramidal neurons of its CA3 area appear uniquely configured for memory storage. However, while the general routes of information flow through the hippocampus have long been understood, little is known about individual cell wiring, which will dictate the rules of information processing. CA3 pyramidal neurons are not uniform – unique subtypes of excitatory pyramidal neurons can be identified by their functional and anatomical properties, with differing levels of input from the dentate gyrus (Hunt et al., 2018, Nat. Neurosci. 21, 985-995; Balleza-Tapia et al., 2022, Progress in Neurobiol. 210, 102213). How these different types of neurons are synaptically connected to each other remains to be determined. To address these questions, we performed simultaneous multi-cellular patch-clamp recordings from up to eight neurons in acute mouse hippocampal slices. To identify neuronal subtypes, electrophysiological recordings were followed by post-hoc morphological analysis. In total, we tested connectivity and synaptic properties in 2892 pairs of CA3 pyramidal neurons, identifying 66 monosynaptically connected pairs. As previously observed in rat (Guzman et al., 2016, Science 353, 1117-1123), synaptic conductances were small (peak conductance , 389 ± 44 pS), but CA3 pyramidal cells showed a non-random circuit architecture containing sub-networks of highly interconnected cells, potentially increasing the ability of the circuit for pattern completion computations. Furthermore, different subtypes followed specific wiring rules, adding a further layer of complexity to the CA3 network. Finally, connectivity between pyramidal neurons and fast-spiking interneurons

was more abundant than principal neuron interconnectivity (19 % connectivity, 67/360 confirmed synaptic connections), with unique synaptic properties that will differentially process excitatory activity in a frequency- and location-dependent manner. Our results shed new light on the microcircuit arrangement of the CA3 network. Non-random synaptic connectivity motifs and cellular heterogeneity will increase the processing capacity of the network, and distinctly interconnected pyramidal subtypes could have specific roles in encoding life experiences within neuronal ensembles.

Disclosures: J.F. Watson: None. **V.M. Vargas-Barroso:** None. **P. Jonas:** None.

Nanosymposium

019. Hippocampal Circuitry and Navigation

Location: SDCC 5

Time: Saturday, November 12, 2022, 1:00 PM – 3:15 PM

Presentation Number: 019.07

Topic: H.08. Learning and Memory

Support: HHMI

Title: Cellular mechanisms of contextual dependent place field in hippocampal CA3

Authors: *Y. LI, J. MAGEE;
Neurosci., Howard Hughes Med. Institute, Baylor Col. Of Med., Houston, TX

Abstract: Hippocampal place cells are believed to encode "where" in spatial navigation and episodic memory. Place cells are not passively driven by the specific content features, but fire in a context dependent manner. That is, the same feature in completely different environments will produce disparate place cells firing, or trigger distinct memory traces. However, how environmental context determines the place field and drives place field remapping is still an open question. Here I am trying to understand the context-dependent place field that is the strongest in hippocampal CA3. I trained head-fixed mice running on a linear treadmill with different feature cues. The corresponding subthreshold membrane potentials and action potentials have been recorded through whole-cell intracellular voltage recordings. I examined the BTSP (behavioral time scale plasticity) in CA3, and found that the dendritic calcium plateau potentials, rather than spike trains, could produce a place field in CA3. The plasticity kernel in CA3 is symmetric compared to the asymmetric one in CA1. And the plasticity is bidirectional that could potentiate and depress the synaptic weight simultaneously. Thus, this symmetric kernel enables CA3 to build an attractor network with equal weights onto neighboring place cells, indicating that the activity could be well-maintained within CA3 attractor network.

Disclosures: Y. Li: None. **J. Magee:** None.

Nanosymposium

019. Hippocampal Circuitry and Navigation

Location: SDCC 5

Time: Saturday, November 12, 2022, 1:00 PM – 3:15 PM

Presentation Number: 019.08

Topic: H.08. Learning and Memory

Support: SfN travel award from SENC
RTI2018-098581-B-100

Title: Quantitative analysis of sharp-wave ripple dynamics across brain states and cognitive demands

Authors: *E. R. SEBASTIAN, J. P. QUINTANILLA, L. MENENDEZ DE LA PRIDA;
Inst. Cajal, Madrid, Spain

Abstract: Hippocampal activity is crucial for navigation and memory abilities. One example is sharp-wave ripple (SWR) underlying memory retrieval and consolidation. Yet, whether changes in SWR follow specific trends in response to learning, novelty and memory remains unclear. Here, we use standard spectral analysis and unsupervised methods to evaluate the intrinsic structure of a wealth of SWRs recorded from freely moving mice exposed to different behavioural tasks. They explored familiar or novel open fields, and learned to run for water reward in linear and circular mazes. SWR were recorded before and after these several tasks, both in awake and sleep condition. We identify specific trends of SWR features influenced by novelty, habituation and learning of different tasks. Novelty and learning had significant impact in modulating SWR frequency and amplitude during wake conditions. These changes however tended to habituate upon repeated expositions to the task. After the first learning session of a linear maze, SWR dynamic was strongly influenced by sleep and exhibited consistent segregation across states. Thus, our approach permits quantifying how the dynamics of SWR under the influences of different cognitive demands.

Disclosures: E. R. Sebastian: None. **J.P. Quintanilla:** None. **L. Menendez de la Prida:** None.

Presentation Number: 019.09

Topic: H.08. Learning and Memory

Title: Flexiblity of functional neuronal assemblies supports human memory

Authors: *G. UMBACH[1,2], R. TAN[4], J. JACOBS[6], B. E. PFEIFFER[3], H. MOORE[2], B. C. LEGA[5];
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Abstract: Episodic memories, or consciously accessible memories of unique events, represent a key aspect of human cognition. Evidence from rodent models suggests that the neural representation of these complex memories requires cooperative firing of groups of neurons on short time scales, organized by gamma oscillations. These co-firing groups, termed "neuronal assemblies," represent a fundamental neurophysiological unit supporting memory. Using microelectrode data from 26 neurosurgical patients, we identify significant counts of neuronal assemblies in the human MTL ($p < 0.001$, permutation testing) for the first time, and show that they exhibit consistent organization in their firing pattern based on gamma phase information. Most neurons within assemblies (63 of 84, $p < 0.001$, binomial test) demonstrated significant phase locking to the underlying gamma oscillation ($p < 0.05$, Rayleigh), but to different phases, giving rise to consistent neuronal firing orders comprising assembly activations ($p < 0.001$, permutation testing). The consistency of this firing order correlated with episodic memory performance ($r = 0.30$, $p = 0.42$, Spearman rank correlation). Finally, we demonstrated that neurons drift in and out of assemblies, meaning that the activity of a given neuron tends to increase or decrease during successive assembly activations (21% of neurons, $p < 0.001$, permutation testing). The degree of neuronal drift positively correlated with memory ($r = 0.47$, $p = 0.0011$, Spearman rank correlation). In sum, our findings provide key evidence linking assemblies to human episodic memory for the first time.
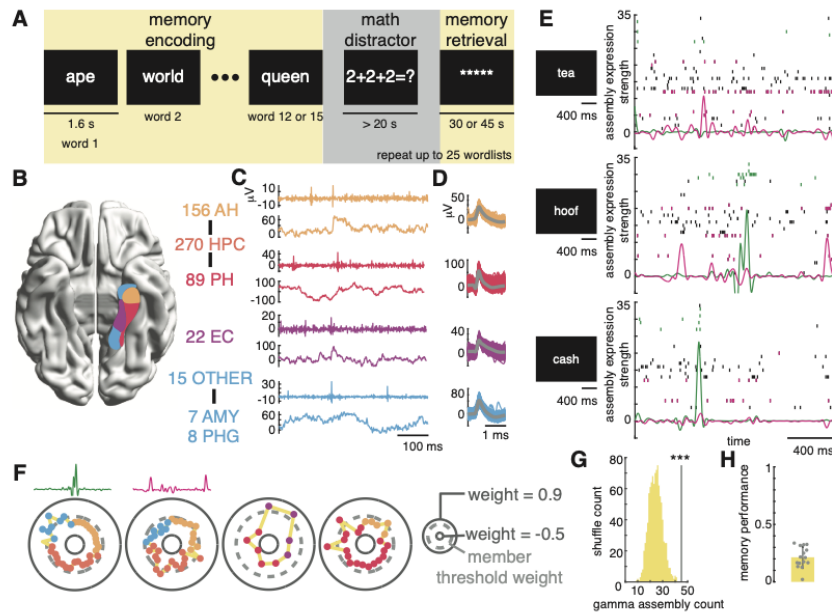
Fig. 1. Significant neuronal assembly identification during an episodic memory task. (A) Schematic of the free recall task. Each displayed word the participant studies represents an "encoding event." **(B)** Unit yield for each brain region included in the study. **(C)** Example denoised high frequency signals from which we isolated unit activity (top rows) and local field potentials (bottom rows) for each brain region. Coloring follows the convention shown in (B). Numbers next to region indicate the number of units isolated from each region and include neurons from all sessions (n = 307), not only those from which assemblies were identified (n = 203). **(D)** Example units from each of the high frequency signals displayed in (C). **(E)** Expression strength of two example assemblies superimposed on the pertinent spike rasters for three example encoding events. Expression strength curves and spike rasters are colored to link them with the assemblies in (F). **(F)** Schematics of four example neuronal assemblies. The first two correspond to the data shown in (E). Each colored data point represents a neuron from that recording session. The further the data point from the circle's center, the greater the contribution of that neuron to the assembly, with member neurons falling outside of the dashed-line circle. The color of each data point represents the region of the neuron, as outlined in (B). **(G)** Comparison of the number of assemblies identified against a null distribution obtained by shuffling the spike trains. **(H)** Average and individual recall fraction of recording sessions with identified assemblies. ***p < 0.001.

Disclosures: G. Umbach: None. **R. Tan:** None. **J. Jacobs:** None. **B.E. Pfeiffer:** None. **H. Moore:** None. **B.C. Lega:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.01

Topic: I.04. Physiological Methods

Support: Max Planck Society

Title: A three-photon head-mounted microscope for imaging all layers of visual cortex in freely moving mice

Authors: A. KLIOUTCHNIKOV[1], D. J. WALLACE[2], J. SAWINSKI[2], K.-M. VOIT[2], Y. GROEMPING[2], *J. N. D. KERR[2];
[2]Behavior and Brain Organization, [1]Max Planck Inst. For Neurobio. Of Behavior, Bonn, Germany

Abstract: Miniaturized head-mounted two-photon microscopes have enabled imaging of activity from fluorescently labelled neuronal populations in the upper cortical layers with single cell resolution in freely moving rodents. Three-photon excitation (3PE) can considerably extend the imaging depth possible in scattering tissue by utilizing longer wavelengths, that decrease excitation light scattering and eliminating the generation of out-of-focus fluorescence. However, 3PE-based head-mounted microscopes have so far been too physically restrictive to take advantage of mouse-based molecular tools. Here we built a 2 gram, 3PE-based microscope, capable of imaging activity from neuronal populations from all cortical layers in the freely moving mouse. This tool is equipped with a z-drive, enabling remote focusing through the whole cortical depth without interfering with the animal's behavior, and resilient miniature detectors that enable imaging in a fully lit environment with high sensitivity. We use it to show that neuronal population activity in cortical layer-4 and layer-6 was differentially modulated by lit and dark conditions during free exploration. As this new microscope design enables animals to naturally explore arenas in any lighting condition while neuronal activity is measured with single cell-resolution from neuronal populations located in any cortical layer, this microscope opens up the possibility to explore the link between neuronal activity and natural behavior.

Disclosures: A. Klioutchnikov: None. **D.J. Wallace:** None. **J. Sawinski:** None. **K. Voit:** None. **Y. Groemping:** None. **J.N.D. Kerr:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.02

Topic: I.04. Physiological Methods

Support: IIS-2011542
               R01 EY030893

Title: Modular functional activity across cortical areas

Authors: *G. SMITH;
Univ. of Minnesota, Minneapolis, MN

Abstract: Columnar organization is a hallmark of visual cortex (V1) in humans, that is shared with other primates and carnivores such as cat and ferret, but is absent in mice. This organization is readily apparent in the responses to visual stimuli, as well as in the patterns of ongoing spontaneous activity. These spontaneous patterns are present early in development, are highly modular, and exhibit millimeter-scale correlations reflecting sensory-evoked networks. However, neural representations vary greatly across cortical areas and this columnar organization is largely thought to be absent from other sensory or association cortices. Thus a fundamental question is whether this diverse array of representations arise developmentally through area-specific mechanisms or rather emerge from a common developmental origin.
To address this, we utilize the ferret, a uniquely powerful model system with both a columnar visual cortex, as well as a highly accessible period of postnatal development. We show that spontaneous activity across the developing ferret cortex including both sensory (V1, A1, and S1) and association cortices (PPC and PFC) is highly modular and exhibits millimeter scale correlations. Two-photon imaging of GcaMP6s reveals a strongly modular activity amongst local clusters of neurons, reflected in high pairwise correlations between nearby cells. We find that in animals a week prior to eye opening, modular patterns of spontaneous activity across all areas were nearly indistinguishable from those seen in V1. This modular organization persisted over the subsequent 3 weeks, including the period in which modular stimulus-evoked orientation maps can first be elicited in V1. Notably, these grating-evoked responses have been shown to exhibit a strong similarity to the modular patters of ongoing spontaneous activity. To determine whether a similar relationship exists with the modular spontaneous activity we find in A1, we presented both simple pure-tone and complex time-varying multi-frequency sounds. We find that as in V1, evoked responses in A1 to both pure tones and complex sounds also showed a modular spatial structure with significant similarity to spontaneous activity. Together, these results demonstrate that modular functional organization of both spontaneous and stimulus-driven activity is a general feature of V1 and A1, and may be shared across all cortical areas. These findings also demonstrate the power of multiphoton imaging in non-murine animal models such as the ferret that share the columnar functional architecture found in humans.

Disclosures: G. Smith: None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.03

Topic: I.04. Physiological Methods

Title: Imaging tonotopic organization in ferret and mouse auditory cortex

Authors: M. PANNIELLO[1], Q. GAUCHER[2], A. Z. IVANOV[3], J. C. DAHMEN[3], A. J. KING[3], *K. WALKER[3];
[1]Italian Inst. Of Technol., Genova, Italy; [2]Ecole Normale Superieure, Paris, France; [3]Univ. of Oxford, Oxford, United Kingdom

Abstract: Primary cortical areas contain spatial maps of sensory features, including sound frequency in primary auditory cortex (A1). Two-photon calcium imaging in mice has confirmed the presence of these global tonotopic maps, while uncovering an unexpected local variability in the stimulus preferences of individual neurons in A1 and other primary regions. Using two-photon calcium imaging in layers 2/3, we show that local variance in frequency preferences is equivalent in adult ferrets and mice. Neurons with multipeaked frequency tuning are less spatially organized than those tuned to a single frequency in both species. We will discuss some of the considerable challenges of in vivo imaging in adult ferrets, as well as some of our solutions. Furthermore, we will show that microelectrode recordings may describe a smoother tonotopic arrangement than two-photon imaging due to a sampling bias towards neurons with simple frequency tuning. These results help explain previous inconsistencies in cortical topography across species and recording techniques.

Disclosures: **M. Panniello:** None. **Q. Gaucher:** None. **A.Z. Ivanov:** None. **J.C. Dahmen:** None. **A.J. King:** None. **K. Walker:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.04

Topic: I.04. Physiological Methods

Title: Three-photon imaging of neurovascular coupling across layers in cat visual cortex to determine the neural basis of fMRI

Authors: *P. KARA, A. LEIKVOLL, C. LIU, A. ROY, D. FARINELLA, H. JAYAKUMAR;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The visual cortex of the non-rodent brain is ideally suited to study the spatial organization of neurovascular coupling at the level of synapses, neurons, individual blood vessels and laminar-resolution fMRI. This is because it is organized in functional neural columns and the array of visual stimuli which are needed to optimally drive neurons are established. Specifically, feature selectivity such as orientation tuning emerges in layer 4 neurons from

untuned thalamic inputs. Tuning is further specialized in layer 2/3 neurons. Thus, in a specific cortical layer, neuro-vascular coupling can be precisely quantified in terms of tuning curves of neural and vascular selectivity. We embarked to determine the extent to which different types of neural (spiking, synaptic) and vascular signals (blood flow from individual vessels) are coupled across cortical layers in cat primary visual cortex. However, critical technical developments were first needed because in non-rodent mammals, 2-photon imaging can only access the most superficial cortical layers. We optimized deep tissue 3-photon imaging and screened for new genetically encoded calcium and glutamate sensors that express in layer 4 neurons. With access to layer 4 in hand, we addressed the following. First, to determine how feature selectivity is spatially organized across input and output layers in cat V1—by imaging spiking and synaptic activity across layer 2/3 and especially layer 4 for the first time. Second, to determine if hemodynamic signals correlate better with synaptic or spiking activity across cortical layers—a major question in the field of NV coupling. Hence, we mapped visually-evoked blood vessel dilation and velocity in layers 2/3 and 4 and related it to neural activity. Our previous published two-photon data in layer 2/3 showed that single blood vessel responses were partially decoupled from spiking and synaptic activity. Specifically, individual blood vessel responses were tuned for stimulus orientation but neural responses were far more selective. Our new three-photon data in layer 4 vessels show that single-vessel hemodynamic responses were completely untuned for stimulus orientation. For hemodynamic responses, both fMRI and optical imaging showed a consistent laminar response pattern in which orientation selectivity in cortical layer 4 was lower compared to layer 2/3. This systematic change in selectivity across cortical layers has a clear underpinning in neural circuitry, as reflected in the spiking vs. synaptic responses we observed in layer 2/3 vs. layer 4.

Disclosures: **P. Kara:** None. **A. Leikvoll:** None. **C. Liu:** None. **A. Roy:** None. **D. Farinella:** None. **H. Jayakumar:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.05

Topic: I.04. Physiological Methods

Title: High fidelity visually-evoked responses with IV injection of AAV.PHP.eB-synapsin-jGCaMP7s

Authors: *A. LEIKVOLL, P. KARA;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Multiphoton imaging of genetically-encoded calcium indicators (GECIs) has traditionally relied on intracranial injections of adeno-associated virus (AAV) or transgenic animals to achieve expression. Intracranial injections require an invasive surgery and result in a relatively small volume of labeling. Transgenic animals can have brain-wide expression, but they

often express GECIs in only a subset of neurons, may have abnormal behavioral phenotypes, and are currently limited to previous generations of GECIs. Additionally, non-rodent transgenic animals expressing GECIs are rarely available. We present an alternative strategy to achieve brain-wide GECI expression suitable for high-fidelity multiphoton imaging: systemic (IV) injections of AAV.PHP.eB. We injected young (P21-P30) C57BL/6J mice with AAV.PHP.eB-Synapsin-jGCaMP7s via the retro-orbital sinus. After allowing 5-8 weeks for expression, we performed 2- and 3-photon imaging of layers 2/3 through 5 of the primary visual cortex. We also performed widefield multiphoton imaging to demonstrate the utility of brain-wide expression. We found robust and reproducible trial-by-trial responses and tuning properties consistent with known selectivity features in the visual cortex, indicating that this method does not interfere with neuronal processing. Histological analysis confirms brain-wide expression of jGCaMP7s, and there is no nuclear expression of jGCaMP7s until at least 10 weeks post-injection. IV injection of AAV.PHP.eB thus yields brain-wide GECI expression suitable for 2- and 3-photon imaging in C57BL/6J mice. Future AAV capsid variants may allow brain-wide GECI expression in non-rodents.

Disclosures: A. Leikvoll: None. **P. Kara:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.06

Topic: I.04. Physiological Methods

Support: NIMH IRP ZIAMH002838
            NIMH IRP ZIAMH002898

Title: Imaging marmoset visual cortex neurons using miniaturized head-mounted microscope during dynamic free viewing

Authors: *S. PARK[1], S. NTI[1], M. MARCELLE[2], E. ESCH[1], A. C. SILVA[3], D. A. LEOPOLD[1];
[1]SCNI, Lab. Of Neuropsychology, NIMH, Bethesda, MD; [2]NINDS, Bethesda, MD; [3]Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Previous single-unit studies in the macaque inferotemporal cortex during video watching revealed that neighboring neurons showed dissimilar video-driven responses to each other (McMahon et al., J Neurosci., 2015) and further covaried with a distinct set of areas across the whole brain (Park et al., Neuron, 2017; Sci. Adv. 2022). These findings raise the question of how the localized regions of the visual cortex are organized, especially under more naturalistic conditions such as free viewing of dynamic videos. Here we addressed this question in the marmoset extrastriate visual cortex. We used microendoscopic calcium imaging with head-mounted miniscopes in awake, behaving marmoset monkeys using an endoscopic PRISM probe to monitor the activity and spatial arrangement of a local population of visual neurons. We

developed a new lens implant that features an injection cannula yoked to a lens ("lennula"), which enables targeted delivery of the genetically encoded calcium indicator directly into the imaging field. We carried out daily recordings in two awake, behaving marmosets, recording from area V3 in one animal and V4 in the other. During each recording session, we imaged neuronal activity from 60 – 130 neurons from the field of view of approximately 800 micrometers in each dimension. From each neuron, we measured calcium responses during both active viewings of videos and periods of quiescent rest. In both areas, we observed that individual neurons exhibited similar response time courses to the repeated presentation of the same videos. The video-driven response correlations across neurons revealed the spatial correlational structure across the imaged field that differed substantially from the one derived from the spontaneous activity at rest. This indicates the local correlational network structure is strongly defined by the visual input. The head-mounted miniscope, in combination with the lennula construct, provides a straightforward way to examine the spatial organization of functional networks over mesopic scales in the nonhuman primate.

Disclosures: S. Park: None. **S. Nti:** None. **M. Marcelle:** None. **E. Esch:** None. **A.C. Silva:** None. **D.A. Leopold:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.07

Topic: I.04. Physiological Methods

Support: U01NS094330

Title: The operating regime of primate sensory cortex

Authors: *J. J. PATTADKAL[1], B. V. ZEMELMAN[1], I. R. FIETE[2], N. J. PRIEBE[1];
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Abstract: Sensory cortical areas amplify relevant features of external stimuli. This neuronal sensitivity and selectivity to our environment arises from the transformation of external inputs by the cortical circuitry. We dissect the circuitry for emergence of amplification using a combination of large-scale simultaneous measurements from single cells in awake marmosets and computational models. In area MT, an area of the neocortex that processes visual motion, even weak motion signals evoke selective output responses in the presence of noise. We also observe tuned patterns of neural activity in the absence of visual motion. This suggests feature-selective amplification within the MT circuit. In addition, responses in MT also display fast dynamics, which poses a critical dynamics constraint on the nature of the underlying amplification mechanisms. We combined multiple cortical models in the literature and identified different regimes capable of generating high amplification in our combined computational

model. These computational regimes exhibit distinct activity signatures which we use to match with physiological responses and distinguish between the regimes. Specifically, we have used the population responses to sudden changes in direction of the input, the responses to sudden changes in stimulus coherence of the moving dots and the expectation for the structure and statistics of spontaneous population activity under the different model regimes. We examined the activity of large populations of awake primate MT neurons across the same conditions and compared the results with the computational models. We find that our recorded responses from MT network match a regime where amplification arises from separate excitatory and inhibitory populations operating in a balanced regime with tuned recurrent interactions. This allows the internal circuitry of the sensory cortex to amplify incoming inputs strongly but transiently, such that it remains sensitive to the input using excitatory amplification and can also rapidly track changes in the input using strong inhibitory contributions that quench amplification (Murphy and Miller, Neuron, 2009). This balanced amplification regime only emerges in models composed of segregated excitatory and inhibitory populations. Our discovery provides a potential explanation for the specialization of neurons into distinct excitatory and inhibitory populations: the fundamental asymmetry that arises from coupling these populations is essential to the generation of large but rapid amplification without response persistence.

Disclosures: J.J. Pattadkal: None. **B.V. Zemelman:** None. **I.R. Fiete:** None. **N.J. Priebe:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.08

Topic: I.04. Physiological Methods

Title: A silent two-photon imaging system for studying auditory neuronal functions in awake marmosets

Authors: *X. SONG[1], Y. GUO[2], C. CHEN[1], X. WANG[3];
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Abstract: Two-photon laser-scanning microscopy has become an essential tool for imaging neuronal functions *in vivo* and has been applied to neural systems, including the auditory system. However, many components of a multi-photon microscope, such as galvanometer-based laser scanners, generate mechanical vibrations and thus acoustic artifacts, making it difficult to interpret auditory responses from recorded neurons. Here, we report the development of a silent

multi-photon imaging system and its applications in the common marmoset (*Callithrix Jacchus*), a non-human primate species sharing a similar hearing range with humans. By utilizing an orthogonal pair of acousto-optical deflectors (AOD), full-frame raster scanning at video rate was achieved without introducing mechanical movement. Imaging depth can be optically controlled by adjusting the chirping speed on the AODs without mechanical motion along the Z-axis. Furthermore, all other sound-generating components of the system were acoustically isolated, leaving the noise floor of the working system below the marmoset's hearing threshold. Imaging with the system in awake marmosets revealed many auditory cortex neurons that exhibited maximal responses at low sound levels, which were not possible to study using traditional two-photon imaging systems. This is the first demonstration of a silent multi-photon imaging system that is capable of imaging auditory neuronal functions *in vivo* without acoustic artifacts. This capacity opens new opportunities for a better understanding of auditory functions in the brain and helps isolate animal behavior from microscope-generated acoustic interference.

Disclosures: X. Song: None. **Y. Guo:** None. **C. Chen:** None. **X. Wang:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.09

Topic: I.04. Physiological Methods

Title: Multi-photon imaging in the visual cortex of the anesthetized macaque

Authors: K. TAKASAKI[1], S. CHATTERJEE[1], C. J. M. DYLLA[2], T. KIM[2], B. MACLENNAN[1], P. BALARAM[1], A. K. PASUPATHY[2], R. C. REID[1], J. WATERS[1], *W. BAIR[2];
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Abstract: A collaboration between laboratories at the Univ. of Washington's National Primate Research Center and groups at the Allen Institute for Brain Science was started several years ago with two major experimental goals for Ca2+ imaging in the nonhuman primate. First we aim to image middle layers in the macaque visual cortex, approaching 1 mm in depth using 3-photon Ca2+ imaging. Second, we aim to image large, contiguous blocks of tissue within the visual cortex to facilitate detailed studies of structure-function relationships using a combination of 2- and 3-photon imaging. Here, we report the following initial results. (1) We have successfully achieved 3-photon imaging and functional characterization of neurons up to 800 um deep in visual cortex of the anesthetized primate. Using drifting sinusoidal gratings, we were able to

image high SNR direction tuning curves of apical dendrites of neurons at 800 um below the pial surface and show consistency in tuning across spatial frequencies. (2) We have directly compared 2-photon (920 nm) and 3-photon (1300 nm) imaging in the same visual cortex imaging session, and we demonstrate how 3P excitation maintains high resolution and image contrast deep in scattering tissue as 2P imaging degrades as a function of depth. (3) We developed a 4-needle "cat's paw" system, derived from Fredericks et al. (2020, J Neurosci Meth), that uses custom sharpened 33 gauge needles (~2 mm apart) to avoid dimpling, graduation marks for controlling injection depth, and a simple titanium mesh grid system for stabilizing the brain during injection. Testing this system across eight hemispheres in four macaques, we drove widespread AAV expression of GcaMP6s in primary and extrastriate visual cortex. (4) We designed and refined a novel and flexible head plate and imaging window system to hold the cortex stable across multiple days and allow access for a variety of multi-photon microscope objectives in the anesthetized monkey. We have successfully used this system to record from areas V1, V2 and V4. (5) We have developed a real-time data analysis system that uses Suite2P and custom lab software to rapidly analyze tuning curves and compute a SNR metric (Pospisil and Bair, 2021, PloS Comput Biol) for optimizing data collection during time-limited recording sessions. We will present our results from four macaque experiments in which we have been able to characterize neurons in terms of their receptive field location and size, simple and complex classification, their tuning for orientation and direction of motion, as well as for color, shape and texture in areas V1, V2 and V4, thereby demonstrating the viability of 3-photon imaging into the middle layers of macaque cortex.

Disclosures: K. Takasaki: None. **S. Chatterjee:** None. **C.J.M. Dylla:** None. **T. Kim:** None. **B. MacLennan:** None. **P. Balaram:** None. **A.K. Pasupathy:** None. **R.C. Reid:** None. **J. Waters:** None. **W. Bair:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.10

Topic: I.04. Physiological Methods

Support: CEA DRF Impulsion
SESAME Ile-de-France
ANR JCJC

Title: Optimization and characterization of three-photon microscopy for reliable and long-term imaging in macaque monkey cortex

Authors: *M. GUILLEMANT[1], M. GAY[1], M. GLATINY[1], J. LEMAITRE[2], B. JARRAYA[1], A. CHENG[3], E. BEAUREPAIRE[4], T. VAN KERKOERLE[1];
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(IDMIT), CEA, Fontenay-aux-roses, France; [3]UCLA Dept Physics and Astronomy, Seattle, WA; [4]Lab. D'optique et biosciences (LOB), Palaiseau, France

Abstract: Neuroscience is currently experiencing a huge development in terms of optical imaging techniques, in particular with the combination of 2-photon imaging and activity-dependent fluorescent sensors that can also provide cell-type specificity, estimates of neurotransmitter release etc. It will be highly relevant to apply these techniques to macaque monkeys because of their close similarity to humans and their ability to be trained on complex cognitive tasks. However, 2-photon imaging in macaque monkeys generally requires the removal of the dura mater, leading to the growth of a layer of granulation tissue that obstructs imaging. We here propose to use 3-photon microscopy to either image through the natural dura or through this layer of granulation tissue, which could make multi-photon imaging more readily accessible to the macaque monkey community.

We developed a 3-photon setup that is optimized for long-term imaging in awake and behaving macaque monkeys. It allows relatively fast imaging (15Hz frame rate) and a large field-of-view (620x620μm²) by combining a high repetition rate laser source (2MHz) and galvo-resonance scanning. We optimized imaging chambers and head-fixation implants for chronic 3-photon imaging in macaque monkeys, and developed viral injection techniques to obtain large and homogeneous expression of fluorescent indicators (bicistronic GcaMP6s and mRuby). The setup has been tested extensively on mice and post-mortem macaque dura tissue, as well as in two macaque monkeys.

In mice, the vascular bed as well as neuronal activity were imaged 1mm into the prefrontal cortex (using a laser power of less than 100mW). The effective attenuation length (EAL) for 1300nm in mouse cortex was estimated to be 245μm, in agreement with the literature. Post-mortem macaque dura was embedded in agarose with 500nm fluorescent beads and a careful comparison was performed between 2 and 3-photon imaging in terms of EAL, signal-to-background ratio (SBR) and point-spread function (PSF). Importantly, while SBR and PSF were significant degraded with 2-photon imaging through the dura, they were not affected with 3-photon imaging. In vivo experiments in two macaque monkeys confirmed that 3-photon microscopy allows long-term robust imaging of the vasculature bed as well as neurons through more than 100μm of granulation tissue and 600μm into the prefrontal cortex (using less than 50mW).

This shows the unique potential of 3-photon microscopy for reliable and long-term imaging in macaque monkeys which could be very exciting to investigate the detailed neural mechanisms of high-level cognitive functions.

Disclosures: M. Guillemant: None. **M. Gay:** None. **M. Glatiny:** None. **J. Lemaitre:** None. **B. Jarraya:** None. **A. Cheng:** None. **E. Beaurepaire:** None. **T. van Kerkoerle:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.11

Topic: I.04. Physiological Methods

Support: U01-NS103518

Title: A robotic platform for multiregional calcium imaging in the non-human primate brain

Authors: *B. PESARAN[1], A. S. CHARLES[3], J. CHOI[2], K. WINGEL[4], J. HAGGERTY[5], H. HAFIZI[5], A. DUBEY[4], M. W. CHOUDHURY[5], R. BAKHSHI[5];
[1]NYU, New York City, NY; [2]NYU, New York, NY; [3]Johns Hopkins, Baltimore, MD; [4]Ctr. For Neural Sci., [5]New York Univ., New York, NY

Abstract: Progress in understanding multiregional networks involves multiregional imaging. In smaller organisms, including C elegans, drosophila, and now in mice, cellular multiphoton imaging of different fluorophores,GFP/BFP/RFP, has resolved activity during behavior across large-scale networks. As Multiphoton imaging expands into NHP, e.g., macaque (macaca mulatta) optical imaging of large-scale networks is facing new fundamental barriers. First, large-scale brain windows must be developed to provide sufficient access to large-sale networks (&gt 5cm). Second, hardware and software must be designed to enable cellular-level imaging at scale across large-scale networks in awake, behaving primates. Specifically, instrumentation must enable experimenters to flexibly target different optically accessible systems in a stable imaging plane and be integrated into an experimental user interface to guide experimentation. Current approaches pre-select an area of interest under the window and finer adjustments to the FOV can be accomplished by optical focusing, e.g., in a mesoscope (sutter-scope). Third, to record activity across cortex, we must be able to elicit large-scale viral expression in vivo. Finally, data processing pipelines must be designed to coregister and identify neurons at scale.
Here, we present solutions to each of these challenges. We engineer a multiplanar primate brain window customized to each experimental subject. We develop approaches to reproducibly and flexibly position and reposition the imaging field of a multiphoton microscope. We elicit viral expression of fluorophores and GECIs (GcaMP7f and GcaMP8f) across centimeter-scale extents using convection-enhanced delivery. We also present algorithms for building volumetric maps of neurons over large areas, registering fields-of-view, and extracting neuronal activity from functional data. These algorithms are integrated into instrumentation and are tuned to the expression and imaging properties in the primate brain. Finally, we provide a user interface to enable experimentalists to navigate the large areas now visible. The results demonstrate a novel approach toward realizing a cellular-resolution multiregional primate brain observatory for research into the neural mechanisms of behavior and cognition.

Disclosures: B. Pesaran: None. **A.S. Charles:** None. **J. Choi:** None. **K. Wingel:** None. **J. Haggerty:** None. **H. Hafizi:** None. **A. Dubey:** None. **M.W. Choudhury:** None. **R. Bakhshi:** None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.12

Topic: I.04. Physiological Methods

Title: Representational geometry of sequence working memory in macaque prefrontal cortex

Authors: *Y. XIE[1], P. HU[1], J. LI[2], J. CHEN[2], S. TANG[3], W. SONG[3], T. YANG[1], S.
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Inspired Technol., Shanghai, China

Abstract: Nonhuman primates can perform well across many kinds of complex cognitive tasks.
These cognitive tasks usually consist of multiple trial types. The prefrontal cortex, regarded as a
central role in complex cognitive tasks, has numerous mixed selective neurons. Multiple trial
types will limit the number of trials sharing same trial type. Mixed selective neurons make single
neuron analysis hard to interpret. Both problems might be solved with simultaneously recorded
high-dimensional neural population data. We injected AAV virus with GcaMP6s and GcaMP6f
in monkeys' lateral prefrontal cortex, then applied two-photon imaging on LPFC neurons.
Around 170 neurons could be recorded simultaneously in a field of view with a size of 512 um ×
512 um. Crucially, most neurons could be recaptured in other recording sessions, which makes it
possible to test if neurons' function would shift at both individual level and population level.
Monkeys were required to do a sequence reproduction task in each imaging session. After head
stabilization and semi-automated/ manual curation, neurons could be imaged at 30 Hz. With the
help of two-photon imaging, we discovered a simple representational geometry underlies
sequence working memory. This kind of neural code was stable across days on single neuron
level. In addition, the anatomical organizations of different functions in LPFC neurons were
revealed.

Disclosures: Y. Xie: None. **P. Hu:** None. **J. Li:** None. **J. Chen:** None. **S. Tang:** None. **W.
Song:** None. **T. Yang:** None. **S. Dehaene:** None. **X. Wang:** None. **B. Min:** None. **L. Wang:**
None.

Nanosymposium

020. Optical Approaches for Large-Scale Cell-Resolved Imaging Across Species

Location: SDCC 25

Time: Saturday, November 12, 2022, 1:00 PM – 4:15 PM

Presentation Number: 020.13

Topic: I.04. Physiological Methods

Support:　　　NIMH (BRAIN) Grant RF1 MH114276-01

Title: Varying patterns of brain-wide neuronal activity underlie correlation structure of low-frequency spontaneous hemodynamic signals

Authors: *S. SHAHSAVARANI[1,2], D. THIBODEAUX[1], F. LODGHER[1], W. XU[1], D. HANDWERKER[2], J. GONZALEZ-CASTILLO[2], P. BANDETTINI[2], E. HILLMAN[1];
[1]Columbia Univ., New York, NY; [2]Section on Functional Imaging Methods, Lab. Of Brain and Cognition, Natl. Inst. Of Mental Hlth., Bethesda, MD

Abstract: Spontaneous or baseline neural activity has been traditionally ascribed to noise. However, analysis of low-frequency fluctuations in spontaneous blood oxygen level-dependent (BOLD) signals has changed this traditional view by showing that the relationship between these baseline fluctuations is not random. Although there is ample evidence for spontaneous BOLD signals being spatiotemporally structured, the functional and behavioral manifestation of this baseline activity is not clear. This is in part due to the dependence of BOLD signals on the hemodynamics of the brain as a proxy for neural activity. The question of what these low-frequency fluctuations in BOLD signals truly reflect still remains unanswered. To address this question, we used a multimodal imaging technique, Wide Field Optical Mapping (WFOM), to simultaneously record neuronal calcium and hemodynamic signals in five thinned-skull adult Thy1- jRGECO1a mice. The advantage of WFOM is twofold; we can directly record cortex-wide, large-scale neuronal activity and estimate their corresponding hemodynamic signals, which helps investigate the relationship between these two modalities. Each recording session was 10 minutes, and mice were allowed to move freely on a rotating wheel with their heads fixed. The mouse locomotion, whisking, and pupil size were monitored in tandem with imaging. We identified five distinct neural correlation patterns, defined as pairwise Pearson's correlation coefficients among brain regions. Using a non-negative least squares fit, we confirmed that the temporal evolution of these patterns was in accordance with mouse behavior. We found that arousal level was different between two distinct correlation patterns occurring while the mice were at rest. Comparing these two resting patterns (Wilcoxon Rank Sum test, p < 0.05 Bonferroni corrected) we revealed that the anterior lateral frontal brain regions were significantly less synchronized with the posterior brain regions during the lower arousal state. Looking further into the neural time courses, we noted that the baseline activity of these frontal regions had higher oscillation than during locomotion activity. This feature seems to drive the anterior-posterior desynchrony during rest, which cannot be detected in trial-averaged data due to the random phase of spontaneous activity. Although hemodynamic signals were not able to capture this intrinsic high variation, they did recapitulate it by reflecting the neural correlation patterns and correlation fluctuations as explained by behavior and arousal states. Our results elucidate the underlying neural basis of correlation structure of spontaneous BOLD signals.

Disclosures: S. Shahsavarani: None. **D. Thibodeaux:** None. **F. Lodgher:** None. **W. Xu:** None. **D. Handwerker:** None. **J. Gonzalez-Castillo:** None. **P. Bandettini:** None. **E. Hillman:** None.

Nanosymposium

100. Autism and Synapse Development

Location: SDCC 1

Time: Sunday, November 13, 2022, 8:00 AM – 10:30 AM

Presentation Number: 100.01

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH U01MH114825

Title: Properties of the synaptic modules in the developing human cortex

Authors: *L. ZHOU[1,2], L. WANG[2], T. MUKHTAR[2], A. BHADURI[4], Q. BI[2], S. WANG[2], G. PANAGIOTAKOS[3], A. R. MOORE[5], A. ALVAREZ-BUYLLA[6], A. R. KRIEGSTEIN[6];
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Abstract:

Properties of synaptic modules in the developing human cortex, Zhou et al.
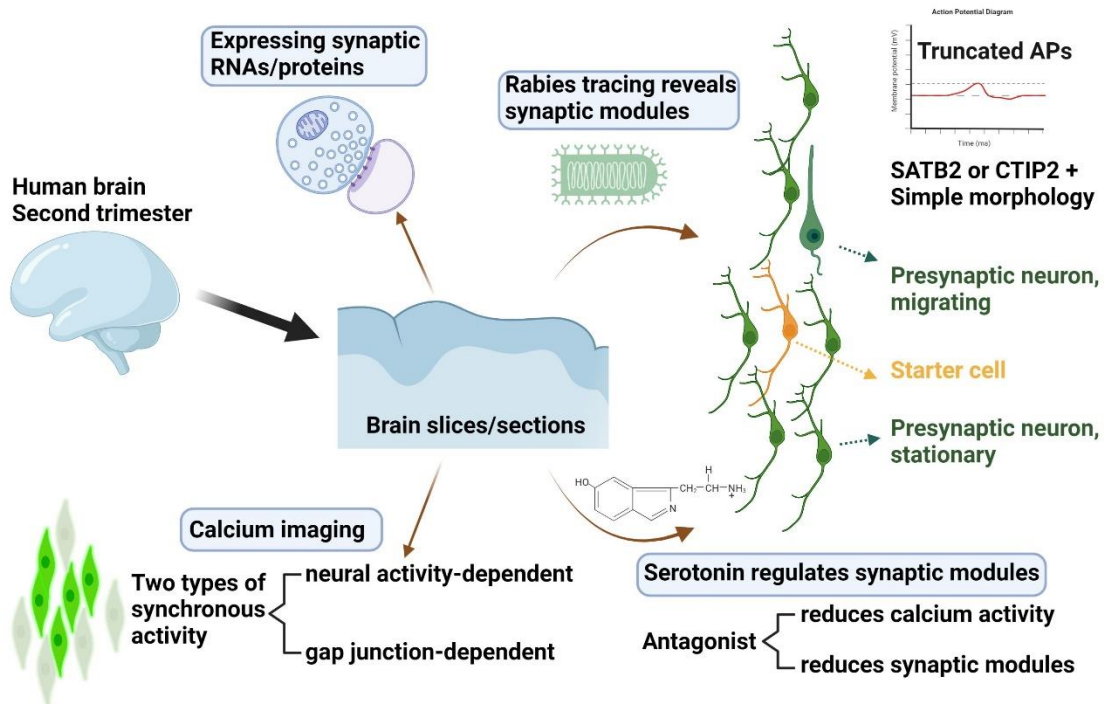
Why the human brain is unique? Learning how it develops helps. Normal brain development requires early circuit activity but little is known about the characteristics of local cortical circuits during human brain development. We explore intracortical connectivity patterns and physiological features during the second trimester of gestation in the developing human cortex, a stage when synapses are beginning to form (gestational weeks 16-24). Single-cell Patch-seq indicates that young cortical neurons at this stage, although electrophysiologically immature and morphologically simple, express genes encoding synaptic components. In organotypic cultures, *ex vivo* rabies tracing reveals the emergence of local synaptic modules in the cortical plate and subplate composed of immature excitatory neurons and migrating cells. These synaptic modules display spontaneous patterns of both individual cellular and synchronous multicellular calcium activity. Synchronous calcium activity is neural activity-dependent and/or activity-independent mediated by gap junctions. Moreover, we find that serotonin signaling can modulate the size of local synaptic modules. The results are from at least three experimental units and/or at least two donors. Understanding the development of early intracortical circuits will deepen our knowledge about how the human brain develops, inform *in vitro* models of human brain development including organoids, and shed light on the etiology of neurodevelopmental disorders.

Nanosymposium

100. Autism and Synapse Development

Location: SDCC 1

Time: Sunday, November 13, 2022, 8:00 AM – 10:30 AM

Presentation Number: 100.02

Topic: A.07. Developmental Disorders

Title: Transcription factor FOXP1 is critical for the cell-type specific development of striatal circuitry

Authors: *N. KHANDELWAL, A. KULKARNI, M. HARPER, G. KONOPKA, J. R. GIBSON;
Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Heterozygous loss-of-function mutations in the transcription factor *FOXP1* are strongly associated with autism. To understand this link, we employed a strategy of electrophysiological and gene-expression methods to study the role of FOXP1 in the

development of striatal circuitry. In a *Foxp1$^{+/-}$* heterozygous mouse, Dopamine receptor 2 expressing (D2) striatal projection neurons (SPNs) show higher intrinsic excitability (Araujo et al., 2015). Moreover, when *Foxp1* is deleted specifically in D2 SPNs using a D2-Cre reporter mouse line (D2 *Foxp1^{cKO}*), mice show autism-like behavioral phenotypes and a reduced number of D2 SPNs (Anderson et al., 2021). The remaining D2 SPNs are hyperexcitable and less conductive due to reduced subthreshold potassium currents (Khandelwal et. Al, 2021). Here, we report that deletion of *Foxp1* in D2 SPNs not only affects their intrinsic properties but also affects their function in maintaining the striatal circuitry. Using channel rhodopsin-mediated optogenetic stimulation of the contralateral cortical inputs, we found a substantial decrease in evoked cortico-striatal synaptic input onto *Foxp1*-deleted D2 SPNs. This was true for both AMPA and NMDA-receptor mediated inputs. Moreover, miniature excitatory post synaptic currents (mEPSCs) in these neurons showed a reduction in their frequency and amplitude. Interestingly, although intrinsically hyperexcitable, *Foxp1*-deleted D2 SPNs displayed reduced excitability in the context of cortico-striatal synapse-driven excitation. This *Foxp1*-dependent regulation of neuronal function is cell-type specific as D1 SPNs did not show this phenotype. The *Foxp1*-deletion phenotype was significantly rescued when *Foxp1*-expressing AAV was injected postnatally into the striatum of D2 *Foxp1^{cKO}* mouse pups. We generated single nuclei RNA-sequencing data from the striatum of the same mice, which suggests that rescued physiological properties of the D2 SPNs are partly mediated by enhanced expression of one of the leak channels that is downregulated in the absence of FOXP1. Our findings decipher a critical and important function of FOXP1 in maintaining functional striatal circuitry and provide new avenues for therapeutics for individuals with FOXP1 syndrome or other forms of autism.

Disclosures: N. Khandelwal: None. **A. Kulkarni:** None. **M. Harper:** None. **G. Konopka:** None. **J.R. Gibson:** None.

Nanosymposium

100. Autism and Synapse Development

Location: SDCC 1

Time: Sunday, November 13, 2022, 8:00 AM – 10:30 AM

Presentation Number: 100.03

Topic: A.07. Developmental Disorders

Support: #632842

Title: Cell type-specific molecular changes are conserved across ASD subtypes

Authors: *Y. PEREZ, D. VELMESHEV, L. WANG, A. R. KRIEGSTEIN;
Neurology/Regeneration medicine, Univ. of California San Francisco, San Francisco, CA

Abstract: Autism spectrum disorder (ASD) is highly heterogenous in clinical manifestations and underlying genetics. In addition to idiopathic cases, ASD is also caused by several genetic syndromes. One of the most common genetic causes of ASD is duplication 15q (dup15q) syndrome. Due to its genetic and phenotypic homogeneity, dup15q provides a well-defined

setting to investigate ASD mechanisms. Previous bulk gene expression studies identified shared transcriptomic changes between idiopathic ASD and dup15q syndrome. However, how specific cell types are affected in different types of ASD is unknown. Previous studies have shown that upper-layer projection neurons are preferentially affected in idiopathic ASD brains. In the current study, we used single cell genomics to study dup15q iPSC derived cortical organoids and post-mortem brains. We discovered convergence of cell-type specific transcriptomic changes between dup15q and idiopathic ASD, especially in L2-3 neurons, indicating that cell-specific molecular changes are conserved between different subtypes of ASD. Moreover, we show that dysregulation of certain early developmental programs of dup15q are maintained in the mature brain. Our study identifies convergent cell type-specific gene expression changes in ASD and highlights molecular and cellular mechanisms that are shared between heterogenous types of ASD.

Disclosures: Y. Perez: None. **D. Velmeshev:** None. **L. Wang:** None. **A.R. Kriegstein:** None.

Nanosymposium

100. Autism and Synapse Development

Location: SDCC 1

Time: Sunday, November 13, 2022, 8:00 AM – 10:30 AM

Presentation Number: 100.04

Topic: A.07. Developmental Disorders

Support: NIH Grant MH111684

Title: Endogenous protein interactomics and spatial proteomics reveal converging mechanisms regulating neural functions in autism spectrum disorder

Authors: *Y. GAO, D. SHONAI, M. TRN, D. VELMESHEV, E. SODERBLOM, S. H. SODERLING;
Duke Univ., Durham, NC

Abstract: Hints of shared biology in autism spectrum disorder (ASD) across genetic risks have been inferred from RNA-level analyses. Yet, it is possible that molecular convergence is best reflected at the protein product level, where they may form as-of-yet unknown physical interactions that may be co-perturbed in disease. Here we report a comparative high-throughput genome-editing-mediated approach to tag brain proteins within mice with a biotin ligase, enabling proximity-based proteomics of endogenous interactomes. We use this new method to uncover protein complexes of prominent ASD-risk targets associated with the synapse, axonal initial segment, and nucleus. We also report co-perturbation of diverse synaptic proteomes in a panel of single-gene ASD models, and identified dysregulated proteins that intersect with the endogenous proximity interactomes. Analysis of these intersections reveals that many ASD-risk proteins interact in unexpected ways. In particular, we identified a previously unknown molecular mechanism that is disrupted in mutations of SYNGAP1, and its functional

consequences. Together, our results reveal protein-level interactomes associated with ASD-risk genes, providing a frame-work to interrogate their functions in ASD neurobiology.

Nanosymposium

100. Autism and Synapse Development

Location: SDCC 1

Time: Sunday, November 13, 2022, 8:00 AM – 10:30 AM

Presentation Number: 100.05

Topic: A.07. Developmental Disorders

Support: NICHD P50 HD093079

Title: Molecular and cellular mechanisms regulating brain overgrowth in autism

Authors: *S. CHETTY;
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Abstract: Approximately 15-20% of individuals with Autism Spectrum Disorder (ASD) have disproportionate megalencephaly (ASD-DM), with disproportionate enlargement in both gray and white matter volume. Individuals with ASD-DM have more severe behavioral and cognitive problems and are less responsive to standard therapeutic interventions, leading to very poor prognoses relative to individuals with ASD and normal head circumferences. Increases in brain size often precede clinical symptoms, suggesting that understanding the underlying mechanisms regulating brain overgrowth could provide a window of opportunity for intervention or mitigation of symptoms. Here, we generated ~40 human iPSC lines from cohorts of children (2-4 years old) with complete clinical and phenotypic data, including A) ASD subjects with disproportionate megalencephaly, ASD-DM; B) ASD subjects with normal sized brains, ASD-N; C) Typically developing (TD) subjects with disproportionate megalencephaly, TD-DM; and and D) TD subjects with normal sized brains, TD-N. We differentiated each of the iPSC lines into neural progenitor cells (NPCs) and oligodendrocyte progenitor cells (OPCs) and investigated changes at the molecular and cellular levels contributing to brain overgrowth. In the differentiated neural and glial progenitor cells, we observe increased proliferation and suppressed phagocytosis of NPCs/OPCs by macrophages in ASD-DM. RNA-sequencing of the differentiated progenitor cells reveals important signaling mechanisms related to the neuroimmune system in regulating cellular phagocytosis. In prior work, we have demonstrated that CD47 (a 'don't eat me' signal) is overexpressed in both NPCs and OPCs in 16p11.2 deletion carriers with macrocephaly contributing to reduced phagocytosis in vitro and in vivo. Treatment

of 16p11.2 deletion NPCs and OPCs with an anti-CD47 antibody to block CD47 restores phagocytosis to control levels in cellular and mouse models. Here, we show that similar neuroimmune mechanisms commonly implicated in cancer regulate cellular homeostasis in idiopathic forms of autism. Furthermore, we highlight new forms of therapy for selected autistic individuals with brain overgrowth early in the disease.

Disclosures: S. Chetty: None.

Nanosymposium

100. Autism and Synapse Development

Location: SDCC 1

Time: Sunday, November 13, 2022, 8:00 AM – 10:30 AM

Presentation Number: 100.06

Topic: A.07. Developmental Disorders

Title: Investigating the synaptic function of the E3 ubiquitin ligase UBE3B in neurodevelopment and disease

Authors: *S. VASHISTH[1], J. BANDOPADHAY[2], K. KAUR[2], M. CHAHROUR[3];
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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired communication, abnormal social behaviors, and restricted and repetitive behaviors. Pathogenic mutations in *UBE3B* result in neurodevelopmental disease, including intellectual disability, lack of speech, and ASD. UBE3B is an E3 ubiquitin ligase that tags substrate proteins with ubiquitin, marking them for proteasomal degradation. The ubiquitin-proteasome system (UPS) is known to regulate several signaling pathways critical for neurodevelopment, including neurogenesis and synaptogenesis, and mutations in various UPS genes have been identified in ASD and related neurodevelopmental disorders. To investigate the function of UBE3B and how its disruption gives rise to neurodevelopmental abnormalities, we generated a brain-specific nestin-Cre conditional knockout (cKO) mouse model and evaluated the resulting neurobehavioral phenotypes. We found that cKO neonates emit significantly fewer and shorter ultrasonic vocalizations (USVs) when separated from their mothers compared to control pups. In a direct social interaction task, cKO juveniles spent less time interacting with unfamiliar conspecifics. In the Morris Water Maze task, cKO mice had a slower latency to the target location than control mice, suggesting that a loss of UBE3B leads to deficits in learning and memory. Golgi-Cox staining showed significantly reduced dendritic complexity, length, and spine density of cortical neurons from cKO mice. To identify neuronal UBE3B substrates, we used stable isotope labeling by amino acids in cell culture (SILAC) of neural stem cells from wild type (WT) and constitutive *Ube3b* knockout (KO) mice followed by mass spectrometry. We

filtered the data for candidate direct substrates that exhibited increased protein level and decreased ubiquitination in KO compared to WT cells. Ontology analyses indicated that many of the identified candidates (~41%) are involved in synaptic development and function. Further, we found that *Ube3b* expression is modulated by neuronal activity, suggesting a potential role in regulating neuronal activity-dependent signaling networks that modulate synaptic development and function, pathways that are known to contribute significantly to ASD pathology. Our findings identify a role for UBE3B in regulating social behavior, learning, memory, and neuronal morphogenesis, and suggest that it may be involved in the synaptic function underlying these behaviors. Ongoing and future studies will further investigate the neuronal substrates of UBE3B and the activity-dependent molecular pathways it regulates.

Disclosures: S. Vashisth: None. **J. Bandopadhay:** None. **K. Kaur:** None. **M. Chahrour:** None.

Nanosymposium

100. Autism and Synapse Development

Location: SDCC 1

Time: Sunday, November 13, 2022, 8:00 AM – 10:30 AM

Presentation Number: 100.07

Topic: A.07. Developmental Disorders

Title: Drug discovery strategy of TAK-418, an LSD1 enzyme activity-specific inhibitor, for potential therapeutics of neurodevelopmental disorders

Authors: *S. MATSUDA, R. BABA, H. KIMURA;
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Abstract: Neurodevelopmental disorders such as autism spectrum disorder (ASD) are lifelong diseases characterized by core symptoms such as social deficits and repetitive behaviors. Heterogeneity and time-dependency of phenotypic traits in ASD are the major hurdles on the developments of effective drugs. Therefore, therapeutic effects of a drug targeting an individual biological signal pathway would be limited, or even if successful only few patients can be improved during a short period. Thus, we focused on epigenetics to produce better therapeutic effects by comprehensive impact on global gene expression. Histone 3 lysine 4 (H3K4) methylation is one of the most important epigenetic pathways that control neural functions including learning and memory. Lysine-specific demethylase 1 (LSD1) regulates H3K4 methylation through its histone demethylation function; however, LSD1 also transduces biological signals through the scaffolding activity of cofactor proteins. We developed small molecule LSD1 inhibitors, TAK-418 and T-448, that specifically inhibit histone demethylation function but not the scaffolding activity. Based on the selective inhibition of LSD1 enzyme activity, both TAK-418 and T-448 did not disrupt LSD1-cofactor interaction and did not cause blood cell toxicity such as thrombocytopenia. Using these inhibitors, we evaluated the therapeutic potential of LSD1 enzyme inhibition in the brain using animal models of neurodevelopmental disorders. As a result, TAK-418 and T-448 improved ASD-like phenotypes

in various rodent neurodevelopmental disorder models with prenatal valproate exposure, prenatal virus infection, and NMDA hypofunction. The efficacy was displayed when the brain LSD1 enzyme activities were fully inhibited. In addition, improving effects of TAK-418 on sociability impairments were observed not only by the treatment in juvenile stages but also in adult stages. The gene expression patterns in the disease models were dysregulated in quite a different manner across the models and across their ages; however, TAK-418 precisely counteracted the dysregulated expression in each condition. From these results, TAK-418 may recover the homeostatic regulation of global gene expression in response to each disease state, rather than control specific biological pathways. As LSD1 inhibition levels in the brain may be a good translation index to the efficacy of LSD1 inhibitors, we developed novel LSD1 PET tracer that has a potential to work as a clinical translation method. TAK-418 warrants further investigation as a novel therapeutic agent for neurodevelopmental disorders.

Nanosymposium

100. Autism and Synapse Development

Location: SDCC 1

Time: Sunday, November 13, 2022, 8:00 AM – 10:30 AM

Presentation Number: 100.08

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant R01 NS117068

Title: Neurodevelopmental role of a tRNA methyltransferase implicated in intellectual disability

Authors: *K. R. MADHWANI[1], S. SAYIED[2], C. H. OGATA[2], C. HOGAN[4], J. L. DUMOUCHEL[3], J. LENTINI[5], D. FU[5], K. M. O'CONNOR-GILES[2,6];
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Abstract: Gene regulation is crucial for neurodevelopment and neurotransmission to occur. Evidence has shown that mutations leading to global misregulation of gene expression disproportionately affect the nervous system resulting in both neurodevelopmental and degenerative disorders. Emerging work demonstrates that posttranscriptional modification of transfer RNAs (tRNAs) regulates tRNA stability, codon-anticodon pairing and translation rate and fidelity. ALKBH8 is one of two metazoan homologs of the yeast tRNA methyltransferase Trm9. Mutations in the highly conserved human ALKBH8 were recently found to cause intellectual disability in four families. However, the neuronal role of ALKBH8 remains unknown. In yeast, Trm9 methylates uridines in the wobble position of the anticodon loop to

reinforce cognate codon-anticodon pairings, resulting in translation of mRNAs enriched for cognate codons. We generated *ALKBH8* null mutants in *Drosophila* and analyzed tRNA posttranscriptional modifications by mass spectrometry. Consistent with prior mammalian findings, we observed a complete loss of wobble uridine methylation in *Drosophila ALKBH8* mutants. We next investigated ALKBH8's role in neurodevelopment and found that ALKBH8 attenuates synapse formation. Additionally, we found that *ALKBH8* mutants exhibit increased levels of reactive oxygen species (ROS). ALKBH8 is required to methylate tRNA-selenocysteine, which promotes the incorporation of selenocysteine into selenoproteins. Selenoproteins are potent regulators of oxidative stress, and we have found that blocking selenoprotein synthesis by independent means also results in synaptic overgrowth. Interestingly, oxidative stress has been shown to manifest in synaptic overgrowth. To determine if increased ROS and synaptic overgrowth in *ALKBH8* mutants are causally linked, we treated *ALKBH8* mutants with the antioxidant N-acetylcysteine amide and observed partial rescue of synaptic overgrowth. To probe the downstream consequences of increased ROS and synaptic overgrowth, we investigated memory formation using a taste aversion assay. This assay measures a sucrose-induced proboscis extension reflex after female flies learn to associate the sweet tastant sucrose with the bitter tastant quinine. We find that *ALKBH8* mutants fail to form gustatory memories, consistent with the observation of human intellectual disability. These findings support a model in which ALKBH8 regulates synaptic growth and memory formation through its role in regulating ROS via methylation of tRNA-selenocysteine, and reveals antioxidants as a potential therapy for individuals with ALKBH8-linked intellectual disability.

Disclosures: K.R. Madhwani: None. **S. Sayied:** None. **C.H. Ogata:** None. **C. Hogan:** None. **J.L. Dumouchel:** None. **J. Lentini:** None. **D. Fu:** None. **K.M. O'Connor-Giles:** None.

Nanosymposium

100. Autism and Synapse Development

Location: SDCC 1

Time: Sunday, November 13, 2022, 8:00 AM – 10:30 AM

Presentation Number: 100.09

Topic: A.07. Developmental Disorders

Support: 5R01MH097236

Title: Age-related changes in inhibitory neuron and synapse density in the basolateral amygdala in Autism Spectrum Disorder

Authors: K. L. HANSON[1], E. L. CARLSON[1], A. SHAH[1], E. HALEY[2], A. OMANSKA[1], K. D. MURRAY[3], C. M. SCHUMANN[1];
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Abstract: Autism spectrum disorder (ASD) is characterized by differences in socioemotional cognition and behavior that persist across the lifespan. Aberrant structure or function of the amygdala, a brain region involved in the processing of salient social and sensory stimuli, has been consistently noted in ASD, and hypothesized to underlie deficits in behavior and cognition. The amygdala in ASD is characterized by an excess of immature neurons in early development, followed by a decline in total neuron numbers into adulthood. It is unknown which neuronal populations may be differentially affected by neuron loss with age in the amygdala. Imbalances in the ratio of excitatory to inhibitory (E/I) neuronal activity are implicated in multiple neurodevelopmental disorders, including ASD. To investigate the hypothesis that neuron loss may disproportionately affect inhibitory GABAergic neurons in the amygdala and contribute to E/I imbalance, we performed immunohistochemical staining for GAD65/67 and parvalbumin (PV) in a unique sample of ASD (n=18) and neurotypical (NT, n=17) cases, ages 2-78, through the rostrocaudal extent of the amygdala, and utilized design-based stereological methods to estimate the total numbers of GAD65/67 and PV-positive interneurons in the lateral, basal, and accessory basal nuclei. Preliminary analyses indicate no significant differences in total GAD or PV neuron numbers or density between ASD and neurotypical cases in the basolateral nuclei. However, an interesting trend emerges with age: the density of PV-positive interneurons in the lateral nucleus of the amygdala may decrease over time in subjects with ASD. We have additionally examined a subset of cases (11 ASD, 11 NT) to measure the density of inhibitory synapses using immunolabeling for Synapsin 1 and GAD65/67, and found evidence for an age-related decline in inhibitory synaptic density in the basal nucleus in ASD. Taken together, these data support the hypothesis that E/I imbalance is a key feature of the pathophysiology of ASD with region-specific effects on microcircuitry that may contribute to progressive socioemotional impairments.

Disclosures: K.L. Hanson: None. **E.L. Carlson:** None. **A. Shah:** None. **E. Haley:** None. **A. Omanska:** None. **K.D. Murray:** None. **C.M. Schumann:** None.

Nanosymposium

100. Autism and Synapse Development

Location: SDCC 1

Time: Sunday, November 13, 2022, 8:00 AM – 10:30 AM

Presentation Number: 100.10

Topic: A.07. Developmental Disorders

Support: Ontario Brain Institute
        Canadian Institute for Health Research

Title: Structural MRI Assessment of the Neuroanatomy in 9 Novel Mouse Models Related to Autism

Authors: *J. ELLEGOOD[1], S. ASSIMOPOULOS[1], V. LAURIN[1], L. NUTTER[1], J. LERCH[1,2];
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Abstract: We have an MRI database of mouse models related to autism for the investigation of neuroanatomy in a model autism population. Recently, we have collaborated with The Centre for Phenogenomics (TCP), a member of the IMPC, to create new models.This study summarizes the differences found in 9 novel models related to autism, *Katnal2, L2hgdh, Nexmif, Otc, Pah, Rab39b, Ranbp17, Upf3b, and Ypel2*.Nine novel loss-of-function mouse lines related to autism were produced in C57BL/6NCrl (B6N) mice. 163 mice were examined for this study, 40 wildtype (WT) and 10-20 from each genotype. The age of the mice was ~P70.

MRI Acquisition – A 7.0 Tesla MRI scanner was used to acquire anatomical images of the brain. A T2-weighted, 3D fast spin-echo sequence was used, which yielded an image with 40 μm isotropic voxels in ~14 h.

Data Analysis – The images were registered together, and volume differences were calculated voxelwise and in 182 different regions (Dorr et al. 2008, Ullmann et al. 2013, and Steadman et al. 2014).

Of the 182 regions examined,15 were found to be significantly different for *Katnal2*, 124 for *L2hgdh*, 4 for *Nexmif*, 0 for *Otc*, 6 for *Pah*, 75 for *Rab39b*, 0 for *Ranbp17*, 112 for *Upf3b*, and 14 for *Ypel2*. *Katnal2* seems to have a specific deficit in CA3, *Nexmif* also shows a hippocampal difference, differences in *Pah* seem to be localized to the motor cortices and the cerebellum, and differences in *Ypel2* are in the striatum. Minimal behavioural findings were reported, and the only relevant behavioural findings were increased hyperactivity and a fear conditioning deficit in the *Upf3b* mice.

Neuroanatomy assessed with MRI provides a first look at the differences caused by autism relevant mutations in mice. These 9 models highlight several different aspects of autism that warrant further investigation, such as total brain volume differences consistent with other mouse models related to autism (Ellegood et al. 2015), and striatal differences possibly relevant to repetitive restricted behaviours.
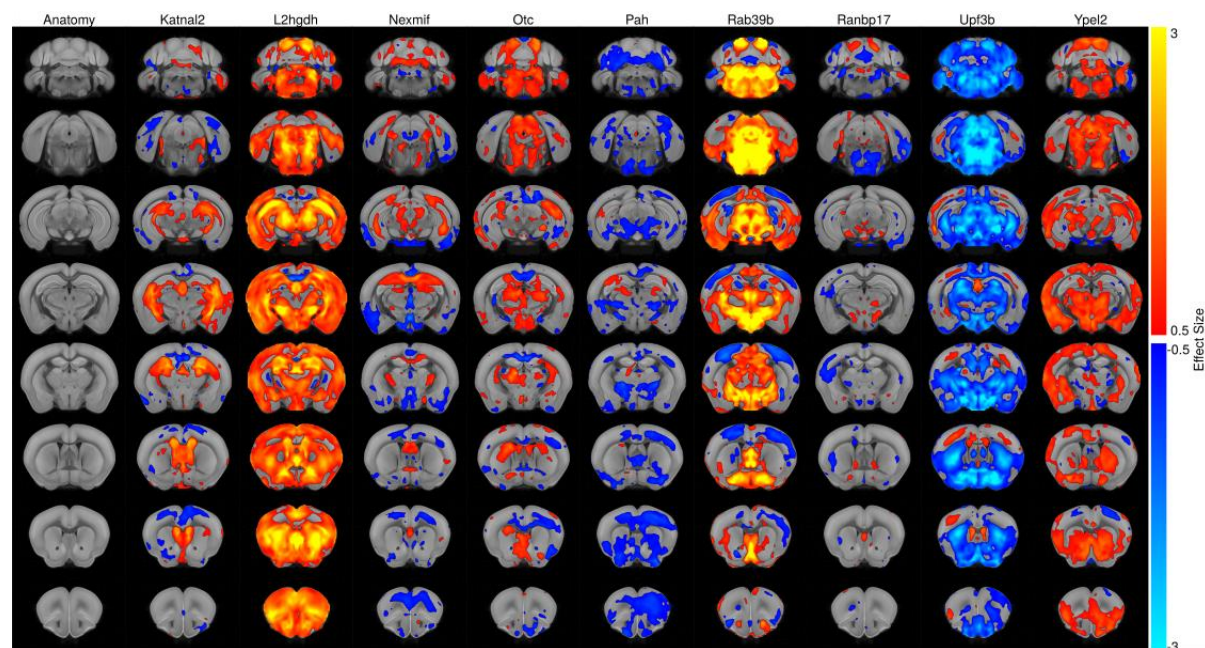
Disclosures: J. Ellegood: None. **S. Assimopoulos:** None. **V. Laurin:** None. **L. Nutter:** None. **J. Lerch:** None.

Nanosymposium

101. Alzheimer's Disease: Biomarkers I

Location: SDCC 7

Time: Sunday, November 13, 2022, 8:00 AM – 9:45 AM

Presentation Number: 101.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DBT govt of India

Title: Novel study for the quantitation of brain and blood glutathione and iron levels and its correlation among various age groups

Authors: *P. K. MANDAL[1,2], D. DWIVEDI[1], A. GOEL[3], Z. AHASAN[3];
[1]Neuroimaging, Natl. Brain Res. Ctr., Gurgaon, India; [2]Florey Inst. Of Neurosci. And Mental Hlth., Melbourne, Australia; [3]NBRC, Gurgaon, India

Abstract: Glutathione (GSH), a major antioxidant involves in thiol-redox balance, is implicated in the neurodegenerative disorders particularly in Alzheimer's disease (AD). Certain bioactive metals like iron are also involved in interplay with antioxidants like GSH in maintaining the redox balance in AD. The significant depletion of GSH level and iron enhancement in the hippocampal area have been reported in Alzheimer's disease. This novel study is directed towards finding the correlation between GSH and iron in brain and blood from 70 healthy individuals in four age groups (Group 1 (18-30Y), Group 2(31-40Y), Group 3 (41-50Y) and Group 4 (51-70Y)). Brain GSH level and metal ions (mainly iron) was measured using state-of-the art MEGA-PRESS and Quantitative Susceptibility Mapping (QSM) imaging modality respectively. Brain GSH and iron levels were processed and quantified using in-house KALPANA and SUMEDHA package respectively. GSH and iron levels in blood were detected using standard kit from sigma Aldrich. Plasma fraction of blood was used for GSH determination and serum fraction was used for iron detection in blood. The GSH and iron in brain was detected from the left hippocampus region from same participants. In this study, we found for the first time that in healthy age groups GSH level did not change in the blood or in the hippocampus region of brain. However, iron level in blood significantly increases in the Group 4 with respect to Group 1 and Group 3 ($p < 0.05$) but iron level in left hippocampus region of brain remains same across all four age groups. The base line GSH and iron detection and its correlation in the brain and blood in these healthy group provide critical information that GSH level is very much preserved in the healthy groups. However, iron level although changes in blood level, but did not vary in the brain. Our study findings will have significant impact in AD, Parkinson's, dementia with Lewy body diseases and in psychiatric disorders.

Figure: (a) GSH conc. in blood plasma (µM/ml), (b) GSH conc. in left hippocampus of brain (mM), (c) Iron conc. in blood serum (ng/µl), (d) Susceptibility (directly proportional to iron conc.) in left hippocampus of brain (ppb)
* p<0.05
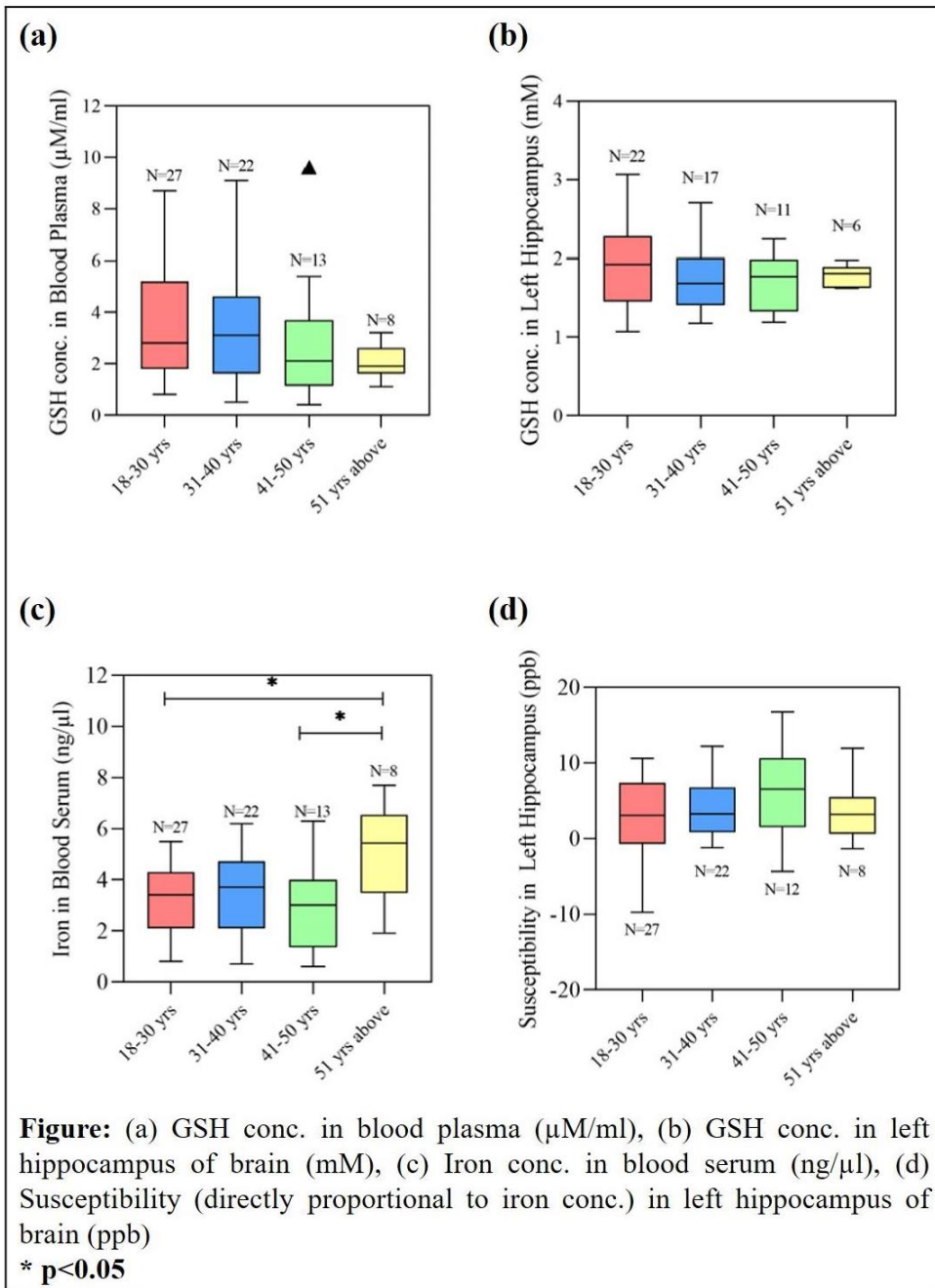
Disclosures: P.K. Mandal: None. **D. Dwivedi:** None. **A. Goel:** None. **Z. Ahasan:** None.

Nanosymposium

101. Alzheimer's Disease: Biomarkers I

Location: SDCC 7

Time: Sunday, November 13, 2022, 8:00 AM – 9:45 AM

Presentation Number: 101.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Physiological kinetics of soluble Amyloid Precursor Protein metabolites in humans and changes in turnover rates that occur in the pathophysiological Alzheimer's disease setting

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[1]Neurol., Northwestern Univ. Feinberg Sch. Of Med., Chicago, IL; [2]Neurol., [3]Med., Washington Univ. Sch. Of Med., Saint Louis, MO

Abstract: The amyloid hypothesis proposes that increased production and/or decreased clearance of amyloid-beta (Aβ) leads to higher order amyloid structures that initiate a cascade of events, culminating in neuronal death manifesting as Alzheimer's disease (AD). Sequential cleavage of Amyloid Precursor Protein (APP) generates Aβ. APP may be processed in one of at least two pathways, initially being cleaved by either α- or β-secretase (BACE1). BACE1 cleavage of APP releases soluble APP-β (sAPPβ) and subsequent cleavage by γ-secretase produces Aβ. Alternatively, α-secretase cleavage of APP precludes Aβ formation and produces soluble APP-α (sAPPα). We hypothesize that a subgroup of the AD and non-demented Amyloid+ populations overproduce Aβ because of increased BACE1 activity. Our objective is to measure CSF sAPPβ and sAPPα turnover rates, as surrogate markers of BACE1 activity, to determine if, and by how much, BACE1 activity is increased.Using stable isotope labeling kinetics/immunoprecipitation/liquid chromatography-tandem mass spectrometry methods, we quantified sAPPβ and sAPPα in CSF from human Amyloid+ (AD) and Amyloid- (control) subjects who had undergone [U-^{13}C$_6$]-leucine labeling and hourly CSF collection. The fraction of metabolite derived from *de novo* synthesis was measured by calculating normalized metabolites' hourly mole fraction labeled (MFL), over 36 hours. A model of sAPP kinetics was derived, which included these subjects' historical Aβ measurements, to study parameters of fractional turnover rates (FTR) within the whole system.Our final model of the complete cohort of 95 subjects will be presented. Modeling efforts indicate sAPPα and sAPPβ turn over slower than Aβ peptides, and sAPPα turns over a little faster than sAPPβ in most subjects (more pronounced in the setting of amyloidosis). In depth results of the Delay Time, Single Compartment FTR and Whole System FTR of the groups will be discussed. We will also assess how these parameters may help to delineate subgroups of AD. Lastly, we will address the implications for the use of these APP metabolites as biomarkers of AD in clinical trials, to both successfully select

individuals for specific trials, as well as assess therapeutic interventions once an individual is enrolled in a trial.

Nanosymposium

101. Alzheimer's Disease: Biomarkers I

Location: SDCC 7

Time: Sunday, November 13, 2022, 8:00 AM – 9:45 AM

Presentation Number: 101.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG055142
NINDS GRT00000131

Title: Noninvasive detection of oxidative stress with [18F]ROStrace predicts advanced progression of Alzheimer's disease pathology in female mice

Authors: C. HSIEH[1], Y. ZHU[2], C. HOU[3], N. KOHLI[4], J. LEE[3], H. LEE[1], S. LI[1], E. GALLAGHER[1], R. H. MACH[5], *__M. J. MCMANUS__[4];
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Abstract: Oxidative stress is implicated in the pathogenesis of the most common neurodegenerative diseases, such as Alzheimer's disease (AD). However, tracking oxidative stress in the brain has proven difficult and impeded its use as a biomarker. Herein, we investigate the utility of a novel positron emission tomography (PET) tracer, [^{18}F]ROStrace, as a biomarker of oxidative stress throughout the course of AD in the well-established APP/PS1 double mutant mouse model. PET imaging studies were conducted in wild-type (WT) and APP/PS1 mice at 3 different time points, representing early (5 mo.), middle (10 mo.), and advanced (16 mo.) life (n = 6-12, per sex). Semi-quantitation SUVRs of the plateau phase (40-60min post-injection; SUVR40-60) of ten brain subregions were designated by the Mirrione atlas and analyzed by Pmod. Statistical parametric mapping (SPM) was used to distinguish brain regions with elevated ROS in APP/PS1 relative to WT in both sexes. The PET studies were validated by ex vivo autoradiography and immunofluorescence with the parent compound, dihydroethidium. The results demonstrate that [^{18}F]ROStrace retention was increased in the APP/PS1 brain compared to age-matched controls by 10 mo. Of age, and preceded the accumulation of oxidative damage in APP/PS1 neurons at 16mo. [^{18}F]ROStrace retention and oxidative damages were higher and occurred earlier in female APP/PS1 mice as measured by PET, autoradiography and immunohistochemistry. [^{18}F]ROStrace differences emerged mid-life, temporally and spatially correlating with increased Aβ burden, which was also greatest in the female brain. In summary, [18F]ROStrace identifies increased oxidative stress and neuroinflammation in APP/PS1 female mice, concurrent with increased amyloid burden mid-life. Differences in oxidative stress during this crucial time may partially explain the sexual dimorphism in AD. [18F]ROStrace may provide a long-awaited tool to stratify at-risk patients who could benefit from antioxidant or hormone replacement therapy prior to irreparable neurodegeneration.

Disclosures: **C. Hsieh:** None. **Y. Zhu:** None. **C. Hou:** None. **N. Kohli:** None. **J. Lee:** None. **H. Lee:** None. **S. Li:** None. **E. Gallagher:** None. **R.H. Mach:** None. **M.J. McManus:** None.

Nanosymposium

101. Alzheimer's Disease: Biomarkers I

Location: SDCC 7

Time: Sunday, November 13, 2022, 8:00 AM – 9:45 AM

Presentation Number: 101.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Skin biomarkers of synucleinopathies and tauopathies

Authors: *Z. WANG[1], B. XU[2], W. ZOU[1];
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Abstract: Synucleinopathies (SOs) and tauopathies (TOs) belong to a group of age-related neurodegenerative diseases. They share prion-like pathogenesis involving propagation and accumulation of different misfolded neurotoxic proteins in the brain. SOs, including Parkinson's disease (PD), Dementia of Lewy bodies (DLB), and multiple system atrophy (MSA), are associated with α-synuclein (αSyn), while TOs consisting of Alzheimer's disease (AD), Pick's disease (PiD), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD) associated with tau. Although they are associated with either misfolded αSyn or tau aggregates, comorbidity of these diseases can be found. For instance, approximately 30-50% of AD patients can have PD pathology while patients with PSP and CBD may manifest SOs-like parkinsonism. Detection of the specific neurotoxic protein aggregates in more accessible peripheral tissues such as skin is important for diagnosis and prognosis and assessing the therapeutic efficacy in clinical trials. Our previous study implied that skin αSyn-seeding activity detected by the ultrasensitive real-time quaking-induced conversion (RT-QuIC) assay is a novel biomarker for diagnosing PD and non-PD synucleinopathies. Here we further validate our initial finding and explore the possible comorbidity of the two types of conditions. We blindly examined autopsy scalp skin samples from a large cohort of neuropathologically confirmed cases including PD (n=40), DLB (n=36), MSA (n=9), AD (n=46), PSP (n=33), CBD (n=4), PiD (n=5), and 46 non-neurodegenerative controls (NC) using RT-QuIC with recombinant human αSyn and tau as substrates. The sensitivity of skin αSyn^P-seeding activity is 82.5% for PD and 70.9% for SOs as a group, while specificity is 89.1% for NC and 84.4% for non-SOs as a whole. Using a truncated tau protein fragment termed 4RCF as substrate, our skin tau RT-QuIC showed that misfolded tau from diseased AD (n=46) and other tauopathies such as PSP (n=33) and CBD (n=4) but not normal controls (n=43) or PiD (n=6) were able to be seeded by 4RCF substrate with an 80.5% sensitivity for AD and a 95.4% specificity for NC. Our study provides the proof-of-concept that the skin seeding activity of misfolded αSyn and tau could be accurate biomarkers in the easily assessable specimen for diagnoses of SOs and TOs. Moreover, our results suggest that skin tau

RT-QuIC assay can differentiate AD from normal controls and discriminate AD from related non-AD TOs such as PiD. Key words: seeding activity, α-synuclein (αSyn), tau, skin, biomarker

Nanosymposium

101. Alzheimer's Disease: Biomarkers I

Location: SDCC 7

Time: Sunday, November 13, 2022, 8:00 AM - 9:45 AM

Presentation Number: 101.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Pattern Dynamics of Brain Waves Altered in Alzheimer's Disease

Authors: *C. HOFFMAN[1], J. CHENG[3], D. JI[4], Y. A. DABAGHIAN[2];
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Abstract: Alzheimer's Disease (AD) is a complex, progressive, and devastating neurodegenerative disease. Its detrimental effects span a variety of neurologic and cognitive functions. AD affects many parts of the brain and manifests at different levels, classically impairing memory, language skills, and muscle coordination, and to date, there is little understanding of AD mechanisms. In our study, we focus on understanding the impact of AD pathology at the neurocircuit level. The main physiological manifestation of circuit activity is the synchronized extracellular field, which gives rise to the recorded local field potential (LFP). These fields are widely studied using a variety of methods, all of which address either time-localized (instantaneous) or the time-averaged characteristics of the brain waves. We propose an alternative approach that focuses on the morphologies of waveforms—the patterns of the brain rhythms' over finite timescales. Specifically, we use two independent methods for quantifying structural regularity and irregularity of the recorded signals and correlate the resulting "stochasticity scores" with behavior. The first method quantifies the pattern's consistency with the underlying mean behavior. The second method measures how "structured" or "orderly" (e.g., periodic-like or time-clustered) the pattern is. Specifically, we studied LFPs recorded from the CA1 area of the hippocampi of WT mice running a U-shaped track with food wells on either end. Our previous work in wild type (WT) mice revealed a curious interrelationship between morphologies of θ-waves, γ-waves, and sharp wave-ripple (SWR) events and parameters of the animal's activity, such as speed and acceleration. We also noticed spatial clustering of waves with different morphology along the animal's trajectory, reminiscent of hippocampal place fields. Based on these observations, we studied circuit activity of hippocampal networks in AD

brains and found a number of alterations in LFP rhythmicity. For example, the coupling of waveforms with speed and acceleration is weaker in AD case. Additionally, the spatial selectivity is lost, suggesting that the damaged synaptic circuits in AD brains compromise wave patterning and information exchange between brain regions. These differences in brain wave pattern can be used to better understand and potentially to detect circuit-level pathologies in AD brains. Overall, these results offer a novel perspective on studying the structure, the dynamics, and the functionality of the brain waves and will provide a deeper understanding of AD at a neurocircuit level.

Nanosymposium

101. Alzheimer's Disease: Biomarkers I

Location: SDCC 7

Time: Sunday, November 13, 2022, 8:00 AM - 9:45 AM

Presentation Number: 101.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Label-free identification of Alzheimer's disease plaques using multiple co-registered nonlinear optical biomarkers

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Abstract: This research presents our discovery of two new nonlinear optical biomarkers of Alzheimer's disease (AD), namely 3-photon autofluorescence (3PAF), and Third Harmonic Generation (THG). AD is a neurological disorder that causes significant impairment of memory, cognition, motor skills, and reduced brain mass. One of the primary pathologies in the disease is the aggregation of the Amyloid-Beta (Aβ) protein and Tau protein, which cause neurotoxicity in the surrounding cells, leading to cell death. Identification of these plaques and analysis of the surrounding cells and tissue is most often done using immunohistochemistry, often with inconsistent results from different stains - depending on their mechanism of binding. For instance, consecutive slices of the same tissue stained with Congo red and Thioflavin S will show different sizes of plaques. In this research, we present the use of nonlinear optical microscopy for label-free identification and analysis of senile plaque consistency. Using simultaneous label-free autofluorescence multi-harmonic (SLAM) microscopy, we discovered that the center of a plaque can be identified by 3PAF potentially discerning neurofibrillary tangles, and the halo surrounding a plaque can be visualized with THG. This is in addition to

validating previously presented second harmonic generation (SHG), which shows the fibrous structure of amyloid, and 2-photon excitation autofluorescence (2PAF), which shows the structural extent of the plaque. Further analysis of the THG images show the cellular networks and the vasculature in the plaque microenvironment. We extended this analysis to brain slices containing hippocampal and cortical regions of 57 mice, including both AD transgenic model (5xFAD) and wild type control mice. Male and female mice were sacrificed and imaged at timepoints from 8 weeks to 52 weeks in age. The brain was excised from the mouse immediately after euthanasia and sectioned coronally at bregma -2 mm. We found that plaques start appearing in the hippocampus beginning at 10 weeks of age, and after 14 weeks, plaques can be found in the cortex and other regions. By 26 weeks, plaques were abundant throughout most of the brain. Our analysis using SLAM imaging explicitly quantifies the extent of neuronal loss in the AD brain independent of stain. Our dataset also enables metabolic measurements on the cellular network (neurons and other glial cells) in the vicinity of the plaques. We confirmed our data with both immunohistochemistry and coherent anti-Stokes Raman spectroscopy. Multimodal SLAM microscopy offers new label-free contrast and optical biomarkers for the detection and tracked progression of AD in this mouse model.

Disclosures: **K. Forouhesh Tehrani:** None. **J. Park:** None. **C. Renteria:** None. **S.A. Boppart:** A. Employment/Salary (full or part-time):; LiveBX, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IP related to SLAM microscopy.

Nanosymposium

101. Alzheimer's Disease: Biomarkers I

Location: SDCC 7

Time: Sunday, November 13, 2022, 8:00 AM - 9:45 AM

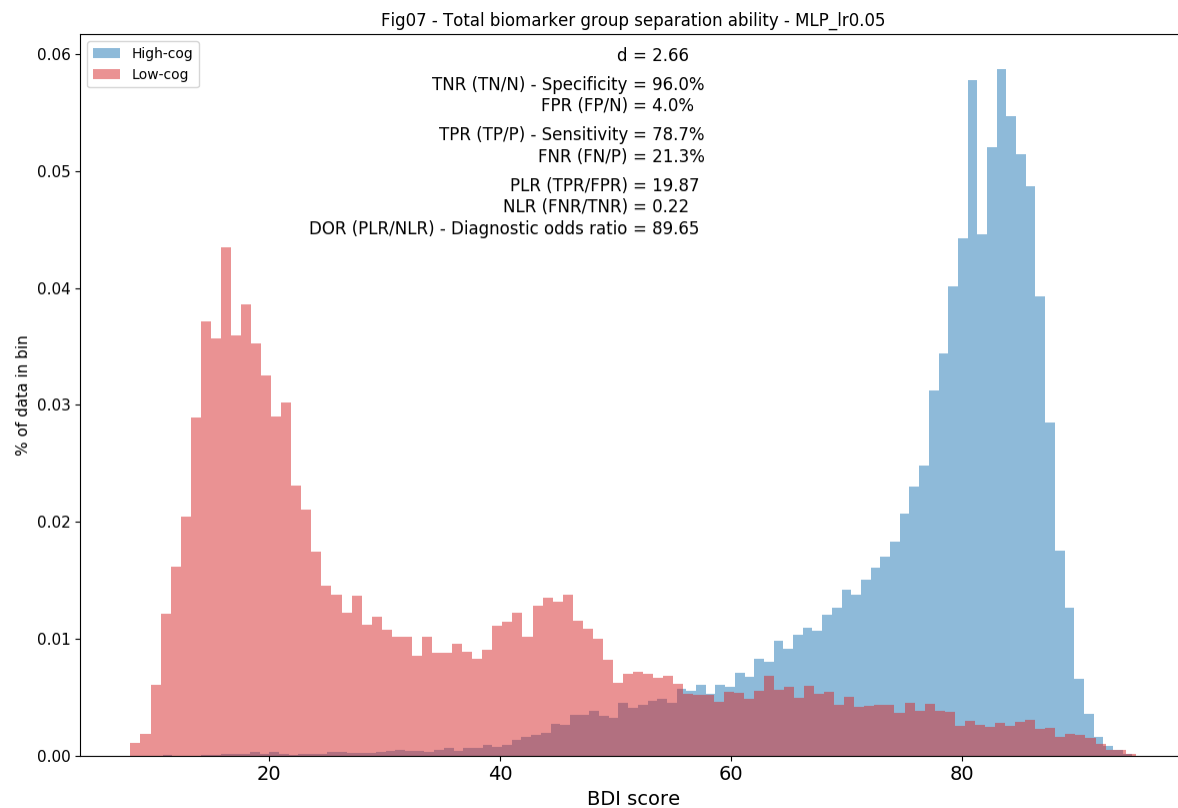**Presentation Number:** 101.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Validation of an EEG based dementia screening test

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Abstract: Dementia is one of the leading causes of impairment in old age with major advocacy groups increasingly promoting populational screening. Early detection coupled with early intervention (pharmacological, social, rehabilitation), even in the absence of a disease-modifying cure, is the only way for patients to slow their decreasing quality of life. However, the tools currently used to diagnose dementia (brain imaging, CSF biomarkers, psychometric testing) are simply not useful for large-scale, cost-effective populational screening. We tested a new approach to screen for dementia based on automatic analysis of EEG signals obtained with a mobile 24-channel amplifier (mBrainTrain), sponge EEG caps (Greentek), and custom software (BDI) for EEG recording and analysis (BrainTrip). The BDI software a) guides minimally-

trained users through the recording, b) provides online data quality assessment, c) performs automated qEEG analysis and returns a 0-100 numerical score indicating general cognitive function. The BDI software automatically detects common EEG artifacts, calculates spectral EEG features known to be associated with poor cognitive performance, and combines them into a prediction model based on density clustering and a MLP classifier. We conducted a large-scale ecological evaluation of the specificity and sensitivity of this EEG tool for cognitive assessment by performing 1344 EEG recordings on 448 seniors recruited from care homes in Slovenia between May 2021 and June 2022. Each senior participated in 3 separate recording sessions with 1-3 weeks between sessions. In each session an 8-min resting EEG (eyes open and closed) was made with the BDI system. Participants' cognitive abilities were tested with five dementia screening tests (MoCA, ADAS-cog, ACE-III, Eurotest, Phototest). The BDI had a specificity of 96% and sensitivity of 79% for detecting cognitive decline/dementia compared to a gold standard of 5 psychometric tests. Based on this performance we conclude that EEG-based assessment of cognitive function is a promising option of screening for dementia.

Fig07 - Total biomarker group separation ability - MLP_lr0.05

d = 2.66
TNR (TN/N) - Specificity = 96.0%
FPR (FP/N) = 4.0%

TPR (TP/P) - Sensitivity = 78.7%
FNR (FN/P) = 21.3%

PLR (TPR/FPR) = 19.87
NLR (FNR/TNR) = 0.22
DOR (PLR/NLR) - Diagnostic odds ratio = 89.65

Nanosymposium

102. Advances in Neuroprosthetics for Control of Motor Behaviors

Location: SDCC 33

Time: Sunday, November 13, 2022, 8:00 AM - 10:15 AM

Presentation Number: 102.01

Topic: E.05. Brain-Machine Interface

Title: Neural representational geometry of finger movements in human pre-motor cortex

Authors: *N. P. SHAH[1], D. AVANSINO[2], F. KAMDAR[3], F. WILLETT[5], L. R. HOCHBERG[6], K. V. SHENOY[7], J. M. HENDERSON[4];
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Abstract: Our ability to make combinatorial movements of multiple effectors reveals the representational capacity and flexibility of the motor cortex. What are the general principles that dictate how the neural representation of homologous movements are related across effectors and how are representations of individual movements combined for simultaneous multi-effector movements?
This question was studied using an intra-cortical Brain Computer Interface (iBCI) consisting of two Utah arrays (192 channels) in the 'hand-knob' area of the premotor cortex of a human participant ("T5") with C4 spinal cord injury as a part of the BrainGate2 clinical trial. Multi-unit neural activity was recorded as T5 attempted different multi-joint postures across fingers, within a limb and across limbs.
Consistent with previous results (Willett*, Deo* et al. 2020), similar conditions (corresponding to each combination of movement and finger) were correlated across the two hands. However, similar conditions (flexion/extension movements) across fingers were correlated only for nearby fingers, suggesting a more complex representation for effectors frequently involved in coordinated movements.
For simultaneous multi-finger movements, the neural activity evolved in a direction largely aligned with predictions from linear summation of neural activity of constituent single finger movements. However unlike a linear model, magnitude of the neural activity did not increase with the number of moving effectors. Population tuning direction for the strongly represented fingers (ex. thumb) was more consistent across conditions compared to weaker fingers (ex.

middle), revealing another deviation from the linear model. Similar phenomena were observed for other multi-joint movements across limbs (ex. wrist movements across left and right hand) and within limbs (shoulder/elbow/wrist movements on right hand). Overall, this suggests that while the relationship between the neural representation of single effector movements differs across effector groups, their combination could be described by a model of linear summation followed by normalization.

CAUTION: Investigational Device. Limited by Federal Law to Investigational Use

Nanosymposium

102. Advances in Neuroprosthetics for Control of Motor Behaviors

Location: SDCC 33

Time: Sunday, November 13, 2022, 8:00 AM - 10:15 AM

Presentation Number: 102.02

Topic: E.05. Brain-Machine Interface

Title: Modulation of the Cortical Grasp Network in a Human with Chronic Tetraplegia

Authors: *A. B. AJIBOYE[1,2], J. KRALL[1], E. CONLAN[1], C. FOLI[1], W. MEMBERG[1], E. L. GRACZYK[1,2], R. F. KIRSCH[1,2], J. P. MILLER[3,2];
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Abstract: Current human brain-machine interface systems, with a few exceptions, record from at most one to two micro-electrode arrays implanted in the primary motor cortex (M1) for decoding arm and hand movements. Investigations in non-human primates suggest that cortical activity corresponding to dexterous hand function is distributed across a cortical grasp network consisting of anterior intraparietal (AIP), inferior frontal gyrus (F5), and primary motor (F1) cortices. However, an analogous grasp network is less established in humans. As part of the Reconnecting the Hand and Arm to the Brain (ReHAB) Pilot Clinical Trial, a human participant with chronic tetraplegia due to high cervical spinal cord injury (SCI - AIS B) received penetrating microelectrode array implants in M1, IFG, AIP, and primary somatosensory (S1) cortices, along with nerve cuff electrodes for reanimating movements of the upper-limb through functional electrical stimulation (FES). This talk focuses on efforts to decode dexterous hand function from arrays implanted in the analogous human cortical grasp network (AIP/IFG/M1). The study participant completed open- (observation only) and closed-loop (user-in-the-loop)

virtual tasks to match grasp states and/or coordinated finger movements to that of a target hand. All movements began with a cued target, followed by a prep period (0.5-1 sec) before the go cue. For closed-loop movements, a standard linear decoder mapped spike threshold crossings and high frequency spike power to grasp state or continuous joint velocities of the thumb and index fingers. We analyzed the modulation of neural features and the contribution to decoding grasp state for each array in the grasp network. We examined how cortical modulation during single vs multi-finger movements could be modeled as a time evolving system following intrinsic dynamical rules. Finally, we examined methods for elucidating connectivity amongst these areas across multiple recorded channels. IFG and M1 arrays showed clear differentiability for grasp state in both prep and go phases, with IFG differentiability most prominent during the prep phase. We observed little modulation from AIP, perhaps due to AIP's deep sulcal location relative to the array placement. Single and multi-finger movements both showed similar low-dimensional rotational dynamical behaviors during the movement phases. Similar projections to the jPCA planes suggest that multi-finger movements may likely arise from simple combinations of the single singer movements. Finally, PCA based methods for elucidating high dimensional functional connectivity suggest strong correlations between these nodes of the grasp network.

Nanosymposium

102. Advances in Neuroprosthetics for Control of Motor Behaviors

Location: SDCC 33

Time: Sunday, November 13, 2022, 8:00 AM - 10:15 AM

Presentation Number: 102.03

Topic: E.05. Brain-Machine Interface

Title: Single hemisphere encoding of 48 right and left hand gestures in human precentral gyrus

Authors: *C. E. VARGAS-IRWIN[1,2,4], T. HOSMAN[3], J. GUSMAN[3,2,4], T. K. PUN[3,2], T. SINGER-CLARK[5], A. KAPITONAVA[5], N. P. SHAH[6,7], F. KAMDAR[8], L. R. HOCHBERG[4,3,5,9,2];
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Abstract: Motivation. Impaired motor function reduces independence, and can make a person fully dependent on caregivers. Emerging brain-computer interface (BCI) technology offers the possibility of building new links between the nervous system and the external world bypassing damaged motor pathways. Surveys of persons with tetraplegia have consistently identified regaining arm/hand function as a top priority towards improvement in quality of life. Understanding the cortical activity patterns driving dexterous upper limb motion has the potential to substantially benefit a broad clinical population living with limited mobility. **Methods.** The present study examines the neural activity of ensembles of human motor cortical neurons across a set of 48 different hand gestures. Our goals are to determine how this wide range of movements is encoded by examining the relationships between neural activity patterns and evaluating their potential as control signals for BCI systems. We generated latent spaces using similarity-based metrics applied to chronic microelectrode array recordings in the 'hand knob' area of precentral gyrus in the dominant hemisphere of two BrainGate participants with cervical spinal cord injury. Neural activity related to intended gestures (including spike counts and field potentials) was captured using an instructed delay paradigm. **Results.** In order to examine the structure of the neural latent space, we generated dendrograms based on the distance between the 48 gesture centroids. Overall, gesture-related neural activity patterns tended to form six main groups according to the type of movement performed: wrist, thumb, finger flexion, finger extension, digit abduction, and grasping actions. We evaluated the separability of individual gestures using nearest neighbor classifiers with 10-fold cross validation. Overall classification accuracy over the full set of 48 gestures exceeded 70% for both participants. On average it was possible to obtain classification of up to 10 different gestures with over 90% accuracy. In one participant right and left hand gestures tended to occupy neighboring regions of the latent space (classification accuracy ~87%), while in the second participant right and left gestures formed distinct clusters with almost no overlap (classification accuracy ~98%). Our results show that single unit ensemble activity recorded in a single hemisphere of human precentral gyrus can encode a wide range of gesture-related signals across both hands, providing an intuitive and diverse set of potential command signals for BCI use.

Disclosures: C.E. Vargas-Irwin: None. **T. Hosman:** None. **J. Gusman:** None. **T.K. Pun:** None. **T. Singer-Clark:** None. **A. Kapitonava:** None. **N.P. Shah:** None. **F. Kamdar:** None. **L.R. Hochberg:** Other; The MGH Translational Research Center has a clinical research support agreement with Neuralink, Paradromics, and Synchron, for which LRH provides consultative input.

Nanosymposium

102. Advances in Neuroprosthetics for Control of Motor Behaviors

Location: SDCC 33

Time: Sunday, November 13, 2022, 8:00 AM - 10:15 AM

Presentation Number: 102.05

Topic: E.05. Brain-Machine Interface

Title: A Long-Term Stable Nerve Interface for Multi-Grasp Prosthetic Control

Authors: *A. K. VASKOV[1], P. P. VU[1], C. LEE[2], D. M. WALLACE[3], A. J. DAVIS[4], T. A. KUNG[1], D. H. GATES[5], P. S. CEDERNA[1], C. A. CHESTEK[2];
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Abstract: State-of-the-art prosthetic limbs and pattern recognition systems can increase functionality for patients with upper limb amputations but rely on inputs with low signal-to-noise ratios (SNRs) and require frequent recalibration (Hargrove et al., 2017). Intramuscular recording electrodes have been shown to improve both control fidelity and reliability (Ortiz-Catalan et al., 2020). However, amputation limits the number of muscles available to provide signals for complex prosthetic functions. In clinical trials, the Regenerative Peripheral Nerve Interface (RPNI) has been shown to amplify efferent nerve action potentials in lieu of missing muscles for prosthetic control (Vu et al., 2020).
RPNIs are created by implanting a severed peripheral nerve into a free muscle graft. Three human participants with transradial amputations (P1, P2, and P3) had intramuscular bipolar electrodes surgically implanted into RPNIs on their Median and Ulnar nerves, and residual muscles. The percutaneous electrodes were connected to a Blackrock neural signal processor and real-time computer which controlled virtual and physical prosthetic hands.
To date, signal-to-noise ratios (SNRs) have been measured approximately monthly across 267 days for P1 (12 sessions), 1178 days for P2 (38 sessions), and 216 days for P3 (10 sessions). Thumb flexion evoked the largest amplitude response from the Median RPNIs with average (mean±std) SNRs of 160±69.0 (P1), 25.4±8.9 (P2), and 275±42.7 (P3). Small finger flexion or wrist flexion evoked the largest response from the Ulnar RPNIs with average SNRs of 35.8±11.0 (P1), 33.0±17.2 (P2), 14.8±3.3 (P3). There have been no significant declines in the SNR of RPNI signals over time. There has been a statistically significant linear increase in SNR over time in both P1's median and ulnar RPNIs and in one of P2's ulnar RPNIs (p < 0.05, F-test).
A Hidden Markov Model (Vaskov et al., 2022) classified grasps using EMG from RPNIs and residual muscles. P1 and P2 controlled fist, pinch, and point of a commercial multi-grip hand, while P3 controlled hand open and close and wrist rotation. These controllers did not require recalibration between experiment sessions. P1 completed a multi-grasp reach and place task with 90% accuracy using a classifier calibrated 30 days prior. P3 used a 48-day old classifier to complete a simulated coffee making task. P2 completed the most extensive controller longevity testing, maintaining > 94% grasp accuracy in a virtual posture matching task across 462 days (11 sessions) and achieving 95% grasp accuracy when completing the coffee task on the last day. Future work involves combining active wrist rotation and multi-grasp control.

Nanosymposium

102. Advances in Neuroprosthetics for Control of Motor Behaviors

Location: SDCC 33

Time: Sunday, November 13, 2022, 8:00 AM - 10:15 AM

Presentation Number: 102.06

Topic: E.05. Brain-Machine Interface

Title: Cortical control of individual fingers and finger combinations

Authors: *B. M. DEKLEVA[1], N. W. BRANTLY[1], A. R. SOBINOV[2], J. E. DOWNEY[3], S. J. BENSMAIA[3], J. L. COLLINGER[1];
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Abstract: One of the distinguishing features of the human motor system is its unparalleled dexterity in controlling the hand and fingers. There are countless tasks and behaviors that rely on individuated finger control, from buttoning a shirt to playing the piano, yet relatively little progress has been made towards incorporating individual finger control into brain-computer interfaces (BCIs). To better understand the cortical underpinnings of finger control, we recorded neural activity from two microelectrode arrays implanted in the motor cortices of two human participants with tetraplegia as part of an ongoing clinical trial conducted under an FDA Investigational Device Exemption. Although the participants do not have overt hand function, they were asked to attempt to flex the fingers on their right hand to perform virtual presses of five on-screen buttons (one button for each finger). The presses throughout a session comprised all five individual fingers as well as a few select multi-finger combinations (e.g., thumb + middle, index + ring). We found that neural population activity contained both finger-specific responses and also separate non-specific transient responses at the onset and offset of intended action, independent of the specific finger(s). As we have done previously for translating attempted grasp into cursor click, we built a binary decoder framework that uses two parallel classifiers to detect these non-specific "onset" and "offset" components. We then incorporated a separate classifier to identify the intended finger. When a nonspecific onset transient is detected, the finger-specific classifier accumulates evidence until it reaches a pre-defined confidence threshold and then outputs the associated button press. This press then persists until the offset transient is detected. Using this decoder, participants were able to achieve high quality control of individual finger presses (subject 1 best session: 97.5% accuracy, 48.5 successes/min; subject 2: 66.2%, 10.1 successes/min). While this individual finger decoding result is promising for eventual dexterous BCI control, the decoder trained only on individual fingers did not generalize to multi-finger combinations. The neural activity was indeed separable across all conditions, and

a decoder could successfully classify all individual and multi-finger presses. However, we found no clear relationship between the activity for combined finger presses and the corresponding individual finger presses. For the purposes of real-world BCI calibration, this suggests that training sets must be exhaustive, and highly dexterous control cannot be "built-up" from individual finger responses.

Disclosures: B.M. Dekleva: None. **N.W. Brantly:** None. **A.R. Sobinov:** None. **J.E. Downey:** None. **S.J. Bensmaia:** None. **J.L. Collinger:** 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.; Blackrock Microsystems.

Nanosymposium

102. Advances in Neuroprosthetics for Control of Motor Behaviors

Location: SDCC 33

Time: Sunday, November 13, 2022, 8:00 AM - 10:15 AM

Presentation Number: 102.07

Topic: E.05. Brain-Machine Interface

Title: Manual force encoding in macaques and humans

Authors: *E. V. OKOROKOVA[1], A. R. SOBINOV[1], J. E. DOWNEY[1], A. VAN DRIESCHE[1], Q. HE[1], C. M. GREENSPON[1], N. G. HATSOPOULOS[2], S. J. BENSMAIA[2];
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Abstract: From prehension to pianism, object interactions require precise control of both the movement of the hand and of the forces it exerts on objects. The time-varying posture of the hand has been shown to be encoded in the activity of populations of neurons in primary motor cortex (M1). Less is known about how manual forces are encoded in M1 because tracking the posture of the hand and the forces it exerts has proven challenging. To fill this gap, we have developed an experimental apparatus that allows us to monitor both hand movements and manual forces. We then recorded the neural activity in M1 as monkeys grasped sensorized objects, identified a population of neurons whose activity tracks manual forces, and characterized the force signal in this population. Next, we applied the insights gleaned from able-bodied macaques to develop manual force decoders in a person with tetraplegia. To this end, we instructed the participant to grasp, in a virtual environment, a set of objects with varying amounts of force while we monitored the motor activity via chronically implanted electrode arrays. We then built decoders that harness force signals in motor cortex to allow the participant to exert forces with his virtual hand. The participant then performed a variety of tasks in VR that required the manual exertion of graded forces on objects. These results pave the way for brain-controlled

bionic hands that allow the user not only to precisely shape the hand but also to apply well-controlled forces with it.

Disclosures: E.V. Okorokova: None. **A.R. Sobinov:** None. **J.E. Downey:** None. **A. Van Driesche:** None. **Q. He:** None. **C.M. Greenspon:** None. **N.G. Hatsopoulos:** None. **S.J. Bensmaia:** None.

Nanosymposium

102. Advances in Neuroprosthetics for Control of Motor Behaviors

Location: SDCC 33

Time: Sunday, November 13, 2022, 8:00 AM - 10:15 AM

Presentation Number: 102.08

Topic: E.05. Brain-Machine Interface

Support: Leibniz Wettbewerb (K265/2019)

Title: Opportunities and limitations of neural representations of observed action

Authors: *J. GOODMAN[1], S. SCHAFFELHOFER[2], H. SCHERBERGER[1];
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Abstract: Cortical areas which control movement are also active during the mere observation of others' movements. This observation-related activity has inspired the successful observation-based calibration of intracortical brain-computer interface (iBCI) decoders. However, a consensus description of several properties of observation-related activity is still lacking, limiting the potential for improvement. To address this, we obtained a dataset with unparalleled coverage of the parietofrontal grasping network, with simultaneous recordings using floating microelectrode arrays (FMAs) in the anterior intraparietal area (AIP), rostroventral premotor cortex (F5), and primary motor cortex (M1), during both the execution and observation of an unparalleled number of grips, enabled by an interchangeable turntable system for the automated presentation of dozens of unique objects. We found no evidence for the classical division of the grasping network into "canonical" and "mirror" neuron subtypes. However, at the population level, AIP and F5 express a similar pattern of activity across execution and observation contexts which explains roughly 20% of the task-conditioned variance in each area, even after accounting for the confounding visual representations of object shape. Nonetheless, different grips are only weakly differentiated from one another during the observation context. This means that observation of action on its own cannot drive observation-based iBCI calibration at the required level of action specificity. A framework for identifying the different levels of engagement that an "observation" context can take may help explain the success of observation-based calibration of iBCI decoders in spite of this.

Disclosures: J. Goodman: None. **S. Schaffelhofer:** None. **H. Scherberger:** None.

Nanosymposium

102. Advances in Neuroprosthetics for Control of Motor Behaviors

Location: SDCC 33

Time: Sunday, November 13, 2022, 8:00 AM - 10:15 AM

Presentation Number: 102.09

Topic: E.05. Brain-Machine Interface

Title: Closed-loop, brain-controlled functional electrical stimulation restores continuous finger function

Authors: *S. R. NASON-TOMASZEWSKI[1], M. J. MENDER[1], E. KENNEDY[1], J. M. LAMBRECHT[9], K. L. KILGORE[9,10,11], S. CHIRAVURI[2], N. GANESH KUMAR[3], T. A. KUNG[3], M. S. WILLSEY[4,1], C. A. CHESTEK[1,5,6,7], P. G. PATIL[4,1,7,8];
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Abstract: Brain-machine interfaces have shown promise in extracting accurate intention information regarding movements of the upper extremity from the thoughts of nonhuman primates and people with tetraplegia. Previous attempts to restore function to the user's own arm and hand have employed functional electrical stimulation (FES) to reanimate the paralyzed musculature. Most upper extremity FES work has investigated the restoration of discrete grasps. Little is known about how well continuous hand and finger movements can be controlled by FES.
Here, we use a brain-controlled functional electrical stimulation (BCFES) system to restore continuous volitional control of the finger positions to a monkey with a temporarily paralyzed hand.
We implanted one nonhuman primate with Utah microelectrode arrays in the hand area of motor-related precentral gyrus and bipolar stimulating electrodes in hand-related forearm muscles. To temporarily paralyze the hand, we applied local-anesthetic block to the median, radial, and ulnar nerves proximal to the elbow, thereby interrupting descending motor signals to the hand. To restore hand and finger function, we predicted intended finger movements using the brain-machine interface and a ReFIT Kalman filter, then used the predictions to control FES activation of the forearm. The brain-machine interface used spiking band power, a low-power signal representing the spiking activity of each electrode's nearest unit, to generate predictions to

control the Networked Neuroprosthesis, an implantable functional electrical stimulation system in a clinical trial.

In a one-dimensional, continuous, finger-related target acquisition task, the monkey improved his success rate to 83% (1.5s median acquisition time) during restoration with the BCFES system, compared to 8.8% (9.5s median acquisition time, near chance) during unrestored motor blockade. When attempting to perform a two-finger continuous target acquisition task in brain-control mode following nerve block, we found acquisition time and success rate dropped substantially (from 0.75s to 1.4s and from 100% to 90%, respectively) compared to before paralysis, likely due to the absence of sensory feedback in motor-related cortex. Interestingly, performance could be completely recovered by performing recalibrated feedback-intention training one additional time following the nerve block (0.81s acquisition time and 100% success rate, p = 0.67 between trials prior to the nerve block and trials using the second-stage ReFIT Kalman filter).

These results suggest that BCFES can restore continuous finger function during temporary paralysis using existing low-power technologies.

Disclosures: S.R. Nason-Tomaszewski: None. **M.J. Mender:** None. **E. Kennedy:** None. **J.M. Lambrecht:** None. **K.L. Kilgore:** None. **S. Chiravuri:** None. **N. Ganesh Kumar:** None. **T.A. Kung:** None. **M.S. Willsey:** None. **C.A. Chestek:** None. **P.G. Patil:** None.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.01

Topic: F.01. Neuroethology

Title: Behavioral strategies and neural mechanisms underlying stay/switch decision-making in Drosophila

Authors: *M. ARAGON[1], M. MURTHY[2];
[1]Princeton Neurosci. Inst., Princeton, NJ; [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Natural environments contain complex sensory information from multiple modalities. Animals must decide what information to attend to in order to choose the correct behavior at each point in time. How the nervous mediates decision-making in natural environments remains largely unknown. Here, we investigate how male fruit flies respond to optogenetically-driven heat pulses during courtship of a female. We developed a closed-loop stimulation paradigm in which the male receives optogenetic stimulation conditioned on his real-time song production. In

response to the stimulus, flies made one of two decisions: a "switch" decision characterized by a U-turn response away from the female, or a "stay" decision characterized by little to no change in the male's rotational speed or male-female distance and a continuation of courtship. Using SLEAP-based automated pose tracking, we quantified multiple dimensions of courtship, and constructed a generalized linear model to predict the male's upcoming decision. We found that among multiple social features, female forward velocity (fFV) has the highest predictive ability (mean cross-validated accuracy = 62%, n=51 flies). The associated fFV filter shape suggests that the male fly integrates fFV information over hundreds of milliseconds, and that high forward velocity leads to "switch" decisions. We next considered how the brain implements stay/switch decisions by recording brain-wide neural activity with 2-photon microscopy in a male fly while he received optogenetic heat pulses during courtship with a virtual female. Preliminary results suggest that low-dimensional neural activity distributed across the brain integrates female motion cues to guide the male's upcoming heat-evoked turn magnitude and direction. Together, these behavioral and neural results demonstrate both the strategies and neural computations underlying decision-making during naturalistic behaviors.

Disclosures: M. Aragon: None. **M. Murthy:** None.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.02

Topic: F.01. Neuroethology

Title: Dynamic visual processing in post-saccadic V1 visual responses of the marmoset monkey

Authors: *Y. ABRHAM[1], J. YATES[2], J. F. MITCHELL[1];
[1]Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY; [2]Vision Sci., Univ. of California at Berkeley, Berkley, CA

Abstract: All animals with image-forming eyes sample visual information through a "saccade and fixate" pattern of eye movements (Land and Nilsson, 2012). This ongoing cycle of saccades and fixations forms the dynamics of natural vision. Here, we investigated how perisaccadic neural responses during free-viewing of natural images relate to the tuning properties of neurons in primary visual cortex (V1). To quantify saccade modulation we recorded from V1 in two marmoset monkeys using silicon linear arrays as they freely viewed full-field natural scenes. We found that on average neural responses time-locked to saccade onset exhibited an early suppression beginning from the onset that was followed by a post-saccadic rebound in excitatory activity. The latency of the post-saccadic rebound varied considerably across the neural population. To determine whether the latency of these saccade dynamics varied with neural

tuning properties we mapped tuning for spatial and temporal frequency, and orientation selectivity, in an independent set of trials. In those trials marmosets free-viewed flashed (60hz) full-field gratings that varied in spatial frequency and orientation. We used linear regression to estimate a spatiotemporal kernel using subspace reverse correlation (Ringach et al., 1997). The preferred spatial frequency was estimated from tuning at the peak temporal lag while temporal frequency preference was estimated by the Fourier transform of the temporal kernel. Among the neurons with significant orientation tuning, we observe a significant correlation between spatial frequency preference and the latency of post-saccadic rebound, with neurons tuned to higher spatial frequencies responding later. The pattern with temporal frequency was less clear (only significant in one of two animals), and reflected that neurons tuned for higher temporal frequencies responded earlier. These results support the idea that neural responses during the saccade-fixation cycle follow coarse-to-fine distinctions proposed to operate in early visual processing (Burr, 1981; Watt, 1987; Hedge, 2008, Boi et al., 2017).

Disclosures: Y. Abrham: None. **J. Yates:** None. **J.F. Mitchell:** None.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.03

Topic: F.01. Neuroethology

Title: Coordinating patterns and directionality in asymmetrical behaviors

Authors: *X. LI, K. THIERINGER, E. IRELAND, M. MURTHY;
Princeton Univ., Princeton, NJ

Abstract: Animals evolved with bilateral symmetry but many behaviors require asymmetric motions and transitions between sides of the body. For example, to hit a tennis ball, players first prepare the stroke by bringing the racket to one side of the body, then accelerate until the racket makes contact with the ball, and finish with a follow-through motion. Meanwhile, the player can choose between using backhand or forehand (making the stroke from the left or right side of the body), and can even switch hands as they follow the ball in the air. We do not know how neural circuits coordinate these two aspects of behavior (motor planning/patterning and directionality) in any system. We take advantage of an asymmetric behavior in Drosophila to study this question. Drosophila melanogaster males are known to sing with one wing at a time, choosing the wing that is best oriented towards the female's ear. Males also pattern their songs into three modes of different spectrotemporal quality and amplitude (Clemens et al., 2018) depending on recent sensory feedback from females (Coen et al., 2014 & 2016). How the two are coordinated (wing choice and song patterning) has not yet been studied.

Here we show using quantitative behavioral analysis that wing choice and song patterning follow distinct timescales. We demonstrate that song continues during wing switches without disruption or alteration of the song pattern, suggesting that song pattern information is provided bilaterally to both wings to ensure smooth transitions during switches. Moreover, we show that female head location is highly predictive of the male wing choice in their monocular zone (when the male fly sees the female head with one only one eye). Intriguingly, when the male fly sees the female head with both eyes, his choice becomes stochastic. We then explore how sensory information from different modalities affects wing dynamics by unilaterally or bilaterally removing sensory input to the male. We show that vision, auditory, mechanosensory, and gustatory inputs play different roles in mediating wing choice, laterality, and preference. Furthermore, using genetic manipulations, optogenetics, and patch-clamp electrophysiology, we explore the roles of individual descending neurons on wing and song pattern choices. Our ongoing study focuses on the computational rules and circuit mechanisms by which ongoing sensory information patterns song sequences and modulates directionality simultaneously. Together, this work provides insights into the neural mechanisms that coordinate the patterning and the directionality of motions in complex behaviors.

Disclosures: X. Li: None. **K. Thieringer:** None. **E. Ireland:** None. **M. Murthy:** None.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.04

Topic: F.01. Neuroethology

Title: Cortical-striatal interactions underlying the selection and performance of naturalistic behavior

Authors: *A. HSU[1], M. A. NICHOLAS[2], E. A. YTTRI[2];
[2]Carnegie Mellon Univ., [1]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: How do the multiple nodes of the motor system work together to create behavior? Despite several studies, most of what is known has come from studies of single brain regions in overtrained behaviors. These factors limit interpretability and create potential confounds. Thus, we chronically implanted Neuropixels electrodes to simultaneously record all layers of motor cortex, dorsal and ventral striatum concurrently with 24/7 video. From the extracted three-dimensional pose estimation, we applied our state-of-the-art unsupervised behavioral identification software, B-SoiD (Nat Comms), to discover and quantify the spontaneous behaviors of the mouse. We discovered robust and distinct neural representations of the naturalistic behaviors, their kinematics, and their state transitions.1) Across the areas we recorded, the majority of neurons in corticostriatal circuit modulated their activity at the onset of the ML-derived behaviors. Across brain areas, both positive and negative modulation was observed, typically starting around ~200ms before the onset of a given behavior. This

modulation was not observed in shuffled data, and similar responses were observed across animals. Across the areas studied, we found that different actions occupied distinct neural subspaces. 2) With a simple random forest classifier, we were able to decode behavior given neural activity. We found that striatum carries more reliable information regarding the different sub-grooms. We also discovered that the decoding accuracy improves more sharply at behavioral onset in motor cortex when compared to striatum.

Disclosures: A. Hsu: None. **M.A. Nicholas:** None. **E.A. Yttri:** None.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.05

Topic: F.01. Neuroethology

Title: Differences in an aversive teaching signal predict and are associated with differences in stress susceptibility

Authors: *A. ZHUKOVSKAYA[1], L. WILLMORE[1], C. A. ZIMMERMAN[1], A. L. FALKNER[2], I. B. WITTEN[1];
[2]Princeton Neurosci. Inst., [1]Princeton Univ., Princeton, NJ

Abstract: Chronic stress can result in a maladaptive state in a subset of individuals, while others are resilient to the same stressor. Pioneering work has uncovered differences across susceptible and resilient mice in the molecular and neural changes resulting from chronic social defeat stress (CSDS). However, it remains unclear what signals are present during the social stress itself could drive these individual differences in response to stress. Do the same neural teaching signals implicated in other forms of aversive learning also drive changes in behavior due to CSDS, and if so, does their presence before or during the stress predict individual differences in learned associations? Using calcium imaging, we recorded activity in the Lateral Habenula (LHb), a region involved in aversive learning, before, during, and after a 10-day chronic social defeat stress paradigm. Behaviorally, as a result of CSDS, susceptible mice form an aversion to the aggressor strain, but an increased preference for their own strain. In contrast, resilient mice display an equal preference for the aggressor strain and their own strain. Consistent with strain-specific learning in susceptible mice, the LHb is active after (but not before) CSDS, when susceptible mice are in the proximity of a conspecific of the aggressor strain, but not of their own strain or of another strain. In contrast, resilient mice show little LHb activity in any case. During CSDS, LHb activity is higher in susceptible mice to the onset of attack by the aggressor, and optogenetically activating the LHb during stress to mimic this pattern biased towards susceptibility. Finally, after defeat, during the elevated plus maze (an assay for anxiety-like behavior), LHb activity is elevated in relation to open arm exits in susceptible mice, suggesting activity in this structure may contribute to an anxiety-like state that generalizes beyond the

aggressor. Taken together, these results point to an aversive teaching signal in the LHb emerging during defeat and contributing to the formation of the susceptibility phenotype.

Disclosures: A. Zhukovskaya: None. **L. Willmore:** None. **C.A. Zimmerman:** None. **A.L. Falkner:** None. **I.B. Witten:** None.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.06

Topic: F.01. Neuroethology

Support: HHMI Janelia Research Campus

Title: Large-scale neural recordings during natural generalization

Authors: M. PACHITARIU[1], L. ZHONG[2], M. A. NUÑEZ[2], C. STRINGER[3];
[1]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA; [2]Janelia Res. Campus, Ashburn, VA; [3]HHMI- JANELIA RESEARCH CAMPUS, ASHBURN, VA

Abstract: Generalization broadly refers to the ability to classify new stimuli based on previous experience with similar stimuli. Different generalization rules may lead to different behaviors even for the same exact stimulus. A simple example would be to classify flowers, which could be done by color or by their number of petals. After seeing enough examples of flowers with their assigned class labels, an observer may infer the correct generalization rule and use it to make predictions. However, in ecological scenarios an animal rarely gets to see many examples before it needs to make decisions. Presented with a single yellow flower with 4 petals, which one is more similar: an orange flower with 10 petals, or a red flower with 5 petals? The answer, of course, is ambiguous. In such cases, the observer may generalize according to some internal, pre-existing rule which we call "natural generalization". We have trained head-fixed mice to perform a variety of tasks that require natural generalization, including a task that requires learning from a single example and a virtual reality task where rewards are delivered stochastically. At the same time, we are recording large-scale neural activity from up to 70,000 neurons simultaneously and from many cortical brain areas, using two-photon calcium imaging with a large field of view mesoscope. We used these recordings to investigate the neural computations happening during the task, and found a rich repertoire of neuronal activity patterns corresponding to different stages of the computation. Furthermore, we investigated the sensory representations that support natural generalization, and tracked their changes from before to after task learning, as well as in dark-reared mice with no prior visual experience. We find that there are complex changes in responses that can be explained by a model which selects between multiple candidate generalization rules based on the available evidence. Looking forward, our approach of combining large-scale neural recordings with flexible behavioral paradigms shows good promise for addressing a variety of cognitive phenomena in mice.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.07

Topic: F.01. Neuroethology

Support: BrainPlay ERC
            DAAD
            HFSP

Title: Unsupervised discovery of behaviorally relevant brain states in rats playing hide-and-seek

Authors: B. BAGI[1], M. BRECHT[2], *****J. I. SANGUINETTI SCHECK**[3];
[1]Humboldt Univ. of Berlin -BCCN, Berlin, Germany; [2]Humboldt University/ BCCN Berlin, Humboldt University/ BCCN Berlin, Berlin, Germany; [3]Harvard Univ., Harvard Univ., Cambridge, MA

Abstract: In classical neuroscience experiments neural activity is measured across many identical trials of animals performing relatively simple tasks and is then analyzed by relating neural responses to pre-defined experimental parameters. This type of analysis is not suitable for patterns of behavior that unfold freely, such as play behavior. Here we attempt an alternative approach for exploratory data analysis on a single-trial level, applicable in more complex and naturalistic behavioral settings, in which no two trials are identical. We analyze neural population activity in prefrontal cortex (PFC) of rats engaging in the well-known game of hide-and-seek and show that it is possible to discover what aspects of the task are reflected in the recorded activity, even with a limited number of simultaneously recorded cells (≤ 31). Using hidden Markov models, we cluster population activity in PFC into a set of neural states, each associated with a pattern of neural activity that reoccurs throughout the experiment. Despite variability in behavior from trial to trial, relating the inferred states to the events of the hide-and-seek game reveals neural states that consistently appear at the same phases of the game. Furthermore, we show that by applying the segmentation inferred from neural data to the animals' behavior we can explore and discover novel correlations between neural activity and behavioral context. Finally, we replicate the results in a second data set, and additionally show that population activity in PFC displays distinct sets of states during playing hide-and-seek and observing others play the game. Overall, our results showcase the applicability of new population analyses in naturalistic neuroscience.

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.08

Topic: F.01. Neuroethology

Title: Disentangling the Neuronal Correlates of Free Behavior

Authors: *B. VOLOH[1], D. MAISSON[3], B. Y. HAYDEN[4], J. ZIMMERMANN[2];
[2]Dept. of Neurosci., [1]Univ. of Minnesota, Minneapolis, MN; [3]Dept. of Neurosci., Univ. of Minnesota Twin Cities Campus: Univ. of Minnesota Twin Cities, Minneapolis, MN; [4]Univ. of Minnesota Twin Cities, Saint Paul, MN

Abstract: Complex behavior is composed of subcomponents that are organized by the brain, and are repeated and configured as required. The relative prominence of such sub-components across tasks and individuals provides a unique "behavioral fingerprint", which can be leveraged to understand the underlying coordination of neural circuits across brain areas. However, in typical electrophysiological tasks, subjects are typically restrained, which necessarily restricts behavioral expression, and thus obfuscates coordination between areas that arise during free behavior. To ascertain the contribution of different brain areas to the expression of free behavior, we recorded synchronized videos with 62 cameras while subjects were free to roam in a large, open enclosure and engage with a standard depleting-rewards foraging task. We performed simultaneous wireless recordings across the frontal expanse, recording ~10000 neurons across two subjects. Body landmarks were reconstructed using OpenMonkeyStudio, an open-source software package our lab has developed. Leveraging embedding approaches and graph-theoretic tools, we developed a novel unsupervised pipeline that extracted distinct postures. Graph-theoretic analysis revealed that postures were organized hierarchically into extended sequences of actions. The temporal extent of organized behavior differed by subjects but not task demands, suggesting subject-specific intrinsic capacity. Building on these results, we found that – after regressing out the effect of subject position and task events – neuronal firing was tuned to postures across the frontal expanse, including cingulate, prefrontal, and motoric subdivisions. Furthermore, the degree of postural tuning differed across a dorsal-ventral axis, with motoric subdivisions showing greatest tuning, and deeper structures such as orbitofrontal cortex exhibiting the weakest tuning. Taken together, these results provide a robust framework for understanding the neuronal circuitry underlying self-directed, free behavior.

Disclosures: B. Voloh: None. **D. Maisson:** None. **B.Y. Hayden:** None. **J. Zimmermann:** None.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.09

Topic: F.01. Neuroethology

Title: Attack motifs vary across sexes and environments in outbred CD1 mice: machine learning approaches to analyzing complex social behavior.

Authors: *N. L. GOODWIN[1], S. R. NILSSON[2], J. CHOONG[2], S. A. GOLDEN[3];
[2]Biol. Structure, [3]Dept. of Biol. Structure, [1]Univ. of Washington, Seattle, WA

Abstract: Background: Variations on chronic social defeat stress (CSDS) and resident intruder (RI) testing are heavily used in behavioral neuroscience, often interchangeably. Consequently, a growing literature demonstrates the importance of social dynamics between dominant and subordinates, and their relation to stress and coping strategies. Due to the technical challenge of manual behavior annotation, results are typically presented as summary statistics of simplified metrics (latency, number of bouts, etc.), which can obfuscate social influences on decision making and nuanced differences in behavioral strategies. The introduction of approaches for automated behavior classification have helped to alleviate this problem; however, as these approaches provide for more granular behavioral information it is more important than ever to establish how experimental parameters influence these classifications. Here, we have used a combination of supervised and unsupervised learning techniques to better understand (1) variation in attack behavior using sex as a biological variable and (2) how environmental context alters attack behavior in males. **Methods**: We used outbred CD1 male and female mice as aggressors in CSDS assays for 5 days (5min/day) against a subordinate same-sex intruder. Additional males underwent home-cage RI assays for 3 days (5min/day). We recorded videos from above and performed pose estimation in DeepLabCut, which we imported into our program Simple Behavioral Analysis. We calculated ~500 features per frame, and used random forest supervised algorithms to analyze attack, defensive, and escape behaviors. We then performed targeted unsupervised analysis on identified attack bouts using UMAP dimensionality reduction and HDBSCAN clustering. We compared male and female mice in CSDS, and male mice across CSDS and RI environmental contexts. **Results**: Behavior in CSDS across sexes, and in males across assays are grossly similar as assessed via standard measures. Unsupervised analysis of attack bouts, however, reveals substantial differences between groups. Across sexes, males demonstrate two attack clusters including and active and stationary attack type, while females exhibit three. Across environmental contexts, males in RI testing exhibit five clusters versus the two in CSDS. RI motifs include "yanking" behavior that was unseen in CSDS. **Conclusions**: Here, we have presented two biological datasets of interest to the aggression field, indicating that machine learning techniques can identify distinct behavioral motifs across sexes and environments which were obfuscated by field-standard measures of behavior duration and number of bouts.

Disclosures: N.L. Goodwin: None. **S.R. Nilsson:** None. **J. Choong:** None. **S.A. Golden:** None.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.10

Topic: F.01. Neuroethology

Title: A dynamic sequence of visual processing initiated by gaze shifts in freely moving mice

Authors: P. R. L. PARKER, D. M. MARTINS, ***E. S. P. LEONARD**, E. T. T. ABE, S. L. SHARP, N. M. CASEY, C. M. NIELL;
Univ. of Oregon Inst. Of Neurosci., Univ. of Oregon, Eugene, OR

Abstract: Animals move their head and eyes both as a result of locomotion through the environment and to sample the visual scene. Previous studies have demonstrated neural correlates of head and eye movements in rodent primary visual cortex (V1), but the sources and computational roles of these signals are unclear. We combined measurement of head and eye movements with high density neural recordings in freely moving mice to show that neurons in V1 respond primarily to gaze shifts, where large amplitude head movements are accompanied by saccadic eye movements, but not to large head movements where compensatory eye movements stabilize gaze. A variety of activity patterns immediately followed gaze shifts, including units with positive, biphasic, or negative responses. These responses were greatly diminished in the dark for the vast majority of units, replaced by a uniform suppression of activity, suggesting that they are largely visually driven. Strikingly, gaze shifts evoked highly similar responses to those evoked by sequentially flashed stimuli in head-fixed conditions, suggesting that gaze shift transients represent the temporal response to the rapid onset of new visual input. Notably, neurons responded in a sequence that matches their temporal frequency tuning – high temporal frequency-tuned neurons responded to stimulus onset, while low temporal frequency-tuned neurons responded to the subsequent sustained input during fixation. Correspondingly, this progression follows low to high spatial frequency tuning, and is consistent with coarse-to-fine processing of the visual scene following each gaze shift. Together, our results show that active vision consists of a dynamic sequence of visual processing that is time-locked to gaze shifts.

Disclosures: P.R.L. Parker: None. **D.M. Martins:** None. **E.S.P. Leonard:** None. **E.T.T. Abe:** None. **S.L. Sharp:** None. **N.M. Casey:** None. **C.M. Niell:** None.

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Presentation Number: 103.11

Topic: F.01. Neuroethology

Support: R37-MH109728

Title: Neural correlates of natural social behavior in freely-moving macaques

Authors: *S. TREMBLAY[1], C. TESTARD[1], F. PARODI[1], R. W. DITULLIO[2], A. ACEVEDO-ITHIER[1], M. L. PLATT[1];
[2]Otorhinolaryngology, [1]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Success in group-living primates, including humans, requires navigating complex and diverse social relationships. Current understanding of the neural mechanisms underlying primate social behavior derives almost exclusively from highly-constrained and scripted laboratory tasks. Precisely how the primate brain dynamically navigates species-typical social interactions, in all their richness and complexity, remains largely unknown. Here we leverage new technology to investigate the neural correlates of an unprecedentedly diverse array of species-typical behaviors in freely-moving, socially-interacting rhesus macaques. We recorded single neurons from the mono-synaptically connected inferior temporal area TEO and prefrontal area 45 in two male macaques (n=6,129 units, 20 sessions) while they interacted with their female partner, and varied the identity of neighboring monkeys. Monkeys exhibited a rich behavioral repertoire of up to 29 different behaviors also observed in the wild, including grooming, foraging, aggression, and mating. The behaviors of the recorded monkeys were reliably decoded on a second-by-second basis from the simultaneous activity of a few hundred neurons (>70% accuracy, chance ~6%) - even during behaviors extending over many minutes where the sensorimotor environment changed considerably. Remarkably, single neurons widely encoded both the subject's behavior (>80% selective units) and the social context in which it occurred; e.g.: whether grooming was reciprocated, initiated, or whether it occurred after a threatening event, the identity and behavior of neighboring monkeys. Moreover, neural activity was also modulated by the actions of the female partner, providing parallel representations of the behavioral states of both self and other. Surprisingly, decoding performance and single unit responses were similar in temporal and prefrontal cortices, with important implications for theories of social information processing. These preliminary results demonstrate that neural ensembles in the prefrontal and inferior temporal cortices of macaques carry information about species-typical social stimuli, behavior, and contexts required for success in the wild.

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Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.12

Topic: F.01. Neuroethology

Title: Gonadal hormone state modifies "behavior space" and coordination of the subcortical social behavior network

Authors: *E. M. GUTHMAN[1], J. IRAVEDRA-GARCÍA[1], L. SIRRS[1], P. WONNENBERG[1], T. D. PEREIRA[2], M. MURTHY[1], A. L. FALKNER[1];
[1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Salk Inst. For Biol. Studies, La Jolla, CA

Abstract: Animals flexibly adjust their social behavior across a variety of external contexts and hormone states. While changes in hormone state are well known to correlate with changes in specific social behaviors (e.g. sex and attack), it is unknown whether they create more global shifts in suites of behavior or how hormones coordinate behavioral change at the neural level. To understand how shifts in adult hormone state change global behavior, we combine unsupervised methods for behavioral discovery with novel methods to record simultaneously from many hormone-sensitive regions in the brain's subcortical "social behavior network (SBN)". To generate behavioral richness, we record mouse behavior and neural dynamics across multiple social contexts (home cage vs partner cage), partners (aggressive male, female, submissive male), and hormone manipulations (intact, gonadectomy [GDX], hormone replacement). We quantify behavior (1.9 M frames) with an unsupervised computational approach, generating low-dimensional representations of "behavior space" from behavior features derived from SLEAP-tracked mouse posture dynamics (extended from Willmore et al. 2022). To induce shifts in hormone state, mice undergo GDX and experiments are repeated in the same mice. Our data show that GDX of males and females flattens observed sex variability in low dimensional behavior space. Specifically, we find that loss of gonadal hormones increases the similarity between male and female behavior towards male partners in their homecage and towards female partners in the partner's cage. In addition, we record neural activity from hormone-sensitive populations in 11 regions of the SBN using dual-color multisite photometry simultaneously from Esr1+ and Esr1- populations (regions: Tenia Tecta, Lateral Septum, Medial Preoptic Area, Bed Nucleus of the Stria Terminalis [BNST], Anterior Hypothalamus, Ventrolateral Ventromedial Hypothalamus [VMHvl], Medial Amygdala, Arcuate Hypothalamus, Ventral Premammillary Hypothalamus [PMv], Posterior Amygdala, and Lateral Periaqueductal Gray [lPAG]). Overall, we find that GDX reduces coordinated activity and changes lead-lag relationships between SBN nodes in a brain region-, partner-, and context-specific manner. These data suggest that gonadal hormones enable cross-talk between regions in this network, creating behavior-specific subnetworks.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.13

Topic: F.01. Neuroethology

Title: Common Sense: Sex-similarity in sensory feedback despite extreme motor connectivity differences in the vocal organ of the sexually dimorphic zebra finch songbird

Authors: *J. V. GOGOLA[1,4], D. MARGOLIASH[1,2], N. B. KASTHURI[3,4];
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Abstract: How songbirds acquire and maintain songs has served as an important model for sensory-guided fine motor skill learning. Much is known about neural circuits mediating this learning, but less is known about how muscles of the syrinx (avian voice box, analogous to larynx) are optimized for song production. Here we leverage the profound sexual dimorphism in the zebra finch: males produce intricate songs; females do not. We hypothesized that motor and sensory connections in male syringes would be fundamentally different from females, reflecting this dimorphic behavior. We analyzed adult male and female syringes (n=3 each) using immunofluorescence for motoneurons (neurofilament-m), neuromuscular junctions (NMJs; α-bungarotoxin), muscle fiber type (Fast vs Superfast; MY-32), and sensory afferents (Substance P), measuring NMJ area, class (stronger en plaque "*enPl*" vs weaker en grappe "*enGr*"), proportions across muscle types, and relationship to muscle diameter. We identified an impressive sexual dimorphism – males have larger, primarily *enPl* NMJs, while females have vastly more small *enGr* NMJs ($p<$1E-25; ANOVA). The difference is not seen in control non-vocal muscles ($p=$0.69; ANOVA), indicating a clear and specific sexual dimorphism in vocal muscles. NMJ area scales with muscle fiber size for *enPl* NMJs regardless of sex ($R^2=$0.65; regression), but *enGr* NMJs primarily appear on smaller muscle fibers ($p<$1E-11; ANOVA). Females have a greater proportion of Fast than Superfast muscle fibers relative to males ($p<$1E-20; ANOVA), where *enGr* NMJs are biased to appear ($p<$1E-4; $\chi2$). *enPl* NMJs in both sexes organize into motor endplate bands, centered slightly posterior, while *enGr* NMJs are more spatially distributed – a classic sign of multiple innervation (multiple motoneurons innervating one NMJ). In marked contrast, sensory afferents distribute similarly between sexes ($p=$0.78; ANOVA), primarily in epithelia and muscle insertions, though also within muscles and nearby NMJs. Preliminary data indicates these sensory fibers may contain mechanosensitive Piezo2, a potential mechanism of sensory feedback from the syrinx (where muscle spindles have never

been found). Thus we conclude that while motor neuron connectivity correlates with sexually dimorphic behavior, sensory innervation does not. Recent work suggests song is actively repressed in zebra finch females, and we postulate these female-biased *enGr* NMJs on the smaller and weaker muscle fibers might be an anatomical correlate of that repression. This data supports the value of considering sex as a biological variable (SABV) for rigorous inquiry, as sexual dimorphisms are not always as obvious as we initially think.

Disclosures: J.V. Gogola: None. **D. Margoliash:** None. **N.B. Kasthuri:** None.

Nanosymposium

103. Network Dynamics During Naturalistic Behavior

Location: SDCC 23

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 103.14

Topic: F.01. Neuroethology

Title: Assessing preferences for different foods in nonhuman primates during unconstrained behavior

Authors: *D. L. BARACK, Y. JIANG, F. PARODI, M. L. PLATT;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Primates including humans and monkeys have a varied diet in the wild. Their diet selection is dictated by the desirability of food items, how much the items are valued, and the availability of food items, how readily accessible the items are. To make these decisions, primates learn to associate the stimulus properties of food items, such as color, texture, and number, with the subjective value experienced by selecting and consuming them. The neural circuits that govern this choice include the lateral prefrontal cortex (LPFC), one of a range of prefrontal areas that have undergone extensive expansion in the primate clade. Imaging, lesion, and electrophysiological evidence suggests a key role for LPFC in representing rules, stimulus-action-outcome representations to guide behavior that encode reward probabilities and must be updated when the environment changes. This function has never been tested in an unconstrained primate model. Here, we report on a project to wirelessly record LPFC activity while nonhuman primates forage in an unconstrained environment. Primates are offered a choice between two patches with varying food identity (different nuts: peanuts or pecans; peanuts are more caloric than pecans per cup) and density (different surface areas). Future trials will feature different numbers of nuts and a greater range of nut identities. These manipulations orthogonalize the availability and desirability of reward. Three primates have performed the behavioral task, one with simultaneous neural recordings in LPFC. All three primates showed a side bias. M1 finished all nuts from both patches on every trial, M2 finished peanuts on 9/9 trials but pecans on 7/9 trials, and M3 finished each nut on 1/3 trials. Next steps include analysis of simultaneously recorded neurophysiology data to examine coding for patch choice in LPFC during diet selection. These preliminary data suggest that unconstrained primates may be sensitive to the

caloric content of different nuts, establishing the viability of our design, and this preference may be present in LPFC activity recorded during free behavior.

Disclosures: **D.L. Barack:** None. **Y. Jiang:** None. **F. Parodi:** None. **M.L. Platt:** None.

Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.01

Topic: G.07. Post-Traumatic Stress Disorder

Support: NIMH-R01-103287
                        Fulbright US-Israel Scholarship

Title: Are We There Yet? Evaluating the Evidence for Neuroimaging-Based Biotypes of Psychiatric Vulnerability in the Acute Aftermath of Trauma

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Abstract: <u>Objective:</u> The weak link between subjective symptom-based diagnostic methods for post-traumatic psychopathology and objectively measured neurobiological indices forms a barrier to the development of effective personalized treatments. In response, recent studies aimed to stratify psychiatric disorders by identifying consistent subgroups based on objective neural markers. Along these lines, a promising study by Stevens et al. (AJP, 2021) identified distinct brain-based biotypes associated with different longitudinal patterns of post-traumatic symptoms. Here, we conducted a conceptual non-exact replication of this work, using a comparable dataset from a multimodal longitudinal study of recent trauma survivors.
<u>Methods:</u> A total of 130 participants (age=33.61±11.21 years, 48% females) admitted to a general hospital emergency department following traumatic exposures underwent demographic, clinical, and neural assessments at 1-, 6-, and 14-months post-trauma. All analyses followed the pipeline as outlined in the original study and in collaboration with its authors.
<u>Results:</u> Task-based functional MRI obtained one-month post-trauma identified four clusters of individuals based on profiles of neural activity reflecting threat and reward reactivity. These clusters were not similar to the previously identified brain-based biotypes and were not associated with prospective symptoms of post-traumatic psychopathology.
<u>Conclusions:</u> We demonstrate that slight variations in the studied population or behavioral tasks

can critically impact the replicability of brain-based biotypes and their association with clinical outcomes. Thus, caution is warranted when attempting to define subtypes of psychiatric vulnerability using neural indices before treatment implications can be fully realized. Future replication studies are needed to identify more stable and generalizable neuroimaging-based biotypes of trauma resilience and psychopathology.

Disclosures: Z. Ben-Zion: None. **T.R. Spiller:** None. **J.N. Keynan:** None. **R. Admon:** None. **I. Levy:** None. **I. Liberzon:** None. **A. Shalev:** None. **T. Hendler:** Other; Chief Medical Officer of GrayMatters Health Co (Haifa Israel). **I. Harpaz-Rotem:** None.

Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.02

Topic: G.07. Post-Traumatic Stress Disorder

Title: Computational mechanisms underlying biases in estimation of uncertainty in generalized anxiety

Authors: *P. PIRAY[1], S. ZOROWITZ[2], N. D. DAW[2];
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Abstract: Computational mechanisms underlying biases in estimation of uncertainty in generalized anxiety
Anxiety disorders have been linked to problems in processing uncertainty, which influences decision making and learning. Previous research has stressed the importance of uncertainty for controlling the speed of learning, and how such control depends on the learner inferring the noise properties of the environment, especially volatility (i.e. the speed of change) and moment-to-moment stochasticity. However, modeling work in computational neuroscience and psychiatry has neglected stochasticity and focused on estimation of volatility. Consequently, this line of work has suggested that anxiety is linked to problems in estimating volatility, but neglected potentially confounding effects of stochasticity. Here, we argue that anxiety is primarily related to problems in estimating stochasticity. We first present a new computational model of anxiety that is built on our recent theoretical work: a Bayesian account that infers both volatility and stochasticity simultaneously from experiences (Piray and Daw, 2021). We then introduce a new task that systematically manipulates both volatility and stochasticity. We show that human subjects (n=223, 134 women, age=29.1±10.4) adjust their learning rate according to theoretical expectations simulated by the model. We further present preliminary data in healthy human subjects, which shows that trait anxiety (GAD-7: (Spitzer et al., 2006)) is selectively associated with deficient estimation of true stochasticity in the environment. These results shed light on computational mechanisms underlying learning in anxiety. Moreover, the task and the modeling approach are also potentially useful for studying reinforcement learning and decision-making problems seen across various psychiatric disorders.

References:Piray P, Daw ND (2021) A model for learning based on the joint estimation of stochasticity and volatility. Nat Commun 12:6587.Spitzer RL, Kroenke K, Williams JBW, Löwe B (2006) A brief measure for assessing generalized anxiety disorder: the GAD-7. Arch Intern Med 166:1092–1097.

Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.03

Topic: G.07. Post-Traumatic Stress Disorder

Title: Computations of rewards and punishment in learning and decision-making and associations with state and trait anxiety

Authors: *C. Y. XU, I. LEVY;
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Abstract: Anxiety is thought to alter value-based learning and decision making, yet its effectsremain unclear. Previous studies have assessed these processes by both taskperformance and post-task preferences, which contributes to the mixed literature.Furthermore, nuances in behavioral flexibility, or switching between habitual and goal-directed decisions, affected by anxiety would be lost in these measurements. Here wecombine learning in the absence of decision, decision in the absence of learning, andboth together to disentangle features of learning performance, post-learning preference,and learning flexibility. Since anxiety may also differentially bias learning in a valence-dependent manner, we include both rewarding and punishing outcomes. Thus, theobjective of this proposal is to understand how expectations of rewards andpunishments are learned, how these values guide decision-making, how they areflexibly adapted under changing contingencies, and how processes are modulated bystate and trait anxiety. To do this, we developed a novel three-part task consisting ofpassive probabilistic learning, decision making, and active reversal learning.102 menand women were recruited on Amazon Mechanical Turk and completed the task inaddition to a series of behavioral surveys. Anxiety as measured by the State-TraitAnxiety Inventory (STAI) averaged a state anxiety score of 38.9 ± 12.6 and a traitanxiety score of 40.1 ± 11.9. Our preliminary model-free analyses demonstrate negativeassociations between acquisition strength of cue-outcome associations and state andtrait anxiety during initial learning, while similar associations were found solely in theloss domain following reversal learning. We also use two types of reinforcementlearning (RL) models for granulated analysis of learning parameters for each participantwhile accounting for behavioral tendencies such as perseveration. Together, these dataprovide novel insights into the

basic processes of value computation for gains andlosses and the contribution of state and trait anxiety.

Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.04

Topic: G.07. Post-Traumatic Stress Disorder

Title: Cumulative lifetime stress-but not acute stress-is associated with economic tolerance to ambiguity

Authors: *C. RAIO[1], B. LU[2], M. GRUBB[3], G. SHIELDS[4], G. SLAVICH[5], P. GLIMCHER[6];
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Abstract: Stressor exposure is common in daily life where decisions involving uncertainty are made, yet the effects of stress on such decisions are equivocal across the literature. One reason for this is because research rarely dissociates between decisions involving risk (known outcome probabilities) and those involving ambiguity (unknown probabilities). Stress is thought to render appraisals of ambiguity more negative, but little work has examined if real-world stressor exposure differentially affects decisions involving risk vs. ambiguity. To quantify risk and ambiguity tolerance, we used a standard economic approach where participants made 240 binary choices between a certain gain ($5) and a lottery where they could win $0 or more money. Critically, the probability of winning was either stated explicitly (risk) or with some degree of ambiguity. In Study 1 (n=51, Mage=25.9), participants completed the task and returned a week later to repeat the task after undergoing either an acute stress induction (Cold-Pressor Task) or a match control task. In Study 2 (n=58, Mage=25.7; in-lab) and Study 3 (n=188, Mage=39.81; online), we used the Stress and Adversity Inventory for Adults to comprehensively measure cumulative lifetime stressor exposure after the decision-making task was complete. In Study 1, we observed no between- or within-group effects of acute stress exposure on risk or ambiguity tolerance. In Study 2, lifetime stressor count was negatively correlated with the proportion of ambiguous lottery choices individuals were willing to accept (r = -0.33, p = 0.01). In contrast, no

relation emerged for risky choices (r = 0.002, p = 0.98). These ambiguity-selective effects were replicated in a subsequent online study (Study 3) using linear regression while controlling for age, gender, income and mental health (ß = -0.25, SE = 0.011). Thus, while a single exposure to an acute stressor did not affect economic tolerance for risk or ambiguity, participants experiencing greater stressor exposure over the lifetime revealed higher aversion to ambiguity (but not risk). Our findings suggest that cumulative exposure to acute stressors may foster adaptions based on experience that inform future decision-making.

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Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.05

Topic: G.07. Post-Traumatic Stress Disorder

Title: Amygdala response to pain is associated with emotional numbing in PTSD

Authors: *N. KOREM[1], O. DUEK[2], A. N. KACZKURKIN[4], S. LISSEK[5], T. OREDERU[6], D. SCHILLER[6], I. HARPAZ-ROTEM[7], I. LEVY[3];
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Abstract: Posttraumatic stress disorder (PTSD) is linked to altered pain perception, specifically with increased pain detection threshold and enhanced pain response. While pain encompasses both physiological and affective elements, the latter is often overlooked. The "increased detection threshold-increased response pattern" is also apparent in response to emotional stimuli, a phenomenon referred to as emotional numbing. Both emotional numbing and emotional aspects of pain are thought to converge in the amygdala. Here, we aim to investigate if the emotional processing of pain is correlated with emotional numbing symptoms during a fear conditioning task in PTSD. To that aim, we contrasted the response to the reinforced CS+ (CS+US) with the unreinforced CS+ in the amygdala in two independent samples of combat exposed veterans (N1:44, PTSD:20; N2:40, PTSD:20). To control for the physiological effects of

pain, we used the insula, the most consistent locus of pain processing in the brain, as a control region. In both samples, the PTSD group showed robust reduction in the amygdala response to pain, compared to the combat controls (1: β = -.2; 89% HPDi = [-.4, -.02]; 2: β = -.16; 89% HPDi = [-.29, -.02]). This response was further positively correlated with emotional numbing (1: ϱ = -3.6, 89% HPDi = [-6.1, -1.2]; 2: ϱ = -4.2, 89% HPD = [-8.7, -.00]). In contrast, we found no evidence of group difference in the insula response to pain or a correlation with emotional numbing. In conclusion, decreased amygdala activation to pain is linked to difficulty experiencing emotions (i.e., emotional numbing) in two independent samples of combat-exposed veterans. These findings suggest a common mechanism for numbing of pain and numbing of emotions in PTSD.

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Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.06

Topic: G.07. Post-Traumatic Stress Disorder

Support: NIH grant ZIA-MH002957-01

Title: The temporal representation of experiences in mood

Authors: *H. KEREN;
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Abstract: We experience mood transitions in response to our environment all the time. These mood transitions influence our cognition and behavior, yet we do not know how the relative timing of past events shapes how we feel in the moment. Here, we investigated the relationship between the timing of previous experiences and mood by combining a novel closed-loop mood controller alongside computational modeling and neural data.
Participants conducted a mood transitions task, where they gained or lostpoints. The number of points possible to win or lose during the game was programmed with an engineering-based controller (the Mood-Machine-Interface) to influence mood either upwards or downwards continuously. This method allowed us to individualize rewards in real-time and generate substantial mood transitions, across healthy as well as depressed, adolescents and adults.
We found that early experiences have a larger effect on mood than recent ones, and that the longer one is exposed to a given context, the harder it is for new events to change mood. In addition, we found that neural activity at the ACC region underlies this influence of early experiences on mood.
Overall, we show a computational model by which earliest events have the strongest effect on mood. Moreover, we present a closed-loop task for generating artificial mood transitions, which

validates that the modeling results are not caused by adaptation. This provides a neuro-computational account of mood regulation by early events and suggests new directions for individualized treatments for low mood.

Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.07

Topic: G.06. Anxiety Disorders

Title: Identification of molecular biomarkers predicting anxiety disorder in a Brazilian cohort of elderly individuals

Authors: *G. B. DE FREITAS[1], R. L. D. DA SILVA[2], F. K. SUDO[3], M. V. LOURENÇO[2], P. E. MATTOS[4], F. TOVAR-MOLL[3], D. P. MUNOZ[1], S. T. FERREIRA[5], F. G. DE FELICE[6];
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Abstract: Anxiety disorders (AD) are highly prevalent in the elderly and are characterized by fear, nervousness, apprehension, and panic. These symptoms are thought to be associated with pathophysiological alterations in the central nervous system leading to functional impairment and reduced quality of life. First-line treatments have delayed actions and undesired side effects that result in treatment withdrawal and reduced therapeutic outcomes. Developing novel and effective therapeutic approaches for AD remains a challenge, partly due to the lack of consensus on potential biomarkers' relevance and associations. Identification of biomarkers that precede or

develop alongside the onset of AD may improve diagnostic and preventive strategies, and may reveal cognitive, behavioral, and pharmacological treatment targets. Here, we analyzed a panel of biomarkers associated with AD in a Brazilian cohort of elderly individuals including controls (N = 34, 71.12 ± 6.46 y/o, geriatric anxiety index [GAI] ≤ 9) and subjects with AD (N = 25, 70.32 ± 6.68 y/o, GAI ≥ 10), either with or without dementia. We investigated CSF biomarkers and neuropsychological indices in this cohort using forward stepwise regression modeling. Demographic and cognitive parameters examined included sex, age, Rey auditory learning test – A7/A5 ratio, MMSE, geriatric depression scale (GDS), and body mass index (BMI). CSF biomarkers analyzed included BDNF, irisin, FABP3, FABP4, oxytocin, leptin, IL6, IL8, IP10, MCP1, MIP1b, RANTES, VEGF, ApoE(pan), ApoE4, ApoE4/ApoE, Ab40, Ab42, Ab42/Ab40, total tau, noradrenaline, L-dopa, DOPAC, dopamine, 5HIAA, HVA, serotonin, DOPAC/DA, HVADA, DOPAC/HVADA, 5HIAA/5HT, glutamate, glutamine, taurine, arginine, GABA, glutamate/GABA, glutamine/glutamate, glutamine/GABA, GABA/glutamate, Lipoxin A4, cys-LT, LXA4/cys-LT. Plasma glycated hemoglobin, HDL, triglycerides and plasma irisin were also analyzed. Our results indicate that CSF BDNF and Lipoxin A4 are highly relevant for our model, and their levels are correlated in control subjects but not in AD patients. Reduced CSF BDNF and Lipoxin A4 were associated with AD (GAI score). The combination of these two biomarkers in a receiver operating characteristic analysis shows high sensitivity and specificity for AD. Our results suggest that BDNF and Lipoxin A4 are possible biomarkers for anxiety disorders in elderly patients with or without dementia.

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Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.08

Topic: G.06. Anxiety Disorders

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BBRF NARSAD YI (NLB; 2018)
BBRF NARSAD YI (NLB; 2021)

Title: Threat of shock increases parietal TMS-evoked BOLD responses contralaterally.

Authors: *N. L. BALDERSTON[1], M. TEFERI[2], W. MAKHOUL[2], D. OATHES[2], Y. SHELINE[2];
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Abstract: It is known that anxiety can impact attention control and that anxiety patients have trouble focusing attention. This deficit manifests in a variety of cognitive tasks, including

working memory. Threat-related hypervigilance is a prominent symptom of clinical anxiety, cutting across multiple diagnoses, and this hypervigilance may be mediated by hyperactive orienting processes mediated by the intraparietal sulcus (IPS). In recent work, we have shown that threat of unpredictable shock increases both excitability and functional connectivity in the IPS, and that online 1 Hz rTMS reduces anxiety potentiated startle in healthy participants. Together these results point to the IPS as a key node for anxiety expression and attention control deficits, making it a prime target for neuromodulatory intervention. However, it is unclear how TMS to the IPS affects downstream regions important for the expression of fear and anxiety. To investigate this, we used interleaved TMS/fMRI during threat to understand how parietal stimulation affects the brain during periods of elevated anxiety. Subjects completed a version of the NPU task while brain activity was recorded with fMRI. The NPU threat task consists of Neutral, Predictable, and Unpredictable periods. During the neutral periods, subjects are not at risk for shock. During the predictable periods, subjects are only at risk for receiving a shock when there is a cue is on the screen. During the unpredictable periods, subjects are at risk throughout. In a typical version of the NPU task, subjects would receive bursts of white noise, designed to elicit a blink response. In the current version, these white noise presentations were replaced by single presentations of a 3-pulse 50 Hz TMS burst. We administered the TMS bursts to the right IPS during unpredictable threat, and recorded brain activity with fMRI. This interleaved TMS/fMRI study is ongoing, and we have 6 subjects with data from the IPS stimulation site. When we extract BOLD responses from the left IPS, a measure of down-stream activity, we find marginally greater TMS-evoked BOLD responses during the threat periods compared to the safe periods. These results show the feasibility of targeting the IPS during the fMRI session. However, these results should be considered preliminary results due to the small sample size (N = 6). Although preliminary, these results show that unpredictable threat increases inter-hemisphere communication, which is possibly driven by parietal hyperexcitability. These results suggest that it may be possible to develop clinical trials using inhibitory TMS protocols to reduce anxiety and improve attention control in anxiety patients.

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Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.09

Topic: G.04. Emotion

Support: ERC-2015-CoG 682591

Title: Unraveling the neurocognitive mechanisms underlying counterconditioning in humans

Authors: M. C. HOUTEKAMER[1], L. WIRZ[1,2], J. DE VOS[1,3], J. E. DUNSMOOR[4], J. HOMBERG[1], M. J. A. G. HENCKENS[1], *****E. J. HERMANS**[1];

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Abstract: Ever since the emergence of the Pavlovian threat conditioning paradigm 100 years ago, it has been suggested that extinction of conditioned threat responses could be enhanced by coupling conditioned stimuli to rewards. Both clinical and experimental work has shown that this form of rewarded extinction, known as counterconditioning, can indeed enhance extinction learning. While the neurocognitive mechanisms underlying counterconditioning are largely unexplored, previous studies suggest qualitatively different mechanisms from regular extinction. In this functional MRI study, we compared neural mechanisms underlying counterconditioning and regular extinction in a between-subjects design (N=48), and investigated their efficacy in preventing spontaneous recovery by assessing physiological threat responses, valence and arousal ratings, and recognition memory. All participants underwent differential categorical threat conditioning, in which CS+s were reinforced by mild electrical shock. Subsequently, half of participants underwent regular extinction through repeated exposure to unreinforced CS+s. The other half underwent counterconditioning: repeated exposure to CS+s integrated with a monetary incentive delay (MID) task, in which participants obtained rewards by responding to targets superimposed onto category exemplars. MID tasks are known to reliably engage mesolimbic reward pathways, including nucleus accumbens. Recovery of differential conditioned threat responses was assessed the next day. We indeed observed spontaneous recovery of pupil dilation responses after regular extinction, but not after counterconditioning. Interestingly, counterconditioning not only strengthened recognition memory for CS+ exemplars presented during counterconditioning, but also retroactively strengthened recognition memory for CS+ items from the threat conditioning phase. In line with earlier work, the ventromedial prefrontal cortex (vmPFC) was progressively more activated over the course of regular extinction learning. By contrast, participants undergoing counterconditioning showed persistent CS+-specific deactivations in the vmPFC and hippocampus, as well as activation of the nucleus accumbens. This indicates that counterconditioning may yield stronger safety memory retention via a neural mechanism that is qualitatively different from regular extinction. Our findings of decreased engagement of (ventromedial) PFC furthermore suggest that counterconditioning may be particularly effective in treatment of fear-related disorders that are associated with dysfunctions in this brain region.

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Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.10

Topic: G.04. Emotion

Title: Heightened dorsal amygdala activation during acute defensive reactions predicts development of trauma-related symptoms: a prospective study

Authors: *L. DE VOOGD[1], M. M. HASHEMI[1], W. ZHANG[1], R. KALDEWAIJ[3], S. B. KOCH[1], V. A. VAN AST[4], F. KLUMPERS[2], K. ROELOFS[2];
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Abstract: Humans show large inter-individual differences in resilience for developing stress symptoms after trauma exposure. This has rendered the identification of neurocognitive risk markers for post-traumatic stress symptoms (PTSD) an important objective, especially for high-risk professions. Therefore, well-powered prospective studies are much needed. Previous evidence and theoretical models point to exaggerated acute responses to threat as vulnerability marker for long-term trauma development. We tested for the first time whether neural signatures during various stages of the acute defense cascade predict early PTSD symptom development. In a well-powered study (N=214), we investigated police recruits at the beginning of their police training and 16 months after their first emergency aid services. We combined functional magnetic resonance imaging (fMRI), heart rate, and posturographic measurements (outside the MRI) while police recruits performed a shooting task under threat of shock. This task was previously shown to elicit a switch from freezing to defensive action (Hashemi et al. 2019). We found that heightened dorsal amygdala activations across different stages of defensive reactions predicted later developed PSTD symptoms (increase in PCL-5 scores from baseline to follow-up) after exposure to traumatic events. These findings suggest that initial dorsal amygdala upregulation, which controls a cascade of defensive reactions, confers risk of developing trauma symptoms after trauma exposure. Therefore, downregulating dorsal amygdala hyperactivity may be considered as a potential prevention target.

Disclosures: L. De Voogd: None. **M.M. Hashemi:** None. **W. Zhang:** None. **R. Kaldewaij:** None. **S.B. Koch:** None. **V.A. van Ast:** None. **F. Klumpers:** None. **K. Roelofs:** None.

Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.11

Topic: G.04. Emotion

Title: Temporal dynamics of fear memory accuracy vs. generalization

Authors: *L. WIRZ[1,2], A. PROPOSITO[1,2], A. PÜTZER[2], B. ROOZENDAAL[1], O. T. WOLF[2], E. J. HERMANS[1];
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Abstract: Research has shown that fear memories transform over time. However, there is ongoing debate on how memory representations in the brain change over time and how this relates to memory quality. Theories suggest that memories become less accurate as they transform from specific, hippocampus-dependent, toward more gist-like, neocortical representations. It remains unclear how this process may be different for fear memories. Using a fear conditioning paradigm with unique exemplars of two categories (CS+/CS-: animals/objects), two spatial contexts as occasion setters (threat and safe), and two different test intervals (1 day vs. 21 days), we investigated long-term changes in fear memory quality. Fifty-three participants (27 female, 26 male) completed fear acquisition (75% reinforcement), and a fear retrieval and item (CS+, CS- exemplars) memory recognition test after 1d (N=26) or 21d (N=27). Functional MRI data, shock expectancy ratings, skin conductance (SCRs) and pupil dilation responses (PDRs) were acquired. Analyses of expectancy ratings, SCRs and PDRs showed successful and context-specific fear acquisition. Specifically, responses to CS+ compared to CS- items were larger in the threat vs. the safe context (all F>36, all p<.001). Item recognition memory performance (d') was higher for CS+ vs. CS- items (F=5, p=.03), and lower after 21d vs. 1d (F=34, p<.001). This memory performance decrease over time was caused by a lower hit rate for CS- items (t=5,p<.001), whereas for CS+ items, specifically the false alarm rate was higher (t=-2,p=.03). This is in line with a more liberal response bias for CS+ vs. CS- items after 21d (t=-3, p=.02). The fMRI data showed stronger vmPFC activation for CS+ false alarms vs. hits after 21d vs. 1d (t=4, cluster $p_{FWE-corr}$=.003) during the item memory recognition test. This suggests that more generalized memories for CS+ items over time more strongly recruit the vmPFC. In line with this, preliminary findings show stronger activation of general category-processing regions over time (fusiform gyrus: t=5, cluster $p_{FWE-corr}$ < .001). Follow-up analyses will examine time-dependent changes in activation of category-specific processing regions, which we identify individually using a category-based functional localizer task. In sum, for safe stimuli (CS-), memory strength decreases over time (less hits), whereas for threat stimuli (CS+), memory specificity decreases over time (more false alarms, more liberal response bias, stronger vmPFC activation). These findings suggest that for threat compared to safe memories, the time-dependent incorporation into neocortical networks is accelerated, resulting in more generalized memories.

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Nanosymposium

104. Emotional State Influences on Brain Function

Location: SDCC 24

Time: Sunday, November 13, 2022, 8:00 AM – 11:00 AM

Presentation Number: 104.12

Topic: G.04. Emotion

Title: Effects of unilateral amygdala resections on event-related potentials during emotional face and word processing

Authors: *J. KISSLER[1], M. MIELKE[2], L.-M. REISCH[2], S. SCHINDLER[4], C. G. BIEN[3];
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Abstract: Medial temporal lobe structures, particularly the amygdalae, are thought to upregulate cortical processing of emotional visual stimuli. Therefore, amygdala loss should reduce commonly observed emotion enhancements in visually evoked event-related potentials (ERPs). Assuming hemispheric specializations for face and language processing, such reductions may be hemisphere- or stimulus-specific in people with unilateral amygdala damage. Therefore, we investigated the impact of left (lTLR) and right (rTLR) unilateral anteromedial temporal lobe resections including the amygdala on event-related potentials (ERPs) during attentive viewing of fearful and neutral faces as well as negative and neutral words. We also assessed appraisal of and recognition memory for the stimuli. Stimulus appraisals and recognition memory did not differ between lTLR (N=17) or rTLR (N=19) groups and healthy controls (N=20). During both face and word processing, the rTLR group lacked the emotion enhancement in the P1 component (75-125 ms) found in the other groups. In the N1 (120-180 ms) all groups showed emotion enhancements for face processing. They were largest and bilateral for lTLR patients, left-lateralized in rTLR patients and right-lateralized in the controls. For words, only lTLR patients showed an N1 emotion enhancement. In the early posterior negativity (EPN: 200 – 400 ms) all groups showed emotion enhancements for both faces and words which were particularly large in lTLR patients. For faces, EPN was large and bilateral in lTLR, right-lateralized in controls and left-lateralized in rTLR patients. For words, EPN was bilateral in lTLR but left lateralized in controls and rTLR. In the late positive potential (LPP: 400 – 800 ms), emotion enhancements were found across groups for both faces and words. They were most pronounced in lTLR patients. This pattern of ERP effects indicates that the right medial temporal lobe, including the amygdala, critically contributes to rapid emotion processing in both faces and words. Its resection abolishes early (P1) and shifts mid-latency processing (N1, EPN) processing to the contra-lesional side. The left medial temporal lobe plays a more pronounced role in sustained emotion processing and likely also in emotion regulation since its resection results in larger and more bilateral ERPs than normal. Data specify bilateral interactive processing of emotion in words and faces and underscore that multiple mechanisms contribute to emotion processing, resulting in largely normal behavioral performance after unilateral loss. This work helps specify neural mechanisms underlying the commonly seen emotion enhancements in vision.

Disclosures: J. Kissler: None. **M. Mielke:** None. **L. Reisch:** None. **S. Schindler:** None. **C.G. Bien:** None.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.01

Topic: H.03. Decision Making

Title: Direct observation of the neural computations underlying a single decision

Authors: *N. STEINEMANN[1], G. M. STINE[1], E. M. TRAUTMANN[1], A. ZYLBERBERG[1], D. M. WOLPERT[1], M. N. SHADLEN[1,2];
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Abstract: The neural computations underlying perceptual decisions have been inferred from single neuron responses averaged over many decisions. These endeavors have provided support for the deterministic rise in activity to a termination bound, predicted by drift-diffusion models of decision making. However, such averaging over many decisions (experimental trials) suppresses the stochastic, diffusion component of the decision variable that is thought to confer variability in both choice and reaction time. To assess diffusion on single trials, we used a nonhuman primate optimized version of Neuropixels probes to record from hundreds of neurons simultaneously in the lateral intraparietal area (LIP) of monkeys as they made decisions about the net direction of random dot motion.
These large-scale Neuropixels recordings furnish an estimate of the signal that gives rise to choice and reaction time on single decisions. We identified a subset of neurons, previously studied singly, that had response fields overlapping the contralateral choice target (T_{in} neurons). We found that the average firing rate of these neurons on single trials represents a combination of deterministic drift and stochastic diffusion—the integral of noisy evidence—and this signal explains much of the variability in choice and reaction times. A drift-diffusion signal is also identified by several supervised and unsupervised dimensionality-reduction methods applied to the full neuronal state space. We show that this signal is redundant with, and depends on, the activity of T_{in} neurons. Despite this redundancy, the dimensionality-reduction methods do not identify the distinct contribution of T_{in} neurons and would therefore not detect the spatially-organized structure of the population, absent a clear hypothesis. The results provide direct support for the hypothesis that a drift-diffusion signal is the quantity responsible for the variability in choice and reaction time, and they highlight important limitations of data-driven, neural state-space approaches.

Disclosures: N. Steinemann: None. **G.M. Stine:** None. **E.M. Trautmann:** None. **A. Zylberberg:** None. **D.M. Wolpert:** None. **M.N. Shadlen:** None.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.02

Topic: H.03. Decision Making

Title: The neural encoding and causal contributions of FEF, LIP, and SC to rapid categorical decisions

Authors: *O. ZHU[1], **V. SHIRHATTI**[1], **O. GOZEL**[2], **S. DAVID**[1], **S. CHANG**[1], **A. MEDOFF**[1], **B. DOIRON**[2], **D. J. FREEDMAN**[3];
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Abstract: Our ability to rapidly categorize incoming stimuli is an essential cognitive process that imbues our world with meaning and guides our behavior. Here, we designed an urgent saccade-based, motion categorization task where the monkey is presented with a random dot motion stimulus and must saccade to a color target corresponding to a learned motion category. During task performance, we recorded populations of neurons using up to six linear microelectrode arrays (Plexon V-Probes) simultaneously targeting the lateral intraparietal area (LIP), frontal eye field (FEF), and superior colliculus (SC). On average, each session yielded around 50 well-isolated single units from each area. Using linear support vector machines, we found that the population responses of each area reliably encoded task information such as the category identity, target configuration, and saccade direction prior to saccade onset. However, the magnitude and latencies of encoding were very similar in each population. To distinguish their contributions to rapid categorical decisions, we reversibly inactivated each area with muscimol while we simultaneously recorded the other two areas with linear arrays. We also used two stimulus-target configurations such that either the motion stimulus or a saccade target was placed in the inactivated visual field (IF). When the motion stimulus was presented in the IF, we found significant behavioral deficits after inactivating each area, with the strongest accuracy deficits associated with FEF and SC inactivation. When a saccade target was presented in the IF, we only found significant behavioral deficits during FEF and SC inactivation. Consistent with these behavioral deficits, we also found a reduced magnitude and delayed latency of encoding in the population responses of other areas after inactivation. Our results suggest that FEF and SC play a causal role in both motion categorization and target selection during this rapid categorization task, whereas LIP is more engaged in the categorization of motion stimuli rather than saccade planning. Ongoing analyses aim to quantify communication subspaces between areas to measure the magnitude and directionality of interactions between each area and test whether these interactions change after inactivation.

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Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.03

Topic: H.03. Decision Making

Title: From encoding subspaces to communication subspaces, to computation subspaces: Neural populations and the effective number of shared dimensions

Authors: *M. AOI[1], M. BOUASSAMI[2];
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Abstract: The development of high volume, and multi-region neural recordings have made possible recent algorithmic descriptions of neural computations, especially in the areas of perception and decision making. Most frequently, neural population activity is summarized using dimensionality reduction techniques, reducing high-dimensional time series into low-dimensional trajectories. Making sense of these trajectories and what they say about the computations that a network performs has posed a challenge to the systems neuroscience community.
Here, I describe an approach to characterizing what these trajectories say about their corresponding computational roles by quantifying the dimensionality of shared functional subspaces. First, I describe a new test statistic, the effective number of shared dimensions (ENSD), which is a measure of shared variability between two matrices. I show that this statistic can identify the dimension of shared subspaces of variability, such as the "communication subspace" between two populations, without explicit modeling fitting. I then show that this metric can similarly identify the dimensionality of shared computational spaces, such as the encoding subspaces of sensory evidence and choice in a neural population. I use this method, in conjunction with a new data analysis method that generalizes model-based targeted dimensionality reduction; a combination of regression and dimensionality reduction that allows for demixing of the effects of different task variables on the activity of neural populations. Using these two approaches, I identify "computational subspaces", that is a computational annogue of the communication subspace between two regions.

Disclosures: M. Aoi: None. **M. Bouassami:** None.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.04

Topic: H.03. Decision Making

Title: Population activity in secondary motor cortex and striatum is category-free during multisensory perceptual decision making

Authors: *C. YIN[1,2], J. COUTO[1], A. KHANAL[1], A. K. CHURCHLAND[1];
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Abstract: A long-standing question in systems neuroscience is whether neurons can be classified into categories according to their response property. The importance of addressing this question grows with the development of large-scale neural recording techniques and algorithms for understanding population activity. When a neural population is category-free, the features of population response are randomly distributed across neurons. This confers the flexibility of using the same neurons in different ways. Category-free encoding has been observed in the rat posterior parietal cortex. The encoding strategy for frontal areas and striatum is unresolved.To further address this question, we recorded electrophysiological signals in secondary motor cortex (M2) and striatum with Neuropixels probes. The activities of 180 M2 neurons and 340 striatum neurons were measured in 6 rats trained to do a two-alternative multisensory decision-making task. Neurons in both regions showed selectivity to multiple task variables, such as choice, sensory modality, and choice history. Our analyses suggested that a significant fraction of neurons encode more than one variable, that is, they have mixed selectivity. We next applied linear regression to measure the task-variable-encoding weights of each neuron. Clustering tests found no cluster in either the firing rates or the regression weights of the neurons. Projection angle index of response similarity analysis (PAIRS) further showed that the neuronal activity is largely category-free. These results indicate that the neurons in M2 and striatum are category-free in terms of information encoding. Overall, we propose that, during multisensory perceptual decision making, the neurons in M2 and striatum have mixed selectivity to multiple task variables and are category-free. This result expands our understanding of how the information is encoded in M2 and striatum at population level. It also constrains the models of M2/striatum activity.

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Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.05

Topic: H.03. Decision Making

Title: Dynamic navigation decisions employ shared and specialized cortical representations

Authors: *S.-Y. TSENG, S. N. CHETTIH, C. D. HARVEY;
Neurobio., Harvard Med. Sch., BOSTON, MA

Abstract: During navigation in dynamic environments, an animal adaptively incorporates sensory information into a plan to guide its movements. The neural underpinning of this behavior must integrate sensory processing, navigation planning, and motor execution, and furthermore adapt the rules governing their integration based on experience. Here we investigated the organizing principles of neural representations in mouse posterior cortex during dynamic navigation decisions. We used two-photon calcium imaging to densely sample activity from large populations of layer 2/3 neurons across posterior cortex in mice performing a virtual navigation task based on rule switches. We analyzed the distribution of single-neuron encoding of various sensory, motor, and cognitive variables across multiple areas. We found that, while average encoding was quantitatively distinguishable between areas, representations were highly distributed across cortical space. Neural encoding in posterior cortex was well-described with three spatially distinct gradients for visual cue, spatial position plus choice, and locomotion, with peaks in primary visual cortex, retrosplenial cortex, and posterior parietal cortex, respectively. Next, we compared the conjunctive structures of single-neuron encoding and the population geometry of neural representations for multiple variables across areas to test whether these areas specialize in the ways they combine different variables to serve distinct computations. Surprisingly, all areas combined variables similarly instead of creating unique conjunctions of variables, resulting in a high-dimensional, complex representation of variable conjunctions shared across areas. We propose that for navigation posterior cortical areas are functionally organized not in a hierarchy but in parallel, where areas are specialized to handle streams of information for distinct modalities but work coherently to synthesize a general-purpose state representation of the environment and behavior that can guide dynamic navigation decisions. We are currently analyzing layer 5 neurons to see if similar organizing principles apply, as well as comparing the encoding in projection pathways to understand whether posterior cortical areas convey similar conjunctive representations to different downstream targets including anterior cingular cortex, orbitofrontal cortex, and striatum.

Disclosures: S. Tseng: None. **S.N. Chettih:** None. **C.D. Harvey:** None.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.06

Topic: H.03. Decision Making

Title: A general framework for modeling neural dynamics during decision-making with extensions to neural populations

Authors: *D. M. ZOLTOWSKI[1], J. W. PILLOW[1], S. W. LINDERMAN[2];
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Abstract: Characterizing the single-trial dynamics of neural activity during decision-making can help identify the mechanisms used to make decisions. In particular, directly incorporating decision-making theories into statistical models of neural dynamics both provides a quantitative test of how well a proposed theory explains neural responses and enables model comparison between alternative decision-making processes. However, current approaches are limited in the number of decision-making models that can be fit to neural data. Here we propose a recurrent switching dynamical systems framework for modeling neural activity during decision-making. The framework includes the canonical drift-diffusion model and enables extensions such as multi-dimensional accumulators, variable and collapsing boundaries, and discrete jumps. We demonstrate our approach in simulation and with two proof-of-concept analyses performed on recordings of parietal cortex neurons during motion-direction discrimination tasks. First, we found that a set of six simultaneous recorded neurons were better described by two-dimensional accumulation rather than one-dimensional accumulation. Next, we identified a variable lower decision-boundary in the responses of a parietal cortex neuron. Notably, our approach also enables adapting the decision-making dynamics models to neural populations with a variety of model extensions. We conclude by proposing non-accumulation dimensions to capture dynamics not directly related to the decision-making process and temporal filters on inputs, among other extensions. We expect this framework will be useful for modeling neural dynamics in a variety of decision-making settings.

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Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.07

Topic: H.03. Decision Making

Title: Neuronal representations of the decision variable underlying perceptual choices are time-varying

Authors: *T. Z. LUO[1], **T. D. KIM**[1], **B. D. DEPASQUALE**[1], **C. D. BRODY**[2];
[1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Princeton Neurosci. Inst., Princeton Univ. / HHMI, Princeton, NJ

Abstract: Decisions based on noisy evidence are well described using a framework in which momentary evidence is integrated over time through a latent "decision variable" (DV; i.e., the accumulated evidence). While it is appreciated that the DV changes over the course of each decision, the neural representation of the DV is widely assumed to be fixed. However, it is unclear whether the representation changes within a single decision, and whether the change is deterministic or stochastic. To address this uncertainty, we carried out Neuropixels recordings in dorsomedial frontal frontex (dmFC) of rats while they made auditory decisions. Optogenetic or chronic perturbations concurrent with our recordings show dmFC to be causally involved in evidence accumulation. We developed a new model that quantifies deterministic changes in the encoding as neuron-specific time courses of coupling weights that do not vary across trials, and quantifies stochastic changes as state-dependent transitions in the degree of coupling of the entire population to the DV. The model leverages the simultaneity in our spike trains to concurrently infer the dynamics of the DV and its encoding. We found that dmFC neurons differ in their encoding dynamics and tend to be most sensitive to the DV at the beginning of the decision. Moreover, model comparison rejects stochastic coupling to the DV. Together with similar findings from striatum and motor cortex, our results indicate that the neural subspace representing the DV changes deterministically over the course of a decision. Other cognitives variables may also be represented by neural subspaces that change over the course of a behavior.

Disclosures: T.Z. Luo: None. **T.D. Kim:** None. **B.D. DePasquale:** None. **C.D. Brody:** None.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.08

Topic: H.03. Decision Making

Title: Dynamic task-belief flexibly modulates decision-related information

Authors: *C. XUE[1,2], **S. MARKMAN**[1], **R. CHEN**[3], **L. KRAMER**[1], **M. R. COHEN**[1,2];
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Abstract: Natural decisions involve two conceptually hierarchical processes: 1) inferring the most relevant task to perform in an uncertain environment (task belief / confidence), and 2) making a decision on the believed-relevant task (decision-making). Traditionally, the two cognitive processes have been studied as separate cognitive functions, mostly in research on task-switching and perceptual decision-making. Here we present multiple lines of evidence from monkey electrophysiology, human psychophysics, and artificial neural networks to demonstrate how the confidence about the relevance of a task and the confidence of perceptual decisions in the respective task are mutually dependent. We designed a behavioral task for monkey and human subjects to easily measure and manipulate the subjects' belief about the relevant task while they make perceptual decisions. Concurrent neuronal recordings in monkey cortical areas 7a and V1 allowed trial by trial decoding of task and perceptual information, respectively. From behavioral and neuronal data, we found that more confident belief of the relevance of a task is associated with better perceptual performance on the task. Inspired by these results, we proposed a possible mechanism that selectively maintains information depending on the belief about its behavioral relevance. Computational modelling with recurrent neural networks shows that the stability at which perceptual information is represented over time scales with the confidence that the information is behaviorally relevant. The model leads to a number of testable behavioral and neuronal predictions which are confirmed by further behavioral data from humans and neuronal results from monkeys. On the other hand, we also showed that trial to trial fluctuations in perception can, in turn, affect how task-belief is updated. Overall, these results show that task belief and decision making belong to one integrated system distributed across multiple brain areas. Such a system should be studied as a whole to reach a more holistic understanding of cognitive flexibility and decision-making, in both health and disease.

Disclosures: C. Xue: None. **S. Markman:** None. **R. Chen:** None. **L. Kramer:** None. **M.R. Cohen:** None.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.09

Topic: H.03. Decision Making

Title: A threshold mechanism in the primate superior colliculus for terminating perceptual decisions

Authors: *G. M. STINE[1], **E. M. TRAUTMANN**[1], **D. JEURISSEN**[1], **M. N. SHADLEN**[1,2];
[1]Columbia Univ., Columbia Univ., New York, NY; [2]HHMI, New York, NY

Abstract: Many decisions are formed by accumulating evidence until there is enough to commit to a choice. When decisions are communicated with an eye movement, neurons in the lateral intraparietal area (LIP) represent the accumulation of evidence. It has long been hypothesized that downstream areas terminate the decision process when LIP activity reaches a threshold level. We recorded from neurons in the superior colliculus (SC), a primary downstream target of LIP, while monkeys performed a reaction-time, motion discrimination task. Simultaneously, we recorded from hundreds of LIP neurons using Neuropixels probes optimized for use in non-human primates. We identified a subpopulation of LIP neurons with spatial selectivity that matched that of the SC neurons, allowing access to ensemble firing rates from LIP and SC on single trials.
Single-trial analyses revealed distinct computations in LIP and SC that were not evident in trial-averaged data. We found that single-trial activity in LIP reflects drift-diffusion dynamics, as previously inferred. In contrast, single-trial dynamics in SC manifest as quiescence and bursts—one immediately before the saccade, preceded by smaller, non-saccadic bursts on 10% of trials. Analysis of LIP activity aligned to SC bursts suggests that SC bursts are the result of a threshold computation, involved in terminating the decision, that is applied to the combination of the LIP firing rate and its derivative.
We confirmed this hypothesis by focally inactivating SC with muscimol. The combined effect of SC inactivation on the monkeys' choices and reaction-times is diagnostic of an impaired threshold mechanism for committing to a contralateral choice. Recordings in LIP during SC inactivation further support this interpretation. SC inactivation prolonged the build-up of activity such that higher LIP firing rates were required to commit to a contralateral choice. The results demonstrate modular computation in two interconnected brain regions and reveal a neural implementation of the decision threshold predicted by sequential-sampling models of decision making.

Disclosures: G.M. Stine: None. **E.M. Trautmann:** None. **D. Jeurissen:** None. **M.N. Shadlen:** None.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

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Presentation Number: 105.10

Topic: H.03. Decision Making

Title: Behavioral state is observable through task-independent movements and modulates cortical activity in a rodent decision making task

Authors: *M. D. MELIN[1], C. YIN[1], G. ROJAS-BOWE[1], A. KOSTIUK[1], S. MUSALL[2], X. R. SUN[3], S. GLUF[4], A. K. CHURCHLAND[1];
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Abstract: Neuroscientists have long assumed that a behavioral strategy remains fixed once animals reach expert levels of task performance. With the development and application of behavioral models to detect rodent decision-making strategies, we now appreciate that decisions are governed by multiple underlying strategies, or states, that are present within a single training session. However, little is known about the neural underpinnings of these decision-making states, and the behavioral features associated with them.
These behavioral states are typically thought to be latent. However, we find that uninstructed movements lend a great deal of insight into behavioral state. Using DeepLabCut (DLC) to label the position of 28 body parts, we compute the "task-independent variance" (TIV): the variance in DLC labels remaining after task-related movement information is removed via regression. TIV is inversely correlated with task performance. Additionally, we fit a generalized linear model-hidden Markov model (GLM-HMM) to estimate behavioral states. Although the GLM-HMM only makes use of stimulus information and the mouse's decision, we find a robust correlation between the GLM-HMM state predictions and TIV. This indicates that behavioral states, previously assumed to be latent, are observable in movement data. To examine cortex-wide, state-dependent changes in neural activity, we compared neural activity from widefield imaging corresponding to different behavioral strategies. We find increased baseline activity in secondary motor and retrosplenial cortex when animals are actively engaged in the task versus when they are biased and disengaged. During trial initiation and stimulus presentation, the engaged state shows increased activity in frontal areas, however, during the conclusion of the trial, the engaged state is characterized by increased activity in parietal and posterior regions when compared to disengaged states.
Taken together, these observations suggest that behavioral strategies can be inferred from task-independent movements, and that the neural signature of behavioral state varies across time and space. It is unclear what drives these differences in activity, since behavioral state is collinear with many variables, such as reward, pupil size, and movement. Thus, future work will focus on identifying the source of state-dependent activity differences. Understanding the neural underpinnings of behavioral state will enable a more nuanced view of decision making by uncovering the role of brain regions recruited during different behavioral strategies.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.11

Topic: H.03. Decision Making

Title: A distributed and efficient population code of mixed selectivity neurons for flexible navigation decisions

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Abstract: Flexible behavior often requires rapid switching of associations between sensory cues and actions based on behavioral objectives stored in memory. A key computation for such rapid switching is the integration of sensory signals with short-term memory. Here we aimed to reveal the cortical areas and neural mechanisms central to this integration, and thus flexible decision-making, during spatial navigation. We trained mice to make flexible decisions in a delayed match-to-sample (DMS) task in a virtual reality T-maze. As a mouse navigated through the maze, it sequentially observed two cues separated by a short maze segment, which created 1-2 s of delay between the cues. Mice switched, on a trial-to-trial basis, their navigation toward or away from a second visual cue depending on its match to the remembered first cue. To systematically screen for cortical areas that are involved in flexible navigation decisions, we bilaterally inhibited different sites across the dorsal cortical surface by optogenetically activating inhibitory neurons in VGAT-ChR2 mice. Inhibition of V1, posterior parietal cortex (PPC), or retrosplenial cortex (RSC) induced a large decrease in the task performance. Two-photon calcium imaging revealed neurons that can mediate rapid sensorimotor switching by encoding a conjunction of a current and remembered visual cue that predicted the mouse's navigational choice from trial-to-trial. These mixed selectivity neurons formed efficient population codes that appeared to guide accurate decision-making because they were informative before correct choices but degenerated during errors. Surprisingly, these neurons were distributed across posterior cortex, even V1, but were densest in RSC and sparsest in PPC. The mixed selectivity neurons were rare in naïve mice that ran through the identical maze, indicating that these neurons emerged through learning of the DMS task. Together, we propose that the flexibility of navigation decisions arises from neurons that develop mixed selectivity over learning to integrate visual and memory information within a visual-parietal-retrosplenial network, centered in RSC.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.12

Topic: H.03. Decision Making

Title: Auditory Evidence Accumulation Involves Multiple Striatal Subcircuits

Authors: *A. G. BONDY[1], T. Z. LUO[1], D. GUPTA[1], V. ELLIOTT[1], C. KOPEC[1], C. D. BRODY[1,2];
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Abstract: The ability to use sensory information to guide decisions—and to accumulate that information over time—is a critical cognitive ability. In rats, inactivation of two distinct subregions of the dorsal striatum, the anterior dorsal striatum (ADS) and the posterior tail of the striatum (TS), impair performance in tasks requiring accumulation of auditory evidence, suggesting that multiple striatal pathways may be involved. ADS neurons have "ramping" firing rates that change at a rate determined by the strength of evidence favoring their preferred choice. The second area, TS, receives dense input from auditory structures, but its encoding properties during evidence accumulation is not known. To directly compare the role of striatal subregions during auditory decision making, we carried out a detailed survey of encoding in single neurons using high-yield silicon (Neuropixels) probes implanted at four sites spanning the anteroposterior striatal axis while rats performed a task in which they accumulated pulses of auditory evidence. Sites 1 and 4 corresponded to ADS and TS while sites 2 and 3 corresponded to anatomically intermediate subregions whose role during perceptual decision making has not been established. We used a generalized linear model (GLM) to describe neuronal firing rates as a combination of temporal kernels corresponding to different task events (e.g. individual evidence pulses, choice, reward, etc.). This revealed a novel and highly systematic set of encoding differences across the four striatal sites. First, we found that sites 1 and 2 (the most anterior sites) more strongly encoded the animal's choice than the two posterior sites, both during the evidence accumulation period and during choice execution. However, only site 1 (ADS) had strong, long-lasting responses to the evidence pulses, an encoding feature necessary for gradual accumulation of evidence. This suggests choice encoding in site 2 is primarily related to motor execution rather than decision formation. Site 4 (TS) was unique in responding strongly, but transiently, to the individual evidence pulses. Lastly, we found that the axis in neural state space encoding sensory evidence was well aligned to the choice axis in ADS but not in TS. Taken together, these results

suggest that auditory evidence accumulation involves multiple cortico-basal ganglia loops, each subserving computationally distinct roles. The most anterior subregion of striatum encodes the accumulated evidence at a long timescale and drives choice while the most posterior subregion appears to play an important role in the lower-level processing of decision-relevant sensory information.

Disclosures: A.G. Bondy: None. **T.Z. Luo:** None. **D. Gupta:** None. **V. Elliott:** None. **C. Kopec:** None. **C.D. Brody:** None.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.13

Topic: H.03. Decision Making

Title: Integrating information and reward into subjective value: humans, monkeys, and the lateral habenula

Authors: *E. BROMBERG-MARTIN[1], Y.-Y. FENG[1], T. OGASAWARA[1], J. K. WHITE[1], K. ZHANG[1], I. E. MONOSOV[1,2,3,4,5];
[1]Neurosci., [2]Neurosurg., [3]Pain Ctr., Washington Univ. Sch. Of Med., Saint Louis, MO; [4]Biomed. Engin., [5]Electrical Engin., Washington Univ., Saint Louis, MO

Abstract: Humans and several animal species including monkeys, rats, and pigeons, are strongly motivated to seek information about uncertain future rewards. Remarkably, they seek information even when it has no objective value for controlling the outcome, suggesting that information has subjective value of its own. In recent years there has been an explosion of research on information seeking in humans and animal models. However, a critical unanswered question is whether the computations that assign subjective value to information are conserved between humans and other species. If so, we could leverage animal models to uncover the neuronal populations that are responsible for conserved information value computations and their causal influence on decisions. To address this, we designed analogous multi-attribute information choice tasks for humans and monkeys. Individuals choose between options with multiple attributes, including cues that are either informative or non-informative about future outcomes, and different probability distributions of rewards (money for humans, juice for monkeys). This let us measure and model the subjective value individuals assign to information; how they compute information value using reward uncertainty, expected reward, and other attributes; and integrate information and reward into the total value of an option. We find human

and monkey value computations are remarkably similar on all these fronts. For example, both species computed the value of information based on the amount of uncertainty it would resolve, and both species were best fit as computing this "uncertainty" with a specific family of mathematical uncertainty measures. We then investigated the neuronal networks responsible for these value computations by recording in monkeys in two interconnected areas with information-related activity, the anterior/ventral pallidum (Pal) and lateral habenula (LHb). We find that both areas respond to all attributes needed for decisions, but only LHb neurons predominantly integrate information and reward to reflect the subjective value of options. Further, trial-to-trial fluctuations in LHb value signals predict ongoing decisions, while electrical stimulation coincident with LHb value signals modifies ongoing decisions. Our data thus implicate the LHb in conserved information value computations that guide online decisions. Furthermore, this data and approach provide promise for computational psychiatry by dissecting the motivational algorithms for information- and reward-seeking behavior, identifying the underlying neuronal circuits, and using them to perform targeted modulation of behavior.

Disclosures: E. Bromberg-Martin: None. **Y. Feng:** None. **T. Ogasawara:** None. **J.K. White:** None. **K. Zhang:** None. **I.E. Monosov:** None.

Nanosymposium

105. Neuronal Mechanisms of Decision Making

Location: SDCC 5

Time: Sunday, November 13, 2022, 8:00 AM – 11:30 AM

Presentation Number: 105.14

Topic: H.03. Decision Making

Support: The Natural Sciences and Engineering Research Council of Canada (NSERC)
Canada Research Chairs Program
IVADO

Title: Distinct trajectories in low-dimensional neural oscillation state space track dynamic decision-making in humans

Authors: T. THIERY[1], P. RAINVILLE[2], P. E. CISEK[3], *****K. JERBI[4];
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Abstract: The brain evolved to govern behavior in a dynamic world, in which pertinent information about choices is often in flux. Thus, the commitment to an action choice must reflect a balance between monitoring that information and the necessity to act before opportunities are lost. Here, we investigate the mechanisms of dynamic decision-making in humans using low dimensional space representation of brain wide magnetoencephalography recordings. We show that the principal components (PCs) of alpha (9-13 Hz) and beta power (16-24 Hz) are involved in tracking sensory information evolving over time in the sensorimotor and visual cortex. We

also found that alpha PCs reflect the commitment to a particular choice, while beta PCs reflect motor execution. Furthermore, higher frequency components in subcortical areas reflect the adjustment of speed-accuracy tradeoff policies. These results provide a new detailed characterization of the distributed oscillatory brain processes underlying dynamic decision-making in humans. Finally, it is important to note that many of the PCs obtained in our study were remarkably similar to those found when dimensionality reduction was applied to the single-unit data from monkey PMd, M1, dlPFC, and pallidum. This supports the proposal that similar mechanisms are involved when decisions are made by highly trained monkeys versus untrained human subjects, and that the prospects are very promising for unifying the conclusions of single-unit studies in animals with whole-brain human magnetoencephalography.

Disclosures: T. Thiery: None. **P. Rainville:** None. **P.E. Cisek:** None. **K. Jerbi:** None.

Nanosymposium

106. Novel Tools for Understanding Neuronal Signaling, Activity, and Gene Expression

Location: SDCC 25

Time: Sunday, November 13, 2022, 8:00 AM – 10:15 AM

Presentation Number: 106.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Multielectrode Array Biosensor for Multiplexing Neurochemical Measurements

Authors: A. G. ZESTOS, I. R. BROWN;
Chem., American Univ., Washington, DC

Abstract: FSCV, or fast scan cyclic voltammetry, is an electrochemical technique that measures the oxidation and reduction of various molecules and proteins. Analytes are oxidized/reduced at specific voltages which serve as a "molecular fingerprint" for detection. Carbon fiber microelectrodes (CFMEs) have been extensively used to measure neurotransmitters with FSCV due to their ability to adsorb cationic monoamine neurotransmitters such as dopamine and serotonin, which are increased extracellularly by cocaine and amphetamine. Although this method provides high temporal and spatial resolution, only single channel potentiostats and electrodes have been primarily utilized. More recently, the need and use of carbon fiber multielectrode arrays has risen to target multiple brain regions simultaneously. Previously, we characterized a novel carbon fiber multielectrode array (MEA), a 4-channel electrode, and found it comparable to the single channel CFME in sensitivity and selectivity. The MEA, along with a commercial potentiostat, had the additional capability of multiplexing neurotransmitter measurements in-vitro by multi-waveform application. We utilized the multielectrode array and four-channel potentiostat to measure potassium chloride (KCl) stimulated release in the caudate putamen in coronal mouse brain slices *ex-vivo*. This method yielded a sensitivity of 3.33 nA/µM with concentrations detected as low as 100 nM. In the present study we used the MEA to detect

neurotransmitters in the brain using electrical stimulation and potassium-chloride induced neurotransmitter release. We mapped out endogenous levels of neurochemicals such as dopamine, serotonin, and adenosine in regions including the ventral tegmental area, caudate putamen, raphe nuclei, and substantia nigra. This was accomplished by targeting the areas using mouse brain atlas coordinates and inserting the electrode into different brain slices. Successful sampling of neurotransmitter concentrations will aide in understanding complex brain heterogeneity, the dynamic neurochemical environment, complex behaviors, and how disease states or drugs affect separate brain areas concurrently. Specifically, we will use this method to measure the effects of psychostimulants such as cocaine and amphetamine on the release of dopamine and other monoamine neurotransmitters.

Disclosures: A.G. Zestos: None. **I.R. Brown:** None.

Nanosymposium

106. Novel Tools for Understanding Neuronal Signaling, Activity, and Gene Expression

Location: SDCC 25

Time: Sunday, November 13, 2022, 8:00 AM – 10:15 AM

Presentation Number: 106.02

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: A tool kit of genetically encoded biosensors for monitoring opioids in vitro, ex vivo and in vivo

Authors: *C. DONG[1], R. GOWRISHANKAR[2], J. HE[3], H. WANG[4], K. MAHE[5], R. FLORESGARCIA[4], A. LAYDEN[3], D. JOHNSON[3], N. S. ATASOY[6], A. GUPTA[7], H. TAJEDA[4], A. DENIZ[6], I. GOMES[7], L. DEVI[7], M. BRUCHAS[2], M. BANGHART[3], L. TIAN[8];
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Abstract: Like monoamine neuromodulators, neuropeptides act on G-protein coupled receptors to actively shape the synaptic strength, post-synaptic neuronal activity and circuit dynamics. Among a hundred neuropeptides (NP) discovered, opioids are most clinically relevant as the major targets for effective analgesic treatments to date, but drugs targeting opioid systems also lead to epidemic of abuse and overdose. To develop effective treatment with unwanted side effects, we must achieve a mechanistic and theoretic understanding of neuropeptide signaling. However, it is largely unknown about their dynamics, such as when, where, and how long they are released, and how the release is altered with pharmacological intervention. Here, we

developed a class of genetically encoded biosensors based on three opioid receptors for detecting endogenous opioids with sub-second temporal resolution. We extensively characterized the sensors' properties to maximize signal to noise. We used the sensor to record the sub-second endogenous opioid NP release at a sub-regional level during aversion across multiple brain regions. In addition, these biosensors predict the pharmacological profiles of endogenous peptides-like compounds, which could facilitate identifying biased opioid ligands capable of eliciting selective functional responses with fluorescence.

Disclosures: C. Dong: None. **R. Gowrishankar:** None. **K. Mahe:** None. **L. Tian:** None.

Nanosymposium

106. Novel Tools for Understanding Neuronal Signaling, Activity, and Gene Expression

Location: SDCC 25

Time: Sunday, November 13, 2022, 8:00 AM – 10:15 AM

Presentation Number: 106.03

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant NS111358
        Alzheimer's Association 2019-AARG-NFT-640971

Title: Live-cell imaging and targeted degradation of RNA-binding proteins using nanobodies

Authors: *Y. CHO[1], A. PIRHANOV[2];
[1]Chem. And Biol. Engin., [2]Univ. of Connecticut, Storrs, CT

Abstract: RNA-binding proteins (RBP) are a primary component of stress granules, a key indicator of various cell stress responses. Recently, RBPs including the TIA1 and hnRNPA2/B1 emerged as a source of neurotoxicity in Alzheimer's disease and other neurodegeneration. TIA1 interacts with tau and promotes its liquid-liquid phase separation and oligomerization. Proteomics studies revealed hnRNPA2/B1 as a primary protein that preferentially interacts with tau oligomers than tau monomers. Stress granules composed of these RBPs initially form a dynamic non-membrane bound liquid phase but over time turns into a gel-like structure. As the phase separation is thought to be dependent on the intracellular concentration of RBPs, the use of ectopically expressed RBP-fluorescent protein fusions in majority of these studies raises questions on its physiological relevance. Here we report nanobodies as a tool to study the dynamics of RBPs TIA1 and hnRNPA2/B1. Since the intrinsically disordered regions of RBPs have homology and repetitive sequences, we carefully selected target epitopes to develop highly specific binders. We demonstrate live cell imaging of these RBPs using nanobodies fused to fluorescent proteins. We also show that by fusing the nanobodies with ubiquitin ligase adaptors domains, the level of hnRNPA2/B1 can be significantly reduced. These novel tools will greatly aid the study of RBP dynamics in their native context.

Disclosures: Y. Cho: None. **A. Pirhanov:** None.

Nanosymposium

106. Novel Tools for Understanding Neuronal Signaling, Activity, and Gene Expression

Location: SDCC 25

Time: Sunday, November 13, 2022, 8:00 AM – 10:15 AM

Presentation Number: 106.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Split-type genetically encoded neurotransmitter sensors for reconstitution between synaptic partners

Authors: *Y. SHINDO[1], K. ASHIDA[1], K. MASAMOTO[2], H. TAKUWA[3], M. TAKAHASHI[3], M. HIGUCHI[3], R. IDE[1], K. HOTTA[1], K. OKA[1,4,5];
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Abstract: To comprehend information processing within the nervous system, it is necessary to understand the connections and transmission among neurons. Exhaustive research using electron microscopy achieved detailed connectome mapping of neuronal circuits in various animals. Fluorescent labeling using the GFP Reconstitution Across Synaptic Partners (GRASP) system has also been used to detect synaptic connections between specific neurons. On the other hand, functional connectivity and neurotransmission were estimated using Ca^{2+} or neurotransmitter imaging with genetically encoded sensors. However, it is still challenging to simultaneously evaluate both the synaptic connections and transmissions in the nervous system *in vivo*.Here, we developed a method to visualize neurotransmission between specific pre- and postsynaptic neurons named Split Protein Hemispheres for Reconstitution (SPHERE) by combining the features of the GRASP system and neurotransmitter sensor. In our method, by splitting a sensor into two fragments and expressing them in pre- and postsynaptic neurons separately, functional neurotransmitter sensors can be reconstituted between those neurons. First, we developed a SPHERE-SF-iGluSnFR to measure glutamate transmission and confirmed its function in cultured cells. In the mixed culture of each fragment expressing cells, the sensor fluorescence was observed only at the attachment sites of those cells, and the fluorescence of the reconstituted sensor increased upon application of glutamate. The localization and function of SPHERE-SF-iGluSnFR were also confirmed in *C. elegans*. SPHERE-SF-iGluSnFR localized to the attachment sites between specific pre- (AWC) and post- (AIY) synaptic neurons expressing each fragment of the sensor and detected the odor-induced change in glutamate transmission between those neurons. These results indicate that concept of our method was successfully achieved. Moreover, SPHERE-SF-iGluSnFR was applied to *in vivo* imaging of mouse brains. An adeno-associated virus (AAV) vector encoding each fragment of SPHERE-SF-iGluSnFR was injected near areas within the cortex, and sensors reconstituted at connections among local interneurons. Anesthesia induced a reduction in glutamate transmission among those neurons. This indicates that our method can be applied to detect neurotransmission among specific neurons in vivo. In addition, SPHERE technique can be applied to other color variants and other neurotransmitter sensors. Therefore, we believe that this method would be a powerful tool for *in vivo* neuroimaging.

Nanosymposium

106. Novel Tools for Understanding Neuronal Signaling, Activity, and Gene Expression

Location: SDCC 25

Time: Sunday, November 13, 2022, 8:00 AM – 10:15 AM

Presentation Number: 106.05

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Optogenetic sensors and tools for studying brain signaling and neurodegenerative diseases

Authors: *W. WANG[1], Y. R. BUTLER[1], K. KRONING[2];
[1]Home, [2]Univ. of Michigan, Univ. of Michigan, Ann Arbor, MI

Abstract: Genetically-encoded sensors and tools have been instrumental in studying various signaling processes. Our lab uses protein engineering to design a range of sensors and tools to investigate neuronal signaling and neurodegenerative diseases. We are developing a new class of irreversible fluorescent sensors to map opioid molecules across the animal tissues[1]. We are also designing fibril-specific nanobodies to inhibit the alpha-synuclein pathology development in Parkinson's disease mouse models[2]. I will show the development of these sensors and tools and their proof-of-principle applications.
1.Kroning, K. E., Wang, W.*, Design of genetically encoded single-chain fluorescent reporters for detecting opioids. *Angew Chem Int Ed, 60,* 13358 (2021).
2.Riani, Y. D.[#], Liu, Y.[#], Kumbhar, R.[#], Li, Y., Mao.*, Wang, W*. Disulfide bond-free and fibril-specific nanobodies prevent the prion-like α-synuclein spreading. *Nat Comm.* Accepted.

Nanosymposium

106. Novel Tools for Understanding Neuronal Signaling, Activity, and Gene Expression

Location: SDCC 25

Time: Sunday, November 13, 2022, 8:00 AM – 10:15 AM

Presentation Number: 106.06

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Real-time visualization of mRNA synthesis during memory formation in live mice

Authors: B. LEE[1], J. SHIM[1], H. MOON[1], D. KIM[1], J. KIM[2], J. YOOK[2], J. KIM[2], ***H. PARK**[3,1];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Korea Inst. Of Sci. and Technol., Seoul, Korea, Republic of; [3]Univ. of Minnesota, Minneapolis, MN

Abstract: Memories are thought to be encoded in populations of neurons called memory trace or engram cells. However, little is known about the dynamics of these cells because of the difficulty in real-time monitoring of them over long periods of time *in vivo*. To overcome this limitation, we present a genetically-encoded RNA indicator (GERI) mouse for intravital chronic imaging of endogenous *Arc* mRNA—a popular marker for memory trace cells. We used our GERI to identify *Arc*-positive neurons in real time without the delay associated with reporter protein expression in conventional approaches. We found that the *Arc*-positive neuronal populations rapidly turned over within two days in the hippocampal CA1 region, whereas ~4% of neurons in the retrosplenial cortex (RSC) consistently expressed *Arc* following contextual fear conditioning and repeated memory retrievals. Dual imaging of GERI and a calcium indicator in CA1 of mice navigating a virtual reality environment revealed that only the population of neurons expressing *Arc* during both encoding and retrieval exhibited relatively high calcium activity in a context-specific manner. This *in vivo* RNA imaging approach opens the possibility of unraveling the dynamics of the neuronal population underlying various learning and memory processes.

Disclosures: B. Lee: None. **J. Shim:** None. **H. Moon:** None. **D. Kim:** None. **J. Kim:** None. **J. Yook:** None. **J. Kim:** None. **H. Park:** None.

Nanosymposium

106. Novel Tools for Understanding Neuronal Signaling, Activity, and Gene Expression

Location: SDCC 25

Time: Sunday, November 13, 2022, 8:00 AM – 10:15 AM

Presentation Number: 106.07

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Evaluation of Upstream Activator Sequences (UAS) promoter copy numbers in transgene expression in Drosophila sensory neurons

Authors: *M. SINGH[1], J. KIM[2];
[1]Biol., UNIVERSITY OF NEVADA, RENO, RENO, NV; [2]Biol., Univ. of Nevada, Reno, Reno, NV

Abstract: Abstract: In *Drosophila*, the GAL4/upstream activating sequence (UAS) system is one of the most efficient tools for targeted gene expression. In the present study we sought to modulate expression levels of transgenes by varying the number of UAS sites carried by the

pUASTattB plasmid that is most widely used in *Drosophila* genetics. We verified that the expression levels of transgenes were dependent on the number of UAS in both cultured *Drosophila* S2 cells and larval sensory neurons. Down syndrome cell adhesion molecule (DSCAM) expression levels determine the size of the presynaptic arbors. We further show that axon arborization levels well correlates with the expression levels of the Dscam transgenes. Our new tool allows fine-tuning of transgene expression for desired levels in GAL4/UAS system.

Disclosures: M. Singh: None. **J. Kim:** None.

Nanosymposium

106. Novel Tools for Understanding Neuronal Signaling, Activity, and Gene Expression

Location: SDCC 25

Time: Sunday, November 13, 2022, 8:00 AM – 10:15 AM

Presentation Number: 106.08

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Multiplexed tropism profiling of systemic AAVs via spatial transcriptomics

Authors: *M. JANG, G. M. COUGHLIN, C. R. JACKSON, X. CHEN, M. R. E. CHUAPOCO, J. L. VENDEMIATTI, A. Z. WANG, V. GRADINARU;
Caltech, Pasadena, CA

Abstract: Targeted genetic access to the cell type of interest is essential to dissect heterogenous brain circuits but mostly requires transgenic animals. Intersectional approaches using efficient viral vectors, such as adeno-associated viruses (AAVs), and cargos with cell-type specific regulatory sequences have arisen as alternatives, although currently limited to few populations. To expand targeted gene delivery toolkits, high-throughput AAV screening platforms have been developed and revealed a few variants via subsequent histology-based characterization. However, limited multiplexability and availability of antibodies makes it challenging to align AAV tropism to a variety of genetically defined cell types with high throughput. To address this, we developed a highly sensitive, spatial transcriptomics method, named ultrasensitive sequential fluorescence in situ hybridization (USeqFISH) for profiling pooled AAVs along with endogenous gene expression in intact tissue. This method achieves high sensitivity by combining two signal amplification strategies, rolling circle amplification (RCA) and hybridization chain reaction (HCR). This high sensitivity of USeqFISH allows us to distinguish 14-nucleotide (nt) difference in viral genomes in cell cultures and 40-nt in tissue, enabling short barcoding of AAVs. With an RNA-retaining passive tissue clearing and a two-step signal quenching method, we established USeqFISH available for detecting ~50 genes via sequential labeling in intact

tissue volume. Using USeqFISH, we profiled the transduction and relative tropism of six systemic AAVs, including previously reported AAV-PHP.eB, AAV.CAP-B10, AAV-PHP.N/V1/B8, and a new variant engineered from AAV-PHP.eB, AAV-PHP.AX, across 10-26 cell types in diverse mouse brain regions. In addition to recapitulating our prior knowledge with high throughput, USeqFISH profiling revealed new tropism of each one across region-specific cell types that have limited choices of antibodies. USeqFISH also provided in-depth characterization of AAV-PHP.AX, with a reasonable transduction efficiency, broad coverage across neuronal subtypes and astrocytes, and a higher tropism-tuning capacity coupled with gene regulatory elements. Lastly, we demonstrate the applicability of USeqFISH to the non-human primate (NHP) brains, showing the potential translation of USeqFISH into in situ AAV profiling and multi-modal single cell, intact-tissue analysis in NHPs. By bringing spatial transcriptomics to the AAV engineering field, we believe USeqFISH will accelerate viral tool engineering for targeted gene delivery and its broader use in basic and pre-clinical research.

Disclosures: M. Jang: None. **G.M. Coughlin:** None. **C.R. Jackson:** None. **X. Chen:** None. **M.R.E. Chuapoco:** None. **J.L. Vendemiatti:** None. **A.Z. Wang:** None. **V. Gradinaru:** None.

Nanosymposium

106. Novel Tools for Understanding Neuronal Signaling, Activity, and Gene Expression

Location: SDCC 25

Time: Sunday, November 13, 2022, 8:00 AM – 10:15 AM

Presentation Number: 106.09

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Brainwide gene transfer in infant Old World primates via intravenous AAV

Authors: *M. E. CHUAPOCO[1], N. FLYTZANIS[2], N. GOEDEN[2], J. C. OCTEAU[2], K. M. ROXAS[2], K. Y. CHAN[3], J. SCHERRER[2], J. WINCHESTER[2], R. J. BLACKBURN[2], L. J. CAMPOS[4], T. F. MILES[1], J. SUN[4], K.-N. M. MAN[4], A. LEFEVRE[5], C. M. AROKIARAJ[1], M. ARTIUKHOVA[1], M. J. JANG[1], J. VENDEMIATTI[1], C. T. MILLER[5], B. E. DEVERMAN[3], J. PICKEL[6], L. TIAN[4], A. S. FOX[4], V. GRADINARU[1];
[1]Caltech, Pasadena, CA; [2]Capsida Biotherapeutics, Thousand Oaks, CA; [3]Broad Inst. Of MIT and Harvard, Cambridge, MA; [4]Univ. of California-Davis, Davis, CA; [5]Univ. of California-San Diego, La Jolla, CA; [6]NIH, NIH, Bethesda, MD

Abstract: Adeno-associated viruses (AAVs) are dependable and ubiquitous gene-transfer tools. Clinicians have safely used AAVs in hundreds of gene therapy clinical trials to date, and neuroscientists frequently use AAVs *in vivo* to deliver genetically-encoded tools. Recently, the

field has focused on engineering novel AAV capsid variants that traverse the blood-brain-barrier. While variants now exist that enable systemic gene transfer to the rodent central nervous system (e.g. AAV-PHP.B), direct efforts in non-human primates (NHPs) are still sparse. As such, BRAIN 2.0 has emphasized that there is a major need to "improve tools for studying primate brains" due to the translational impact that NHP research has on demystifying human neurobiology. Several engineered capsids (e.g. AAV.CAP-B10) now enable systemic gene transfer to the brain of common marmosets (*Callithrix jacchus*), a New World primate species. But few comparable options exist for Old World primates (OWPs), which are more evolutionarily related to humans compared to marmosets, and are well-established animal models of human cognition, neurodevelopment, neuroanatomy, and physiology. To meet this need, it is imperative that we advance AAV development for systemic gene transfer to the brains of OWPs.

Here, we present AAV.CAP-Mac, an engineered AAV9 variant that efficiently transduces cortical and subcortical brain regions in infant OWPs after intravenous (IV) administration. We identified CAP-Mac after screening an engineered AAV9 capsid library for enriched CNS gene transfer, performing selections in 4 male, adult common marmosets and 2 male, infant rhesus macaques (*Macaca mulatta*). During the selection in macaques, CAP-Mac-delivered transgenes were enriched 10- and 6- times more than those delivered by AAV9 in viral DNA and whole RNA brain extracts, respectively. CAP-Mac tropism is conserved across OWP species, efficiently transducing neurons in both infant macaques and green monkeys (*Chlorocebus sabaeus*) after IV administration. Fluorescent protein expression was more biased in neurons when using CAP-Mac (47% of all GFP+ cells are NeuN+, n=110 fields of view across 2 green monkeys) compared to AAV9 (12% of GFP+ cells, n=108 in 2 green monkeys). This neuronal bias was conserved *in vivo* in macaques (53% of XFP+ cells, n=23 in 1 macaque) and *in vitro* in human stem-cell derived neurons (EC_{50} in CAP-Mac-treated cultures=$10^{3.0}$ vector genomes (vg)/cell vs. $10^{4.7}$ vg/cell in AAV9 cultures). Finally, we used systemically delivered CAP-Mac for Brainbow-like, multicolor labeling and morphological tracing of medium spiny neurons and functional recording of calcium gradients using GcaMP8s *ex vivo*.

Nanosymposium

176. Autism: Genetics to Phenotypes

Location: SDCC 1

Time: Sunday, November 13, 2022, 1:00 PM – 4:00 PM

Presentation Number: 176.01

Topic: A.07. Developmental Disorders

Title: Mapping Autism Spectrum Disorder Behavioral Endophenotypes to Genomic Regions in Thousands of Families

Authors: *N. STOCKHAM, K. PASKOV, B. CHRISMAN, J.-Y. JUNG, D. P. WALL; Stanford Univ., Palo Alto, CA

Abstract: The genetic architecture of Autism Spectrum Disorder (ASD) is multifaceted and unresolved despite consistently high heritability estimates. Recent studies with extremely large sample sizes have associated a few common variants and many high-effect ultra-rare variants with ASD diagnosis status but leave the genetic etiology of the vast majority of ASD diagnoses unexplained. A possible explanation for this lack of discovery is that there are several etiologically distinct forms of ASD and grouping these distinct forms under one diagnosis status obscures the genomic elements relevant to each etiology; therefore, ASD must be studied at the level of detailed psychiatric endophenotype while integrating information across the entire genome. In this work we attempt to map a set of three commonly accepted ASD behavioral endophenotypes to specific genomic regions. These endophenotypes are the three subdomains of the Autism Diagnostic Interview-Revised (ADIR) and the closely related Social Communication Questionnaire (SCQ): Reciprocal Social Interaction (RSI), Restricted Repetitive and Stereotyped Behavior (RRSB), and Communication (COM). Due to recombination, siblings share varying fractions of chromosomal material that are Identical-By-Descent (IBD) from each parent. We hypothesized that increased sibling IBD fraction would entail decreased sibling differences in the

molecular mechanisms relevant to ASD, and therefore decrease the sibling differences in ASD phenotypes. To determine which chromosomes are most relevant to each subdomains, we applied a modified Haseman-Elston regression to seventeen hundred sibling pairs with concordant ASD diagnosis status from the two largest family-based ASD cohorts: the Autism Genetic Research Exchange/Hartwell (AGRE/iHART) collection and Simons Foundation SPARK (SPARK). The RSI endophenotype regression yielded the strongest genetic signal in both SPARK and AGRE/iHART cohorts, with chromosome 15 passing conservative permutation tests in SPARK and AGRE/iHART. In contrast, neither the RRSB nor COM subdomains could be mapped to a chromosome in both cohorts. The highlighting of chromosome 15 is particularly interesting given that chromosome 15q11-13 is the known causal loci for Angelman Syndrome and Prader-Willi Syndrome; genetic imprinting disorders that share many social behavior phenotypes with ASD. By focusing on detailed phenotypic and genomic differences between sibling pairs, we shift focus from searching for causal genetic variants in unrelated ASD cases to discovering the molecular mechanisms most relevant to ASD phenotypes.

Disclosures: N. Stockham: None. **K. Paskov:** None. **B. Chrisman:** None. **J. Jung:** None. **D.P. Wall:** None.

Nanosymposium

176. Autism: Genetics to Phenotypes

Location: SDCC 1

Time: Sunday, November 13, 2022, 1:00 PM – 4:00 PM

Presentation Number: 176.02

Topic: A.07. Developmental Disorders

Title: Machine Learning using Resting-State Connectivity Identifies Brain-Behavior Dimensions That Delineate Autism Spectrum Disorder Subgroups

Authors: *A. M. BUCH[1], P. VERTES[2], J. SEIDLITZ[3,4], S. KIM[1], L. GROSENICK[1], C. M. LISTON[1];
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Abstract: Autism Spectrum Disorder (ASD) describes a diverse group of neurodevelopmental disorders encompassing a wide range of clinical impairments. The two core symptoms that

define ASD are social communication impairments and restricted and repetitive behaviors, and there is a wide range of cognitive and language abilities. How distinct neurobiological substrates give rise to differing clinical symptoms in subsets of ASD patients is unknown. Using a large, publicly available neuroimaging dataset comprising resting state functional magnetic resonance imaging (rsfMRI) scans from N=299 subjects with ASD and N=907 neurotypical controls, we identified three latent dimensions of functional brain network connectivity that predict individual differences in ASD symptoms and behaviors. We show that patients with ASD can be grouped into distinct neurophysiological subgroups based on patterns of dysfunctional connectivity and clinical behaviors. In this cohort, functional connectivity features were extracted from rsfMRI data, regularized canonical correlation analysis was used to identify associations between connectivity features and behavioral data, and ASD subjects were clustered along these dimensions. Cross-validation analyses showed high stability in the brain-behavior dimensions, with replicable clusters in held-out data. Next, we integrated neuroimaging data with gene expression data from the Allen Human Brain Atlas, and found that within each subgroup, ASD-related functional connectivity was explained by regional differences in the expression of distinct gene sets. These were enriched for transcriptionally-regulated and ASD-associated genes along with immune and synaptic signaling pathways. In sum, our results identify discrete ASD subgroups associated with specific ASD behaviors and neurophysiological signatures, and these different forms of ASD implicate distinct genetic mechanisms. The results of this study suggest a promising new approach for understanding the neurobiological substrates of ASD.

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Nanosymposium

176. Autism: Genetics to Phenotypes

Location: SDCC 1

Time: Sunday, November 13, 2022, 1:00 PM – 4:00 PM

Presentation Number: 176.03

Topic: A.07. Developmental Disorders

Title: Modulation of cerebello-neocortical structural covariation in neurotypical development and autism spectrum disorder

Authors: *F. D'OLEIRE UQUILLAS[1], B. LI[1], M. A. TROTTER[1], R. GESUE[1], M. LATIF[1], K. STEELE[1], E. BUEICHEKÚ[2], J. SEIDLITZ[3], V. ZHANG[1], T. FASULO[1], P. M. HOYOS[1], E. DANIEL HERTZ[1], E. SEFIK[1], S. R. GUARIGLIA[1], S. JANARTHANAN[1], J. LEE[1], G. J. BROUSSARD[1], J. L. VERPEUT[4], M. KISLIN[1], H.-J. BOELE[1], J. SEPULCRE[2], S. S.-H. WANG[1], J. GOMEZ[1];
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Abstract: Using structural covariance analysis, here we investigate relationships between cerebellar and cerebral brain volumes in neurotypical and individuals with autism spectrum disorder (ASD) given that cerebellar injury at birth carries the largest non-genetic risk ratio in ASD. We computed individualized MRI volumes in all regions found in the HCP-multimodal brain parcellation and SUIT cerebellar atlas for 993 participants of the ABIDE consortium database (6-30 years old, 28% female). In typically developing (TD) children and young adults (n=512), cerebellar cortex volumes related inversely to deep cerebellar nuclei (DCN) (spearman correlations; $p<0.05$), and DCN volumes related positively to sensorimotor cerebral cortex (SMC) ($p<0.0001$). These results are consistent with the idea that net excitatory drive from cerebellar nuclei plays a positive role in neocortical growth and/or survival, and that inhibitory output of the cerebellar cortex has a negative influence. The magnitude of correlations did not significantly differ in ASD (n=481). Given the focal position of the thalamus in cerebello-neocortical pathways, we next analyzed the influence of sensorimotor thalamic volumes. Volumes of deep cerebellar nuclei were positively related to contralateral thalamic nuclei [$p<0.05$: deep dentate nucleus x thalamic ventromedial (VM), laterodorsal (LD), and lateral posterior (LP) nuclei], except for an inverse correlation between right interpositus deep nucleus and left ventral posterolateral (VPL) thalamic nucleus ($p<0.01$). Thalamic and ipsilateral SMC volumes were similarly positively correlated ($p<0.05$: SMC vs. VM, LD, LP), again with the exception of an inverse relationship with bilateral VPL thalamic nuclei ($p<0.05$). Interaction models testing whether thalamic nuclei modulate the relationship between DCN and SMC revealed that greater thalamic size was associated with a stronger link between DCN and SMC size (dentate nucleus vs. VPL, VM, VLp, LD, and LP models: $p<0.05$; interpositus vs. LD model: $p<0.05$). The role of the thalamus as a moderator differed in ASD in a three-way interaction model ($p=0.02$). Overall, the thalamus seems to play a key role in the anatomical development of cerebello-neocortical regions. We are now further investigating how these effects change with age and in ASD.

Disclosures: F. d'Oleire Uquillas: None. **B. Li:** None. **M.A. Trotter:** None. **R. Gesue:** None. **M. Latif:** None. **K. Steele:** None. **E. Bueichekú:** None. **J. Seidlitz:** None. **V. Zhang:** None. **T. Fasulo:** None. **P.M. Hoyos:** None. **E. Daniel Hertz:** None. **E. Sefik:** None. **S.R. Guariglia:** None. **S. Janarthanan:** None. **J. Lee:** None. **G.J. Broussard:** None. **J.L. Verpeut:** None. **M. Kislin:** None. **H. Boele:** None. **J. Sepulcre:** None. **S.S. Wang:** None. **J. Gomez:** None.

Nanosymposium

176. Autism: Genetics to Phenotypes

Location: SDCC 1

Time: Sunday, November 13, 2022, 1:00 PM – 4:00 PM

Presentation Number: 176.04

Topic: A.07. Developmental Disorders

Title: Links of overconnectivity between sensory and salience network regions with elevated anxiety in ASD

Authors: *N. MEAVE OJEDA[1], K. ALEMU[2], A. SRIDHAR[2], M. WILKINSON[3], A. MANLEY[3], A. LINKE[2], R. JAO KEEHN[3], I. FISHMAN[2], R.-A. MÜLLER[3];
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Abstract: Heightened sensory sensitivity and anxiety symptoms frequently co-occur in people with autism spectrum disorder (ASD). In children with ASD, anxiety symptoms have been associated with impaired executive function, cognitive flexibility, and social functioning. Decreased functional connectivity (FC) between salience and sensory perceptual brain networks has been reported in adolescents with anxiety, and weaker FC in somato-motor and visual networks has been observed in adults with social anxiety disorder. Given the common occurrence of anxiety symptoms in ASD, we aimed to investigate potential links between anxiety symptoms and FC of sensory circuits and salience network within ASD. The Screen for Child Anxiety Related Disorders (SCARED) was completed by caregivers of 37 adolescents with ASD and 22 typically developing (TD) peers (aged 12-21 years). Groups were matched on age, gender, handedness, non-verbal IQ, and in-scanner head motion (indexed with RMSD). Resting state fMRI data were acquired with a multiband-multi echo sequence, which allows for improved denoising. FC between twelve regions of interest (ROIs) in the salience and sensory networks, derived from the Human Connectome Project (HCP) templates, was estimated using Pearson correlations between BOLD signal time series extracted from these regions. Associations between FC and anxiety symptoms (SCARED Total scores) in the ASD group were examined using partial correlations controlling for age and RMSD. Direct comparisons between ASD and TD groups revealed an overall pattern of overconnectivity between sensory regions and the anterior cingulate cortex (ACC) in adolescents with ASD (e.g.,FC between Early Auditory (EA) right and ACC left [MASD=0.15, MTD= -0.02, $t(59)$ = 4.05, pFDR-corrected = 0.04]), as well as a pattern of underconnectivity between visual ROIs (e.g., Early Visual (EV) left and Posterior Opercular (PO) left [MASD= -0.005, MTD= 0.18, $t(59)$ = -3.57, pFDR-corrected =0.07]). Within the ASD group, overconnectivity between EA and ACC (e.g., EA right and ACC right) was positively associated with SCARED Total scores (r = 0.50, $p$uncorrected = 0.003), while FC between sensory areas (e.g., right V1 and Ventral Visual Cortex (VVC) right) was negatively associated with SCARED Total scores (r = -0.40, $p$uncorrected=0.02). The observed links between increased anxiety symptoms and connectivity of sensory and salience network regions suggest that functional connections between these networks may be implicated in anxiety within ASD. Future longitudinal studies are needed to better understand how this relationship between sensory symptoms and anxiety emerges in ASD.

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176. Autism: Genetics to Phenotypes

Location: SDCC 1

Time: Sunday, November 13, 2022, 1:00 PM – 4:00 PM

Presentation Number: 176.05

Topic: A.07. Developmental Disorders

Title: Defining patterns of sex-differential expression in the human cortex during prenatal development and the intersections with Autism Spectrum Disorder

Authors: *L. T. KISSEL[1], D. M. WERLING[2], S. POCHAREDDY[3], J.-Y. AN[4], K. ROEDER[5], N. SESTAN[3], S. J. SANDERS[6];
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Abstract: Autism spectrum disorder (ASD) has a consistent 4:1 male prevalence, suggesting a role for sex-differential biology in ASD risk. Sex differences in neural structure, function, and later behavior may begin to take shape in mid-gestation due to the effects of gonadal hormones and sex chromosomal genes. Previous studies of human adult brain show strong sex biases in expression of sex chromosome genes and limited sex-skewed expression of autosomal genes. However, sex differences during human prenatal cortical development are poorly characterized. To quantify fetal sex-differential gene expression and its relationship to ASD risk, we analyzed male and female expression patterns in a subset of samples from BrainVar, an RNA-sequencing data set of human dorsolateral prefrontal cortex (dlPFC) tissue. For 15,649 expressed genes in 85 samples ranging from 14.4-21 post-conception weeks (PCW; 39 male, 46 female), we used limma-voom to run sex-differential expression analysis, dtangle to estimate cell type proportions, Weighted Gene Co-expression Network Analysis to identify sex-specific co-expression modules, and gene set enrichment analyses to compare identified sex-differential genes with functional annotations, cell type markers, and ASD-associated risk genes.
We observe 69 significantly sex-differentially expressed genes (sex-DEGs, FDR ≤ 0.1: 30 X chr, 19 Y chr, 20 autosomal), with autosomal sex-DEGs showing subtle sex effects (fold change magnitude 1.2-2). We do not find a significant sex difference in the estimated proportion for any cortical cell type assessed. However, in an extended set of sex-DEGs ($p \leq 0.05$ and FC≥1.2), female-skewed genes show enrichment for genes associated with interneurons (OR=34.04,

p=5.22E-08; Fisher's exact test) and radial glia (OR=8.06, p=7.0E-03), and male-skewed genes are enriched for endothelial cell markers (OR=14.63, p=3.55E-05). ASD risk genes from exome sequencing are not enriched among the extended sex-DEGs, but male-skewed DEGs are enriched for ASD-upregulated, glial-associated CTX.M19 module (OR=6.75, p=0.01), implicating a downstream sex difference in transcription that may reflect a common mechanism in ASD pathology and male-skewed neurodevelopment. We also find one co-expression module specific to prenatal males that is enriched for immune processes (GO:0006955, 68 genes overlap, p=1.05E-3). These results align with the largely subtle sex differences reported in adult human cortex and confirm overlap of ASD-up-regulated and fetal male-biased expression patterns, suggestive of a potential sex-differential role for glial- and immune-associated genes in ASD risk or pathology.

Nanosymposium

176. Autism: Genetics to Phenotypes

Location: SDCC 1

Time: Sunday, November 13, 2022, 1:00 PM – 4:00 PM

Presentation Number: 176.06

Topic: A.07. Developmental Disorders

Title: Testing convergent transcriptional pathways across multiple brain regions to investigate postnatal Chd8 gene function

Authors: *C. P. CANALES[1], K. CICHEWICZ[1], S. A. LOZANO[1], W. AMARAL[2], S. FRANK[2], J. BRENNETT[2], N. SEBAN[1], E. FENTON[1], E. SMITH[1], R. ORTIZ[1], J. ZHU[1], M. COREA[1], D. RAHBARIAN[1], D. G. AMARAL[2], A. S. NORD[1];
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Abstract: De novo mutations in the chromatin-remodeling factor *CHD8* (Chromodomain-Helicase DNA-binding protein 8) are strongly associated with autism spectrum disorder (ASD) and more generally with neurodevelopmental disorders (NDDs). Most CHD8 mutations are expected to lead to a loss of functions and carrier patients display, among other manifestations, ASD-like behavior, intellectual disability, and macrocephaly. Mice with heterozygous germline loss-of-function mutation in *Chd8* exhibit genomic, neuroanatomical, and behavioral pathology. Here we aim identifying the molecular consequences of *Chd8* haploinsufficiency in the postnatal brain across regions and cell types. We used bulk RNA-sequencing in cerebral cortex, hippocampus, and cerebellum to compare wild type and heterozygous *Chd8* mutant mice. Meaningful transcriptional changes were validated via qPCR and paired with neuroanatomy and unbiased mouse brain stereology. We identified differentially-expressed genes (DEGs) that were

altered across all three brain regions, as well as region-specific signatures, with the greatest dysregulation identified in the cerebellum. DEG pathways included neuroinflammatory, metabolic, and synaptic processes. We identified a set of genes that were consistently perturbed across brain regions. Male and female DEG signatures showed some differences in DEG pathways and magnitude of effects. Single nuclei resolution experiments via single nucleus (sn)RNA-seq in the cortex revealed cell-specific transcriptional dysregulation that could explain relevant stereology findings. These experiments revealed changes that were present across all neurons, as well as unique signatures within excitatory and inhibitory subsets. Ongoing efforts focus on using additional single-cell approaches to investigate the effects of Chd8 haploinsufficiency with cell-type specificity to investigate and further validate the observed changes at protein level. Overall, our findings show that decreased Chd8 dosage alters gene expression in adult brain both via shared pathways, and in region-specific and sex-specific manners, with some transcriptional aberration that may be attributable to specific cell types in the cortex. Our results present a systems-level characterization of molecular and cellular pathways that are disrupted in adult *Chd8* mutant brain, and potentially captures generalizable dysfunction driven by mutation to chromatin-associated NDD risk genes.

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Nanosymposium

176. Autism: Genetics to Phenotypes

Location: SDCC 1

Time: Sunday, November 13, 2022, 1:00 PM – 4:00 PM

Presentation Number: 176.07

Topic: A.07. Developmental Disorders

Support: NYIT COM internal grant

Title: Haploinsufficiency of Shank3 selectively impairs target recognition in novel background odors

Authors: A. STRASSBURG[1], G. H. OTAZU[2];
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Abstract: The Shank3 protein acts as a scaffolding protein on the post-synaptic membrane of glutamatergic synapses. In humans, deletion of one copy of the Shank3 gene results in Phelan-McDermid Syndrome (PMS). PMS has neurobehavioral characteristics including intellectual disability and autism/autistic-like features that affect social interactions (Phelan et al., 2022). As such, mice that are heterozygous and homozygous for Shank3 are a widely used mouse model of autism (Peça et al., 2011). Considering olfaction in mice is essential for social behaviors, olfactory performance in *Shank3$^{+/-}$* and *Shank3$^{-/-}$* mice has been assessed. *Shank3$^{+/-}$* and *Shank3$^{-/-}$*

mice show no olfactory deficits in discriminating urine versus saline (Wang et al., 2011) nor differences in odor habituation (Yang et al. 2012). We have recently developed an olfactory task using head-fixed water-deprived mice. Mice were trained to detect the same target odors in the presence of known and unknown background odors. The *Cntnap2*^{-/-} mouse model of autism (Poliak et al., 2003) was found to have deficits in the presence of novel background odors but not with the known background odor. We tested 3 *Shank3*^{+/-} mice on this behavior. Learning rates for the *Shank3*^{+/-} mice were similar to the WT mice. Performance on the known background odor was >80% for *Shank3*^{+/-} mice. However, when tested with novel background odors, *Shank3*^{+/-} mice performance dropped to almost chance levels (59.5%). *Shank3*^{+/-} mice performance was similar to the performance of the *Cntnap2*^{-/-} mice (61.1%) and it was significantly lower (p=0.0013, Fisher exact test) than the WT mice (74.1%). Despite the significant difference in performance level, glomerular responses measured with intrinsic imaging were similar between *Shank3*^{+/-} and WT mice which is consistent with previous reports of unaffected olfactory function. We found significant differences in olfactory performance between the *Shank3*^{+/-} mice and their WT counterparts, suggesting that haploinsufficient Shank3 mice show specific deficits in the presence of novel background odors. This deficit of odor detection in novel environments is a common feature affecting the performance of multiple mouse models of autism.

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Nanosymposium

176. Autism: Genetics to Phenotypes

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Presentation Number: 176.08

Topic: A.07. Developmental Disorders

Support: NS118026

Title: Haploinsufficiency of a Circadian Clock GeneBmal1(ArntlorMop3) Causes Brain Wide mTOR Hyperactivation and Autism like Behavioral Phenotypes in Mice

Authors: *A. MISHRA[1], R. SINGLA[1], H. LIN[1], E. LORSANG[1], N. LE[1], S. TIN[1], V. X. JIN[2], R. CAO[1];
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Abstract: Approximately 50-80% of children with autism spectrum disorders (ASDs) exhibit sleep problems, but the contribution of circadian clock dysfunction to the development of ASDs remains largely unknown. The essential clock gene Bmal1 (Arntl or Mop3) has been associated with human sociability, and its missense mutation is found in ASD. Our recent study found that Bmal1-null mice exhibit a variety of autism-like phenotypes. Here, we further investigated whether an incomplete loss of Bmal1 function could cause significant autism-like behavioral changes in mice. Our results demonstrated that heterozygous Bmal1 deletion (Bmal1^{+/−}) reduced

the Bmal1 protein levels by ~50-75%. Reduced Bmal1 expression led to decreased levels of clock proteins, including Per1, Per2, Cry 1, and Clock but increased mTOR activities in the brain. Accordingly, $Bmal1^{+/-}$ mice exhibited aberrant ultrasonic vocalizations during maternal separation, deficits in sociability and social novelty, excessive repetitive behaviors, impairments in motor coordination, as well as increased anxiety-like behavior. The novel object recognition memory remained intact. Together, these results demonstrate that haploinsufficiency of Bmal1 can cause autism-like behavioral changes in mice, akin to those identified in Bmal1-null mice. This study provides further experimental evidence supporting a potential role for disrupted clock gene expression in the development of ASD

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Nanosymposium

176. Autism: Genetics to Phenotypes

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Topic: A.07. Developmental Disorders

Support: NIMH Grant R01 MH113948
Civitan International Research Center Emerging Scholar Award
O'Neal Comprehensive Cancer Center Core Grant P30 CA013148

Title: Effects of Heterozygous Deletion of Autism-related Gene Cullin-3 in Mice

Authors: *Q. XIA[1], A. K. WALKER[2], C. SONG[1], J. WANG[1], A. SINGH[1], J. A. MOBLEY[1], Z. XUAN[1], J. D. SINGER[3], C. M. POWELL[1];
[1]Univ. of Alabama at Birmingham, Birmingham, AL; [2]Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [3]Portland State Univ., Portland, OR

Abstract: Autism Spectrum Disorder (ASD) is a cognitive developmental disorder in which children display repetitive behavior, restricted range of interests, and atypical social interaction and communication. *CUL3*, coding for a Cullin family scaffold protein mediating assembly of ubiquitin ligase complexes through BTB domain substrate-recruiting adaptors, has been identified as a high-risk gene for autism. We performed behavioral assays, morphological analyses, hippocampal slice whole-cell patch-clamp recording and field recording synaptic electrophysiology, and proteomics analyses to characterize the effects of *Cul3* heterozygous deletion in mice. Although complete knockout of *Cul3* results in embryonic lethality, *Cul3* heterozygous mice have reduced 53% (53.1% ± 19.4) CUL3 protein, demonstrate no differences in body weight, and have minimal behavioral differences including decreased spatial object recognition memory. In measures of reciprocal social interaction, *Cul3* heterozygous mice behaved similarly to their wild-type littermates. Within hippocampal CA1 neurons, reduction of *Cul3* significantly increased the mEPSC frequency by 82.3% (p < 0.01) but did not significantly

change the mEPSC amplitude, nor other synaptic properties including baseline evoked synaptic transmission and paired-pulse ratio. Sholl and spine analysis suggests that there is no significant difference in CA1 pyramidal neuron dendritic branching or spine density. Our proteomic analysis identified 133 differentially expressed proteins in the hippocampus upon heterozygous deletion of *Cul3*. We included a q-value (false discovery rate adjusted p-value) between 0.05-0.10 as an additional filter, thus narrowing to 37 differentially expressed proteins. Our proteomic analyses highlight several significant biological processes affected such as cytoskeletal organization, neurotransmitter regulation, cellular component biogenesis and protein localization/transport by Gene ontology analysis. These findings are validated by western blot on two dysregulated cytoskeletal associated proteins, Tropomyosin and Transgelin-2. In summary, our study demonstrates that *Cul3* heterozygous deletion impairs spatial object recognition memory, alters actin cytoskeleton-related and signaling proteins, but does not cause major CA1 neuronal morphology, functional, or behavioral abnormalities in adult global *Cul3* heterozygous mice.

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Nanosymposium

176. Autism: Genetics to Phenotypes

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Presentation Number: 176.10

Topic: A.07. Developmental Disorders

Support: DA-044308

Title: The gut derived short chain fatty acid Acetate reverses social deficits in a Shank3KO mouse model of ASD

Authors: *A. OSMAN[1,2,3], N. L. MERVOSH[5], N. STRAT[3], T. EUSTON[6], G. ZIPURSKY[7], K. R. MECKEL[8], R. POLLAK[9], L. LITICHEVSKIY[10], C. A. THAISS[11], M. S. BREEN[4,2], E. DRAPEAU[12], J. D. BUXBAUM[1,4,3], D. KIRALY[13,14];
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Abstract: Mounting evidence demonstrates a role for the gut microbiome in Autism Spectrum Disorder (ASD), with signaling via microbial produced metabolites as a potential mechanism. The microbiome-produced short chain fatty acids (SCFA) acetate has received attention for its ability to influence brain and behavior. In order to investigate microbiome and metabolite effects in a model for ASD we 175ndersen a gene x microbiome paradigm in which antibiotic (Abx) depletion of the microbiome was combined with acetate replenishment in the Shank3KO model for ASD. To assess the clinical relevance of findings from the mouse model, targeted serum SCFA metabolomic analysis in Phelan McDermid (PMS) patients, who are hemizygous for the Shank3 gene, was conducted. Shank3KO mice and wild-type (Wt) littermates were divided into control, Abx depletion, acetate replenishment and acetate + Abx groups at weaning. On postnatal day 60, animals were subjected to behavioral testing using three-chambered social interaction. Caecal content was collected for 16S sequencing, metagenomic analysis and metabolomic profiling. Medial prefrontal cortex (mPFC) taken for transcriptomic profiling and western blot analysis. Serum from male and female PMS patients and control counterparts was collected for targeted SCFA analysis. Analysis of mouse microbiome contents demonstrated Shank3KO gene effects leading to decreased microbiome diversity, altered Bacteroidetes to Firmicutes ratios, and reduced levels of Lactobacillus genus. Metagenomic profiling revealed altered microbial functional output including fatty acid metabolism, this was confirmed by metabolomic analysis as reduced levels of SCFAs including acetate, effects that were exacerbated by Abx treatment. Behaviorally, control KO mice demonstrated decreased social interaction, a deficit exacerbated by microbiome depletion. RNA-sequencing showed marked changes in gene expression related to epigenetic regulation and synaptic plasticity in Abx+KO mice compared to Wt. Treatment of KO animals with acetate or Abx+acetate reversed the social deficits reported accompanied by changes to acetylation of specific histone marks. Human PMS patients were found to have sex-specific alterations in SCFA levels including acetate, which correlated with clinical behavioral measures. Our rodent data identified an altered gut microbiome and acetate levels in the Shank3KO model which can be targeted to reverse core social deficits possibly via epigenetic mechanisms. Clinical data corroborate findings of altered gut derived metabolites in PMS and add sex differences as a variable for further investigation.

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Nanosymposium

176. Autism: Genetics to Phenotypes

Location: SDCC 1

Time: Sunday, November 13, 2022, 1:00 PM – 4:00 PM

Presentation Number: 176.11

Topic: A.07. Developmental Disorders

Title: Role of autophagic protein degradation in autism-associated cognitive deficits.

Authors: Z. ZHANG[1], M. W. PORCH[3], Y. LI[2], P. S. SINGARAVEL[1], N. BHANDARI[1], B. J. ROSALIA[1], B. COURT-VAZQUEZ[3], W. ZHANG[4], B. SU[2], R. ZUKIN[3], ***J. YAN**[1];
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Abstract: Fragile X syndrome (FXS) is the most common form of heritable intellectual disabilities and a leading genetic cause of autism. However, effective treatment for FXS-induced cognitive deficits remains an unmet medical need. Autophagy is a catabolic process of programmed degradation and recycling of proteins and cellular components via the lysosomal pathway. Our recent findings demonstrated that upregulating autophagy via a genetic method rescued aberrant spine morphology and impaired cognitive behaviors in *Fmr1* KO mice. Further analysis with proteomics revealed that 288 of the 548 overabundant proteins in the hippocampus of *Fmr1* KO mice are targets of autophagic protein degradation. Pharmacological activation of autophagy rescued the aberrant spine morphology and cognitive behaviors, and the rescue effects are largely abolished in neuronal-specific ATG7 knockout mice. Mechanistic studies revealed that eIF4G1 and PSD-95 belong to the identified 288 protein targets and are critical for rescue effects. Overabundant eIF4G1 and PSD-95 induce increased spine density and elevated the percentage of immature spines in CA1 neurons of *Fmr1* KO mice. Autophagy degrades eIF4G1 and PSD-95 proteins, corrects the downstream pathway and F-actin assemble, and rescues the spine deficits. Direct brain infusion of autophagy activator also rescued the synaptic and cognitive deficits of *Fmr1* KO mice. Altogether, our findings identify autophagy as a novel therapeutic target for Fragile X syndrome and revealed the proteins mediating the rescue effects.
Key Words: *Fragile X syndrome, Autophagy, Cognition deficits, Spine morphology*

Nanosymposium

176. Autism: Genetics to Phenotypes

Location: SDCC 1

Time: Sunday, November 13, 2022, 1:00 PM – 4:00 PM

Presentation Number: 176.12

Topic: A.07. Developmental Disorders

Title: A clinically relevant ERK pathway inhibitor reverses core deficits in a mouse model of autism

Authors: K. MURARI, A. ABUSHAIBAH, J. RHO, R. TURNER, ***N. CHENG**;
Univ. of Calgary, Calgary, AB, Canada

Abstract: Extracellular signal-regulated kinase (ERK/MAPK) pathway in the brain is hypothesized to be a critical convergent node in the development of autism spectrum disorder. However, it is not clear whether selectively targeting this pathway is beneficial in autism. Here we tested a clinically relevant, selective inhibitor of ERK signaling in a mouse model of idiopathic autism. We report that treating juvenile mice with this inhibitor reduced ERK pathway activation, dose and duration-dependently reversed core disease-modeling behavioral deficits in sociability, vocalization and repetitive behavior, and reversed abnormal EEG signal. Further analysis revealed that sub-chronic treatment did not affect weight gain, locomotion, or neuronal density in the brain. Together, our data indicate that specifically inhibiting the ERK pathway is beneficial in this model of idiopathic autism, and suggest that selectively targeting ERK pathway could be a new approach for treating autism.

Disclosures: K. Murari: None. **A. Abushaibah:** None. **J. Rho:** None. **R. Turner:** None. **N. Cheng:** None.

Nanosymposium

177. Mechanisms Underlying Synaptic Plasticity: Structural, Metabolic and Gene Regulation Changes

Location: SDCC 23

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 177.01

Topic: B.05. Synaptic Plasticity

Support: Louis D. Srybnik Foundation and FORE Foundation
               Max Planck Society

Title: What fuels memory formation- investigating the energy supplies of synaptic plasticity

Authors: *I. GHOSH, M. SHAH, V. RANGARAJU;
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Abstract: Learning and memory formation consume a large amount of energy. Synapses specifically are hotspots for energy demand. As most synapses are placed far from the cell body, a local energy source is required. We recently showed that dendritic mitochondria form spatially stable compartments to fuel local synaptic protein synthesis during plasticity. However, a direct quantitative estimation of postsynaptic ATP levels and their spatiotemporal regulation is unknown. A major bottleneck has been the lack of reliable methods to quantify ATP in individual spines during synaptic plasticity. We developed a postsynaptic ATP reporter (Spn-ATP) based on an engineered firefly luciferase that produces light in the presence of ATP by bioluminescence. We targeted the luciferase to Homer2 for spine-specific expression and tagged it with mOrange for calibration of expression level and pH. Since luminescence has low photon yield, we built a state-of-the-art imaging system comprising a highly sensitive EM-CCD camera

for capturing the luciferase luminescence, in parallel with a confocal camera for imaging mOrange fluorescence and a two-photon glutamate uncaging system for single-spine stimulation. Our results reveal that at steady-state there are ~10^5 free ATP molecules per spine. At rest, spine-specific energy demands are met by both mitochondrial and glycolytic ATP synthesis. However, upon synaptic plasticity induction, spines exhibit a local increase in ATP, contributed solely by mitochondrial ATP synthesis and not glycolysis. We are currently investigating the underlying molecular mechanisms regulating local mitochondrial ATP synthesis during synaptic plasticity.

Disclosures: I. Ghosh: None. **M. Shah:** None. **V. Rangaraju:** None.

Nanosymposium

177. Mechanisms Underlying Synaptic Plasticity: Structural, Metabolic and Gene Regulation Changes

Location: SDCC 23

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 177.02

Topic: B.05. Synaptic Plasticity

Title: The SNX17-Retriever endomembrane recycling pathway is critical for synaptic function and plasticity

Authors: *P. RIVERO-RÍOS, T. TSUKAHARA, A. CHEN, G. D. CHAVIS, S. IWASE, M. A. SUTTON, L. S. WEISMAN;
Univ. of Michigan, Ann Arbor, MI

Abstract: Trafficking of receptors from endosomes to the cell surface is a key mechanism to regulate synaptic function. In non-neuronal cells, many cell surface proteins recycle from endosomes to the plasma membrane either via the SNX27-Retromer-WASH pathway, or via the recently discovered SNX17-Retriever-CCC-WASH pathway. To date, the only recycling pathway studied in neurons is the SNX27 pathway. However, mutations in the SNX17 pathway cause neurodevelopmental disorders, suggesting that this pathway likely has crucial roles in synaptic function. Here, using cultured hippocampal neurons, we discover that the SNX17-Retriever pathway is a critical regulator of synaptic function and plasticity. SNX17 loss results in decreased dendritic spine density and prevents the long-term potentiation (LTP)-mediated structural changes in spines. Live imaging of GFP-SNX17 revealed that it is rapidly recruited to spines upon LTP induction. Furthermore, we show that SNX17 acts in part by regulating the surface levels of ß1-integrin in neurons. These studies increase our knowledge of endocytic recycling at synapses and uncover key roles for the SNX17-Retriever pathway in neurons.

Disclosures: P. Rivero-Ríos: None. **T. Tsukahara:** None. **A. Chen:** None. **G.D. Chavis:** None. **S. Iwase:** None. **M.A. Sutton:** None. **L.S. Weisman:** None.

Nanosymposium

177. Mechanisms Underlying Synaptic Plasticity: Structural, Metabolic and Gene Regulation Changes

Location: SDCC 23

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 177.03

Topic: B.05. Synaptic Plasticity

Title: Cortical spine dynamics during a single seed grasp motor learning

Authors: J. SOHN[1,2], M. SUZUKI[3,4], M. E. LARKUM[5,3], Y. KAWAGUCHI[1,6,8], *Y. KUBOTA[1,7,9];
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Abstract: Dendritic spine plasticity is thought to be the cellular basis of learning and memory involving a dynamic orchestration of plasticity in existing spines and the formation and elimination of new spines. Motor learning is correlated with a significant increase in formation of new spines on the apical tuft of layer 5 pyramidal cells in the primary motor cortex (M1) (Xu, T. Nature 2009) and selective artificial shrinkage of dendritic spines in M1 that are potentiated during motor learning disrupts an acquired motor skill (Hayashi-Takagi, A. Nature 2015) indicating that the spine plasticity during motor learning is indispensable for an animal's skill acquisition. However despite abundant evidence for the correlation between motor learning and postsynaptic spine plasticity the origin of synaptic inputs to newly-formed spines remain elusive. Spine plasticity depend on the characteristics of presynaptic neural circuits. M1 forms corticocortical networks as well as cortico-subcortical loops of wiring via the thalamus. Understanding network plasticity during learning therefore requires concurrent analysis of postsynaptic spine dynamics and presynaptic cell characteristics. We found that distinct neural circuits are involved in formation of new spines and in their maintenance for motor learning and memory. Post hoc characterization of the presynaptic cell types revealed that motor skill improvement coincided with selective formation of spines innervated by corticocortical axons. Chemogenetic silencing of corticocortical input to the motor cortex impaired both motor learning

and spinogenesis. Fewer thalamocortical synapses were generated during learning but survived longer with increased spine size compared to new corticocortical synapses. These results suggest that pyramidal cell dendrites in motor cortex use a neural circuit division-of-labor during skill learning with dynamic teaching contacts from top-down intracortical axons followed by synaptic memory formation driven by thalamic axons. These results suggest that pyramidal cell dendrites in motor cortex use a neural circuit division-of-labor during skill learning with dynamic teaching contacts from top-down intracortical axons followed by synaptic memory formation driven by thalamic axons. Dual spine supervision may govern diverse skill learning in neocortex.

Disclosures: J. Sohn: None. **M. Suzuki:** None. **M.E. Larkum:** None. **Y. Kawaguchi:** None. **Y. Kubota:** None.

Nanosymposium

177. Mechanisms Underlying Synaptic Plasticity: Structural, Metabolic and Gene Regulation Changes

Location: SDCC 23

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 177.04

Topic: B.05. Synaptic Plasticity

Support: DFG/SFB 1286 (Projects A07 and Z04)

Title: Synaptic plasticity is realized via protein turnover and nanoscale arrangement of PSD95 on a single-synapses level

Authors: C.-M. GÜRTH[1], M. DO REGO BARROS FERNANDES LIMA[1], J. HUBRICH[1], A. CERECEDA DELGADO[1], V. MACARRON PALACIOS[1], N. MOUGIOS[3], F. OPAZO[3], ***E. D'ESTE**[2];
[2]Optical Microscopy Facility, [1]Max Planck Inst. For Med. Res., Heidelberg, Germany; [3]Ctr. For Biostructural Imaging of Neurodegeneration (BIN), Univ. Med. Göttingen, Goettingen, Germany

Abstract: PSD95 is a key component of the postsynaptic density undergoing activity-dependent regulation. In particular, chronic stimulation induces plasticity mechanisms which rely on protein synthesis and structural remodeling. We set out to investigate these mechanisms on a single-synapse level by analyzing how activity influences the integration of new PSD95 molecules at synaptic sites and their rearrangements within individual clusters at molecular resolution.
By combining genome-editing, pulse-chase experiments and STED nanoscopy on primary rat hippocampal neuronal cultures, we revealed that the amount of new PSD95 recruited to individual synapses scales with the synapse size, meaning that smaller synapses integrate a lower amount of new PSD95, while bigger synapses integrate a higher amount of new PSD95. This evidence suggests an activity-dependent regulation, which was confirmed by the bidirectional response observed when applying prolonged excitatory (gabazine) or inhibitory (TTX) stimulation. Indeed, activity inhibition results in a higher amount of new PSD95 incorporated at synapses, while excitation has the opposite effect. Importantly, the co-localization of the old and

new protein pools as observed at STED resolution is only partial, suggesting a distinct function of the two protein pools or a modular process of PSD95 assembly.

To analyze the activity-dependent structural rearrangements of the PSD95 clusters, we used MINFLUX nanoscopy, a technique that delivers single-digit nanometer resolution in 3D. When imaging PSD95 labelled with nanobodies, we observed a clustering behavior of molecules distancing less than ~40 nm from each other in the majority of the synaptic sites. Notably, the distance between the PSD95 nanobody-detected molecules does not correlate with the synapse size, and therefore appears to be a fixed feature of the PSD95 organization. Upon prolonged stimulation, the density of detected molecules in the PSD95 clusters increased for both TTX and gabazine treatment, resulting in smaller and compacter PSD95 clusters.

Concluding, we demonstrate that while excitatory and inhibitory stimulations induce bidirectional changes to PSD95 turnover at individual synapses correlating with the synapse size, the rearrangements in the structural organization of PSD95 molecules, as detected by specific nanobodies, are monodirectional. Altogether, the present work leverages on innovative labeling and imaging technologies to shed new light into the mechanisms underlying plasticity at the individual synapse level adding previously inaccessible information.

Nanosymposium

177. Mechanisms Underlying Synaptic Plasticity: Structural, Metabolic and Gene Regulation Changes

Location: SDCC 23

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 177.05

Topic: B.05. Synaptic Plasticity

Title: Reorganization of Synaptic Vesicles Associated with Mitochondria Following Long-Term Potentiation

Authors: *G. C. GARCIA[1], T. M. BARTOL[1], L. M. KIRK[2], K. M. HARRIS[2], T. J. SEJNOWSKI[1];
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Abstract: Functional and structural elements of synaptic plasticity are tightly coupled, as has been extensively shown for dendritic spines and presynaptic terminals. We interrogated structural features of presynaptic terminals in 3DEM reconstructions from CA1 hippocampal axons that had undergone control stimulation or theta-burst stimulation (TBS) to produce long term-potentiation (LTP). The total volumes of the presynaptic bouton, mitochondria, and synaptic vesicles were measured, in addition to distances between neighboring vesicles and to the active zone. Finally, we computed the vesicle-associated volume, volume and density of the cloud of vesicles. The outcomes revealed that vesicles in the core of the presynaptic vesicle cloud are reduced two hours following the induction of LTP. These findings support the hypothesis that the greater vesicle loss in boutons associated with mitochondria (Smith et al., 2016) occurs at the core of the vesicle cloud, and not restricted to the docked, readily-releasable, or reserve pool of vesicles. Furthermore, the distances between neighboring vesicles were greater in less dense terminals that present greater percentage of mitochondria volume and lower percentage of total vesicle volume. These findings further support the involvement of mitochondria on vesicle mobilization (Smith et al., 2016) and allow the identification of presynaptic terminals with extreme morphometric changes following the induction of LTP. Smith HL, Bourne JN, Cao G, Chirillo MA, Ostroff LE, Watson DJ, and Harris KM (2016) Mitochondrial support of persistent presynaptic vesicle mobilization with age-dependent synaptic growth after LTP. eLife, 5:e15275. PMCID: PMC5235352

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Nanosymposium

177. Mechanisms Underlying Synaptic Plasticity: Structural, Metabolic and Gene Regulation Changes

Location: SDCC 23

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 177.06

Topic: B.05. Synaptic Plasticity

Support: NIH 5RO1NS112492
        NIH R37MH070000

Title: Copia, a Drosophila 182ndersen182ection, regulates structural synaptic plasticity

Authors: *P. M'ANGALE, A. LEMIUEX, A. SIMKIN, C. XIAO, V. BUDNIK, T. THOMSON;
Neurobio., Univ. of Massachusetts, Worcester, Worcester, MA

Abstract: The function of the non-genic DNA often referred to as "junk DNA", of which transposable elements (TE) makes up a large portion, has remained elusive. Previously our lab has shown that a domesticated retrotransposon fragment, *activity regulated cytoskeleton-associated protein 1 (Arc1)*, is important in the regulation of structural synaptic plasticity in the

Drosophila larval Neuromuscular junction (NMJ). Key to the finding was that Arc1 transport its mRNA across the synapse and is required for normal maturation of synaptic boutons. Here, we show that copia, a bona fide TE is enriched at the larval NMJ and is trafficked across synapses in extracellular vesicles. Targeted knockdown of copia[gag] utilizing the bipartite GAL4/UAS genetic system pre-synaptically results in abnormal NMJ development, increased plasticity, and a marked activity derived phenotype. Through digital PCR and immunofluorescence microscopy we determined that there is an enrichment of copia[gag] mRNA and protein in the larval brains when compared to other somatic tissue. We observe that copia[gag] acts antagonistically to Arc1 and together regulate structural synaptic plasticity at the larval NMJ. This, we believe is the first report to document a physiological role for a retrotransposon at synapses, and further corroborates recent findings and data that suggest Tes and potentially other types of "junk DNA" require a closer examination.

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Nanosymposium

177. Mechanisms Underlying Synaptic Plasticity: Structural, Metabolic and Gene Regulation Changes

Location: SDCC 23

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 177.07

Topic: B.05. Synaptic Plasticity

Title: Homeostatic synaptic plasticity recruits epitranscriptomic modifications in mice and humans

Authors: *M. LENZ[1], P. KRUSE[1], A. EICHLER[1], P. STÖHR[1], J. STRAEHLE[2], P. TURKO[3], I. VIDA[3], J. BECK[2,4,5], A. VLACHOS[1,4,5,6];
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Abstract: Homeostatic synaptic plasticity aims at compensating for perturbations in network activity, thereby maintaining neurons in a dynamic functional range. Among the mechanisms that regulate synaptic plasticity, dendritic mRNA translation plays a crucial role for the local control of synaptic properties. Nevertheless, the precise regulatory mechanisms and the relevance of homeostatic plasticity in the human brain warrant further investigation. In this study, we investigated the impact of neural network silencing on functional and structural properties of neurons in murine and human cortical tissue. Pharmacological inhibition of

voltage-gated sodium channels or glutamatergic neurotransmission (i.e., common targets of anticonvulsant substances) in organotypic tissue cultures and adult human neocortical slices demonstrated that network silencing promotes a compensatory functional and structural reorganization of excitatory synapses. Moreover, this form of compensatory excitatory synaptic plasticity was accompanied by (epi)transcriptomic changes. Our findings provide first experimental evidence for homeostatic synaptic plasticity in the adult human neocortex. They suggest an important role for mRNA modifications and protein synthesis in the regulation of synaptic homeostasis in mice and humans.

Disclosures: M. Lenz: None. **P. Kruse:** None. **A. Eichler:** None. **P. Stöhr:** None. **J. Straehle:** None. **P. Turko:** None. **I. Vida:** None. **J. Beck:** None. **A. Vlachos:** None.

Nanosymposium

177. Mechanisms Underlying Synaptic Plasticity: Structural, Metabolic and Gene Regulation Changes

Location: SDCC 23

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 177.08

Topic: B.05. Synaptic Plasticity

Support: Else-Kröner Fresenius Stiftung (EKFS)

Title: Entorhinal cortex lesion induces homeostatic synaptic plasticity of CA3 pyramidal neurons

Authors: *P. KRUSE[1], A. VLACHOS[1,2,3], M. LENZ[1];
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Abstract: A common aspect of many neurological diseases is the denervation of brain regions because of demyelination or cell death. Nonetheless, the underlying mechanisms involved in lesion-induced reorganization of neural networks warrant further investigation. In this study, we assessed the effects of a partial denervation on hippocampal neurons. Lesion of the entorhinal cortex in organotypic entorhino-hippocampal tissue cultures — prepared from mice of both sexes — was used to denervate distal apical dendrites of hippocampal granule cells and CA3 pyramidal neurons. Changes in excitatory neurotransmission were assessed with single and paired whole-cell patch-clamp recordings, and morphological alterations were analyzed with electron microscopy. Moreover, a region specific transcriptome as well as compartmentalized FISH analyses were performed. Partial denervation resulted in homeostatic synaptic adaptions of dentate granule cells and CA3 pyramidal cells. These changes in excitatory neurotransmission occurred predominantly in the strongest synapses as shown by a hierarchical analysis of spontaneous excitatory postsynaptic currents. The homeostatic adjustment was accompanied by characteristic region-specific transcriptomic changes. Consistent with these findings, paired

recordings of dentate granule cells and CA3 pyramidal neurons and ultrastructural analyses of mossy fiber synapses revealed denervation-induced compensatory structural and functional changes at the single synapse level. These homeostatic adjustments seem to depend at all levels on the presence of the actin-binding protein synaptopodin, since they were not evident in synaptopodin-deficient tissue preparations. We conclude that partial denervation of dentate granule cells and CA3 pyramidal neurons leads to synaptopodin-dependent homeostatic hetero- and transsynaptic adaptations in hippocampal networks.

Disclosures: P. Kruse: None. **A. Vlachos:** None. **M. Lenz:** None.

Nanosymposium

177. Mechanisms Underlying Synaptic Plasticity: Structural, Metabolic and Gene Regulation Changes

Location: SDCC 23

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 177.09

Topic: B.05. Synaptic Plasticity

Support: NIH 5P01NS092525

Title: Mitochondrial and Cortical Synaptic Deficits in a Mouse Model of Huntington's Disease: Effects of Brain-Derived Neurotrophic Factor

Authors: *W. MOBLEY[1], **Y. GU**[2], **A. POPE**[3], **C. SMITH-GEATER**[4], **C. CARMONA**[5], **A. JOHNSTONE**[3], **Z. SHI**[3], **X. CHEN**[3], **K. M. KLECZKO**[6], **J. FRYDMAN**[6], **M. W. BERNS**[3], **L. M. THOMPSON**[7], **C. WU**[8];
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Abstract: BACKGROUND: Huntington's disease (HD) is a rare, inherited disease that often causes movement, cognitive and psychiatric deficits. Synaptic alteration of the cortico-striatal circuits is one of the earliest manifestations of neuronal dysfunction in HD. However, the mechanism(s) by which mutant Huntingtin protein (mHTT) impacts synaptic formation and function is not yet well understood. **OBLECTIVE:** To define mitochondrial and synaptic deficits in the BAC mouse model of HD and to investigate the potential rescuing effect of brain-derived neurotropic factor (BDNF). **METHODS**: Here we used the BACHD and the ΔN17-BACHD models to examine cortical synaptic formation and function *in vitro*. We established long-term cortical neuronal cultures up to 35 days in vitro (DIV35). We quantitated mitochondrial morphology and membrane potential and examined synapse formation and maintenance by immunostaining with specific antibodies against Synapsin I, a pre-synaptic

marker, and against the 95 kD post synaptic density protein (PSD95), a post-synaptic marker, at different stages. **RESULTS:** Synapses began to form at DIV14 at which time there was no difference between WT and BACHD and between WT and ΔN17-BACHD cortical neurons. However, starting at DIV21 and continuing to DIV35, BACHD neurons showed a progressive reduction in synapses as compared to WT neurons. Significantly, the synaptic deficits in BACHD neurons were completely mitigated by BDNF treatment. Unlike BACHD neurons, the synapses in ΔN17 BACHD cortical neuronal cultures showed a progressive increase as compared to WT neurons. Similar patterns were also observed for mitochondrial membrane potential using JC-9 at DIV13. The mitochondrial membrane potential was lower in BACHD but higher in ΔN17 BACHD cortical neurons than WT neurons. However, cortical neurons from both models showed a significant mitochondrial fragmentation. **CONCLUSION**: Taken together, our results demonstrate that synapses and mitochondria are abnormal in both BACHD and ΔN17 BACHD cortical neurons, albeit with interesting and significant differences. That BDNF exerts a potent effect in rescuing synaptic deficits as well as some aspects of mitochondrial dysfunction points to BDNF raises the question as to its mechanism(s) of action and points to the possibility of employing it therapeutically.

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Nanosymposium

177. Mechanisms Underlying Synaptic Plasticity: Structural, Metabolic and Gene Regulation Changes

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Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 177.10

Topic: B.05. Synaptic Plasticity

Support: NS115072 (GMT)

Title: New mechanistic insights into acute intermittent hypoxia-induced sympathetic long-term facilitation in mice

Authors: *R. RODRIGUES PERIM, G. M. TONEY;
Cell. And Integrative Physiol., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Sleep apnea (SA) is associated with chronically elevated sympathetic nerve activity (SNA) and hypertension. The sympathetic hyperactivity of SA can be mimicked by exposing animals to acute intermittent hypoxia (AIH). Notably, just a few bouts of AIH are sufficient to elicit a prompt and progressive increase of SNA, referred to as sympathetic long-term facilitation (sLTF). Although sLTF is thought to reflect neuroplasticity, underlying mechanisms are not well understood. Here we tested the hypotheses that: 1) AIH is sufficient to induce sLTF in mice, opening the opportunity to apply transgenic/gene-editing methods along with opto-

/chemogenetic techniques to explore sLTF mechanisms; 2) AIH-induced sLTF in the mouse reflects ionotropic glutamate receptor activation in the hypothalamic paraventricular nucleus (PVN). AIH was performed in vagus nerve-intact, anesthetized, artificially ventilated, and paralyzed male C57Bl/6 mice (20-30g). AIH consisted of 5, 3 min bouts of hypoxia at 0.15 FiO2 interspersed with 3 min bouts of recovery at 1.0 FiO2. Arterial pressure and postganglionic renal SNA were recorded during the AIH protocol and for 60 min thereafter. Control and treated mice received PVN injections of vehicle (aCSF) and the pan-specific ionotropic glutamate receptor antagonist kynurenic acid (KYN, 2 nmol, ~40 nL), respectively, 10 min before the start of AIH (n=7 per group). Renal SNA was similar between groups before and after PVN vehicle/KYN but increased progressively with each bout of hypoxia only in the control group. Immediately after AIH (~ 5 min), when mice were returned to continuous hyperoxic ventilation, SNA was again similar between groups. Over the next 60 min of hyperoxic ventilation, the ramp increase of SNA was significantly greater in PVN vehicle-treated than in KYN-treated mice (P<0.05). In control mice only, the magnitude of sLTF quantified 60 min after AIH was significantly correlated with the amplitude of the SNA response to the last bout of AIH (R=0.85, p=0.015) and with the amplitude ratio of the SNA response to the 5th vs. 1st hypoxic episodes (R=0.75, p=0.0498), indicating that these variables predict sLTF magnitude. Arterial pressure was stable at baseline but dropped in both groups by <15 mmHg over the 60 min post-AIH period. This indicates that differential arterial baroreceptor unloading is unlikely to underlie differential sLTF magnitude between control and PVN KYN-treated mice. We conclude that post-AIH sLTF requires availability of PVN ionotropic glutamate receptors, which supports a model of sLTF in which potentiating glutamatergic plasticity impacting pre-sympathetic neurons contributes to AIH-induced sLTF.

Disclosures: R. Rodrigues Perim: None. **G.M. Toney:** None.

Nanosymposium

178. Novel Therapeutic Interventions for Ischemic Stroke

Location: SDCC 11

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 178.01

Topic: C.09.Stroke

Title: Protecting ischemic penumbra against cerebrovascular injury with novel combinatory treatment LAU-0901 and 187ndersen187ection D1

Authors: *N. BAZAN[1], M. M. REID[2], P. K. MUKHERJEE[2], A. OBENAUS[3], L. KHOUTOROVA[1], K. SHELVIN[1], W. LEWIS[1], L. S. BELAYEV[1];
[1]Neurosci., [2]Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA; [3]Pediatrics, Univ. of California, Irvine, Irvine, CA

Abstract: Acute ischemic stroke lesions encompass the penumbra, an area of hypoperfusion surrounding the core with a limited lifespan of a few hours unless reperfusion is initiated. Despite this short window, the penumbra is potentially salvageable and is a target for neuroprotection. This study aimed to determine the neuroprotective effect of LAU-001 and NPD1 on the ischemic penumbra. Two different types of small bioactive molecules were investigated. The first was LAU-0901, an antagonist of PAF-R that blocks activated pro-inflammatory signaling and has been shown to have promising efficacy in a stroke model. The second product of DHA, a novel synthetic docosanoid 188ndersen188ection D1 (NPD1), activates cell-survival pathways and possesses potent anti-inflammatory and neuroprotective activity in the brain. Male Sprague-Dawley rats (280-355g) were anesthetized with isoflurane/nitrous oxide and received 2h MCAo by intraluminal suture. Neurological status was evaluated at 60 min, and on days 1, 2, 3, or 7; a grading scale of 0-12 was employed. Rats were treated with LAU-0901 (i.p. 60mg/kg at 3h) and NPD1 (i.v. 222 µg/kg at 3h 15 min) after the onset of stroke and vehicle (cyclodextrin and saline at 3h). There were four groups: LAU-0901+NPD1 and vehicle, 3 days' survival, and LAU-0901+NPD1 and vehicle, 7 days' survival. Rats were perfused with 4% Paraformaldehyde on days 3 and 7, and an *ex vivo* MRI of the brain was conducted using 11.7 T MRI. Infarct Hierarchal region splitting was used to distinguish the core from the penumbra. Physiological variables showed no significant differences among groups. No adverse behavioral side effects were observed after the administration of LAU-0901+NPD1. Combinatory treatment with LAU-0901+NPD1 improved behavioral score compared to vehicle-treated rats in both 3-day and 7-day groups. In the 3-day LAU-0901+NPD1 treatment group, the neurological scores improved on days 1, 2, and 3 by 33, 34, and 35% compared to the vehicle group. In the 7-day LAU-0901+NPD1 treatment group, the neurological scores improved on days 1, 2, 3, and 7 by 23, 26, 28, and 34% compared to the vehicle group. MRI analysis of lesion, core, and penumbra volumes in the process. We concluded that combination treatment by the PAF-R antagonist, LAU-0901, plus NPD1 affords synergistic neuroprotection in the post-ischemic brain and open avenues for potential future therapeutics for ischemic stroke. We are currently exploring the molecular mechanisms involved.

Disclosures: N. Bazan: None. **M.M. Reid:** None. **P.K. Mukherjee:** None. **A. Obenaus:** None. **L. Khoutorova:** None. **K. Shelvin:** None. **W. Lewis:** None. **L.S. Belayev:** None.

Nanosymposium

178. Novel Therapeutic Interventions for Ischemic Stroke

Location: SDCC 11

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 178.02

Topic: C.09.Stroke

Support: PON NEON (ARS01_00769) from the Italian Ministry of Research, MIUR, to GP

Title: Plasma Exosomes from Remote Ischemic Post-Conditioned Rats Attenuate Cerebral Ischemia-Reperfusion Injury Through Transferring a Specific Cluster of miRNAs

Authors: O. CUOMO[1], P. CEPPARULO[1], P. BRANCACCIO[1], A. VINCIGUERRA[2], L. ANNUNZIATO[1], *G. PIGNATARO[1];
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Abstract: Remote ischemic conditioning (RIC) represents an innovative and attractive neuroprotective approach in brain ischemia. The purpose of this intervention is to activate endogenous tolerance mechanisms by inflicting a subliminal ischemia injury to the limbs, or to another "remote" region, leading to a protective systemic response against ischemic brain injury. Among the multiple candidates that have been proposed as putative mediators of the protective effect generated by the subthreshold peripheral ischemic insult, it has been hypothesized that microRNAs may play a vital role in the infarct-sparing effect of RIC. Recently, miRNAs have been isolated in secreted vesicles called exosomes. Exosomes, small lipid bilayer vesicles, are part of the transportable cell secretome that can be taken up by nearby recipient cells or can travel through the bloodstream to cells in distant organs. In the light of these premises, the aims of the present study were: 1) To evaluate the effect of the systemic administration of exosomes isolated from plasma of animals subjected to remote ischemic postconditioning (RLIP) on cerebral ischemia-reperfusion injury; 2) To finely dissect exosomes content in terms of miRNAs; 3) To select those regulatory miRNAs specifically expressed in protective exosomes and to identify the molecular pathways involved in their protective effects. Circulating exosomes were isolated from blood of animals exposed to RLIP and administered to animals exposed to tMCAO by intracerebroventricular, intraperitoneal or intranasal routes. miRNA signature in protective exosomes was evaluated by microarray analysis. Exosomes isolated from the plasma of rats exposed to RLIP attenuate cerebral ischemia-reperfusion injury and improved neurological functions. By contrast, exosomes isolated from plasma of animals exposed to tMCAO alone failed to confer neuroprotection. Notably, protective exosomes were characterized by a peculiar miRNA signature. Collectively, the results of the present work demonstrated that exosomes released in plasma of rats after RLIP may transfer a neuroprotective signal to the brain of ischemic animals and represent a potentially translatable therapeutic approach in stroke.

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Nanosymposium

178. Novel Therapeutic Interventions for Ischemic Stroke

Location: SDCC 11

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 178.03

Topic: C.09.Stroke

Title: Hdac9 promotes brain ischemia ferroptotic neuronal death by regulating SP1/GPX4 and HIF-1/TFR1 signaling pathways

Authors: *L. FORMISANO[1], L. SANGUIGNO[1], N. GUIDA[1], O. CUOMO[1], G. PIGNATARO[1], L. ANNUNZIATO[2];
[1]Univ. Degli Studi Di Napoli Federico II, Naples, Italy; [2]IRCCS SDN NAPOLI, Naples, Italy

Abstract: Histone Deacetylase 9 (HDAC9) overexpression has been found neurodetrimental in humans stroke patients. Ferroptosis is a neuronal death pathway characterized by an iron-depenent accumulation of lipid peroxides and its counteraction is an emerging therapeutic target in brain ischemia. Here, we investigated the possible relationship between stroke-induced HDAC9 and ferroptosis in *in vitro* and *in vivo* models of stroke. The results indicated that counteraction of oxygen and glucose deprivation (OGD) plus reoxygenation (RX) -induced HDAC9 increase blocked glutathione peroxidase 4 (GPX4) reduction and transferrin 1 (TfR1) receptor increase. Interestingly, HDAC9 increase occurred in the cytosol compartment but not in the nucleus. Importantly, OGD/Rx reduced specificity protein 1 (Sp1) protein expression and increased hypoxia-inducible factor (HIF-1) (HIF-1) that are transcriptional activators of GPX4 and TfR1 in neurons, respectively. Importantly, Sp1 reduction and HIF-1 increase were partially blocked by pre-treatment with siHDAC9, suggesting the involvement of this HDAC isoform in causing this effect. Interestingly, OGD/Rx-induced Sp1 down-regulation and HIF up-regulation was related to their deacetylation, with consequent ubiquitination for Sp1 and deubiquitination for HIF-1. Co-immunoprecipitation experiments showed that Sp1 and HIF were bound with HDAC9 after OGD/Rx exposure, whereas siHDAC9 reverted OGD/Rx-induced degradation of Sp1 and increase of HIF. Furthermore, rat cortical neurons exposed to OGD/Rx showed an increase of HDAC9, in parallel with a down-regulation of Sp1 and GPX4 and an up-regualtion of HIF and TFR1 proteins, respectively. Importantly, transfection of siRNAs against HDAC9, HIF-1 or TFR1 or transfection of vectors overexpressing Sp1 or GPX4 significantly blocked OGD/Rx-induced increase of the malondialdehyde (MDA) content, a well known marker of lipid peroxidation and consequent cell death in cortical neurons. Notably,, Sp1 recruitment to the GPX4 gene was reduced whereas HIF-1 recruitment to the TFR1 gene was increased in the temporoparietal cortex of rats subjected to transient middle cerebral artery occlusion (tMCAO), with a consequent GPX4 reduction and TFR1 increase, respectively. Conversely, the tMCAO-induced. (1) Sp1 and GPX4 reduction or (2) HIF-1 and TfR1 increase was prevented by intracerebroventricular injection of siHDAC9. These results suggest that HDAC9 knockdown by blocking Sp1 and HIF-1 binding on GPX4 and TfR1 gene promoter sequences respectively, provides protection from stroke-induced ferroptosis.

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Nanosymposium

178. Novel Therapeutic Interventions for Ischemic Stroke

Location: SDCC 11

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 178.04

Topic: C.09.Stroke

Title: Long-lasting neuroprotection and neurological improvement by PAF-receptor antagonist plus a lipid mediator in transient focal cerebral ischemia in rats.

Authors: *L. BELAYEV[1], M. M. REID[1], A. OBENAUS[2], P. K. MUKHERJEE[1], L. KHOUTOROVA[1], K. SHELVIN[1], S. KNOWLES[1], T. MOFFATT[1], R. S. PAYNE[1], J. M. DUGAS[1], N. G. BAZAN[1];
[1]LSU Hlth. Sci. Ctr., New Orleans, LA; [2]Pediatrics, Univ. of California, Irvine, Irvine, CA

Abstract: Neuroprotective efficacy observed during short survival periods may not necessarily apply to more extended survival periods, and some stroke-impaired behaviors recover naturally in rodent models 1-2 weeks after stroke. The objective of the present study was to test the hypothesis that acute LAU-0901 plus NPD1-induced neuroprotection endures in animals allowed to survive for several weeks after focal ischemic insult. Two small bioactive molecules were investigated: LAU-0901, a PAF-R antagonist that blocks pro-inflammatory signaling, and 191ndersen191ection D1 (NPD1), which activates cell-survival pathways, and their combination exerts potent anti-inflammatory activity in the brain. Male Sprague-Dawley rats (290-360g) were subjected to 2h of middle cerebral artery occlusion (MCAo) by the intraluminal filament and treated with vehicle, LAU (60 mg/kg, IP), NPD1 (222 µg/kg, IV), LAU-0901+NPD1 at 3 h after onset of MCAo. Rats received neurobehavioral examinations during MCAo (60 min) and then on days 1, 2, 3, 7, and weekly during eight weeks survival period, followed by *ex vivo* MRI using 11.7 T on weeks 4 and 8. Physiological variables showed no significant differences among groups. No adverse behavioral side effects were observed after the administration of LAU-0901, NPD1, or LAU-0901+NPD1. LAU-0901 and NPD1 treatments alone significantly improved the behavior compared to the vehicle group on day 1 (by 32 and 33%), day 7 (38 and 40%), week 4 (38 and 39%), and persisting throughout eight weeks of survival period (by 58 and 44%, respectively). Combinatory treatment with LAU-0901 plus NPD1 dramatically improved total neurological score on days 1 (by 37), 2 (by 37%), 3 (by 36%), 7 (40%) and on weeks 4 (by 40%), 5 (44%), 6 (44%), 7 (44%) and 8 (41%) compared to vehicle group. MRI analysis in the process. In conclusion, these data suggest that LAU-0901 and NPD1 alone provide high-grade neuroprotection in the MCAo model. In conclusion, combinatory therapy with LAU-0901 plus NPD1 affords synergistic neuroprotection with improved neurological recovery when administered at 3h after focal cerebral ischemia in rats.

Disclosures: L. Belayev: None. **M.M. Reid:** None. **A. Obenaus:** None. **P.K. Mukherjee:** None. **L. Khoutorova:** None. **K. Shelvin:** None. **S. Knowles:** None. **T. Moffatt:** None. **R.S. Payne:** None. **J.M. Dugas:** None. **N.G. Bazan:** None.

Nanosymposium

178. Novel Therapeutic Interventions for Ischemic Stroke

Location: SDCC 11

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 178.05

Topic: C.09.Stroke

Title: A Novel Method of Delivering a Therapeutic Combination of Vasculogenic Transcription Factor Genes in Stroke via Myeloid-Derived Suppressor Cells

Authors: *D. J. DODD[1], L. LEMMERMAN[2], A. PANIC[2], D. ALZATE-CORREA[2], S. DUARTE-SANMIGUEL[2], S. PIATKOWSKI[2], M. NG[3], R. TURAGA[2], W. E. CARSON[4], N. HIGUITA-CASTRO[2,4,5], D. GALLEGO-PEREZ[2,4];
[1]Biomed. Sci. Grad. Program, [2]Biomed. Engin., [3]Microbial Infection and Immunity, [4]Surgery, [5]Biophysics, Ohio State Univ., Columbus, OH

Abstract: A major goal of clinical applications in cerebral vascular disorders, primarily stroke, is rehabilitation at the molecular and cellular level. This involves reconstruction of vascular networks using pro-angiogenic therapies to recover neural functionality. On this front, nanotechnology-based methods have made big strides in recovering vascular networks using pro-angiogenic molecules. Previously, our lab has successfully shown that nanotransfection of fibroblasts with a DNA cocktail for pro-vasculogenic transcription factors can mediate an increase in perfusion in the brain following an ischemic stroke [1]. Plasmid DNA for transcription factors Etv2, Fli1, and Foxc2 (EFF) were transfected into murine fibroblasts, which were intracranially delivered to the site of the infarct in an induced MCAO model of stroke. After a 21-day period, our results showed an increase in biomarkers for both vascularity and neural cellularity. Furthermore, imaging showed increased vascularity, decreased infarct size, and decreased glial scarring. Finally, behavioral analysis of mice showed a marked recovery of motor function in the weeks following treatment. Altogether, these results suggested that our plasmid DNA cocktail might serve as a viable candidate for re-vascularization therapy. Despite this promise, however, the proposed therapy needed to be precisely delivered to the infarct region, which can prove to be difficult and risky. Thus, our focus turned to finding a new candidate delivery vessel for our gene therapy cocktail. Myeloid-derived suppressor cells (MDSCs), a population of immature myeloid cells, showed promise due to the tendency of MDSCs to naturally accumulate in chronic disease tissues (e.g., stroke). Thus, we used an electroporation-based transfection technique to transfect MSC-2 immature myeloid cells with the EFF cocktail, thus engineering them to express a pro-angiogenic phenotype. Moreover, we looked at the role which biological nanoparticles, known as extracellular vesicles (Evs), secreted from the transfected cells played. We show that engineered MDSCs (eMDSCs) and their secreted Evs show significant EFF cargo loading of mRNA transcripts. We also show that secreted Evs and proteins from eMDSCs are bioactive via normalized uptake experiments. In this regard, eMDSC-derived conditioned media drove the formation of a highly proliferative tubular network of endothelial cells that is indicative of angiogenesis by numerous factors related to network growth and maturity in vitro. Ongoing studies aim to demonstrate the migration and therapeutic potential of eMDSCs in an induced MCAO model.
1. Lemmerman et al. Sci Adv, 7(12): eabd4735 (2021).

Nanosymposium

178. Novel Therapeutic Interventions for Ischemic Stroke

Location: SDCC 11

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 178.06

Topic: C.09.Stroke

Title: Reduction Of Behavior Deficits Following Delivery Of HSOX2 Gene To Stroke Brain Using PAMAM Dendrimer Nanomolecules

Authors: *B. SRINAGESHWAR[1,2,7], J. STADLER[1,2,7], M. M.-M. ANDREWS[2,7,1], B. MACDONALD[3,1,7], A. TOTH[2,7,1], A. POUDEL[2,1,7], R. SCHALAU[2,7], J. EVERS-SMITH[2,7], N. SINGH[2,7], J. SWIONTEK[2,7], P. OTERO[2,7], L. MERSINO[2,7], C. MALKOWSKI[2,7], D. SWANSON[4], A. SHARMA[4], G. L. DUNBAR[3,7,5], J. ROSSIGNOL[6,2,7];
[1]Col. Of Med., [2]Program in Neurosci., Central Michigan Univ., Mount Pleasasnt, MI; [3]Program in Neurosci., Central Michigan Univ., Mount Pleasant, MI; [4]Chem. And Biochem., [5]Psychology, Central Michigan Univ., Mount Pleasasnt, MI; [6]Col. Of Med., Central Michigan Univ., Mount Pleasant, MI; [7]Field Neurosciences Inst. Lab. For Restorative Neurol., Mount Pleasasnt, MI

Abstract: PAMAM dendrimers are 3-dimensional nanomolecules which can deliver DNA/RNA and drugs to different cells types *in vitro* and *in vivo*. In the past, we used the generation-4 (G4) PAMAM dendrimers with modified surface having 90% hydroxyl groups and 10% amine groups (known as G4-90/10). They can blood-brain barrier and deliver the cargo into the cells *in vitro* and *in vivo*. Unlike some of the viral vectors, these dendrimers can carry plasmids that are more than 10 kb in size. In the current study, we utilized G4-90/10 PAMAM dendrimers tagged with Cy5.5 fluorescent dye to deliver a gene that encodes a transcription factor hSOX2 to the infarct region in a stroke brain in rats. Following a stroke event, reactive astrocytes are recruited at the stroke region to perform their normal maintenance functions, creating severe inflammation. The goal of this study was to reprogram a fraction of these astrocytes into neurons and repopulate the infarct region in the stroke brain. The hSOX2 gene was delivered to the stroke brain complexed with the G4-90/10 dendrimers. Our initial behavior analysis as evidenced by the ladder test, indicated that, following delivery of hSOX2 by the dendrimers, there was recovery of behavioral functioning in the stroked rats compared to the vehicle-treated stroked rats and sham-operated

controls. Overall, these results show that PAMAM dendrimers have significant potential delivering therapeutic molecules for treating stroke and other neurological conditions.

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178. Novel Therapeutic Interventions for Ischemic Stroke

Location: SDCC 11

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 178.07

Topic: C.09.Stroke

Title: Stroke induced somatosensory loss impairs motor recovery in survivors using a Multisensory BCI-FES intervention

Authors: A. B. REMSIK[1], T. HOSSEINI[1], P. L. VAN KAN[3], *N. ADLURU[2], V. A. NAIR[4], J. WILLIAMS[1], V. PRABHAKARAN[5];
[1]UW-Madison, Madison, WI; [2]UW-Madison, Verona, WI; [3]Dept. of Kinesiology, Univ. of Wisconsin-Madison, Madison, WI; [4]UW-MADISON, Madison, WI; [5]Dept Neurosci, Univ. of Wisconsin, Madison, WI

Abstract: Objective The degree to which somatosensory loss resulting from stroke insult impairs motor function and recovery is not well understood. We seek to determine whether stroke-induced somatosensory impairment limits perceived recovery, as measured by Stroke Impact Scale-hand function (SIShf) subdomain, during BCI-FES intervention.
Methods Stroke participants (n=35, 16 female, M age 62yrs, ±12.8yrs) completed <30hrs of BCI-FES intervention with neuropsychology testing at pre (M1), and post intervention (M3). Participants were grouped *post hoc* on presence or absence of cutaneous impairment (n=20 with cutaneous impairment, n = 15 without cutaneous impairment), as measured by the NIH Stroke Scale (NIHSS) sensory domain and into a group with (n=25) and without poststroke proprioceptive impairment (n=10), as measured by the NIHSS motor arm domain and change in SIShf (M1–M3) for each grouping were compared using independent samples *t*-tests. **Results** Over time, participants with cutaneous sensory impairments perceived greater recovery (SIShf

mean difference =1.60 ±3.48) than those without (SIShf mean difference =0.067, ±1.87) and these mean differences were significantly different (p=0.016, *t* = -1.54, df =33). Participants without proprioceptive motor impairments resulting from stroke, perceived more recovery (SIShf group mean difference =2.20, ±4.21) than those with (SIShf group mean difference =0.44, ±2.22) and the mean differences were significant (p=0.50, *t*= 1.62, df =33).**Discussion** Integrity of the sensorimotor system is important for perceived motor recovery poststroke and those with proprioceptive sensory impairments perceive less recovery following BCI. Those without perceived less recovery than those with cutaneous loss, suggesting a greater limit of proprioceptive somatosensation with respect to motor rehabilitation poststroke.
Conclusion Proprioceptive loss as a result of stroke is prohibitive for perceiving motor recovery in a stroke-impaired hand following multimodal BCI-FES intervention.

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Nanosymposium

178. Novel Therapeutic Interventions for Ischemic Stroke

Location: SDCC 11

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 178.08

Topic: C.09.Stroke

Title: Inducible miR-1224 is Angiogenic and Restores Blood Flow to the Stroke-Affected Site of the Brain

Authors: R. PALAKURTI, N. BISWAS, S. ROY, S. C. GNYAWALI, M. MITHUN SINHA, K. SINGH, S. GHATAK, C. K. SEN, *****S. KHANNA**;
Indiana Univ., Indianapolis, IN

Abstract: α-Tocotrienol (TCT) form of natural vitamin E is more potent than better known α-tocopherol against experimental stroke. Angiographic studies of canine stroke revealed beneficial cerebrovascular effects of TCT. This work sought to understand the molecular basis of such effect. In mice, TCT supplementation improved perfusion at the stroke-affected site by inducing miR-1224. miRNA profiling of laser capture microdissected stroke-affected brain site identified miR-1224 as the only vascular miR induced. Lentiviral knockdown of miR-1224 significantly blunted the otherwise beneficial effects of TCT on stroke outcomes. Studies on primary brain microvascular endothelial cells revealed direct angiogenic properties of miR-1224. In mice not treated with TCT, advance stereotaxic delivery of miR-1224 mimic to the area at risk for stroke markedly improved stroke outcomes. Mechanistic studies identified Serpine1 as a novel target of miR-1224. Down-regulation of Serpine1 augmented the angiogenic response of

miR-1224 mimic in the brain endothelial cells. The inhibition of Serpine1, by dietary TCT and pharmacologically, increased cerebrovascular blood flow at the stroke-affected site and protected against stroke. This work assigns Serpine1, otherwise known to be of critical significance in stroke, a novel cerebrovascular function that can worsen stroke outcomes. miR-1224 dependent inhibition of Serpine1 can be achieved by dietary TCT as well as by the small molecule inhibitor TM5441.

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Nanosymposium

178. Novel Therapeutic Interventions for Ischemic Stroke

Location: SDCC 11

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 178.09

Topic: C.09.Stroke

Title: Analysis of Behavioral Deficits in Stroke Rats following Treatment with G4 PAMAM Dendrimer-encapsulated Curcumin

Authors: *J. ROSSIGNOL[1,2,3], J. STADLER[1,2,3], D. DOYLE[1,2], L. GARMO[1,2], G. RICHARDSON[1,2], M. KOPKA[1,2], A. POUDEL[1,2,3], B. SRINAGESHWAR[1,2,3], D. SWANSON[4], A. SHARMA[4], G. L. DUNBAR[2,3,5];
[1]Col. Of Med., [2]FNI lab for Restorative Neurol., [3]Program in Neurosci., [4]Chem. & Biochem. Dept., [5]Psychology Dept., Central Michigan Univ., Mount Pleasant, MI

Abstract: As ischemic strokes account for roughly 85% of strokes and current methods inadequately provide for the intense inflammation inherent to stroke, anti-inflammatory treatments provide potential alternative therapy for stroke patients. In combining cutting-edge nanoparticles with traditional turmeric (Curcumin) with anti-inflammatory properties, unique supportive therapies may be possible. The purpose of this study was to analyze behavior outcomes such treatments with anti-inflammatory properties injected systemically within an ischemic stroke model in rats. The curcumin encapsulated with the surface-modified PAMAM dendrimers were injected intraperitoneally for a week into the stroke rats following 90-min occlusion of middle cerebral artery. The rats underwent a battery of behavior tests such as Paw-lateralization index and neuro scoring. Average lateralization scores for each postoperative day within the stroke group demonstrated ipsilateral dominance, whereas lateralization was mixed within the sham group. One-way ANOVA within the stroke group demonstrated significant

differences in lateralization scores collected on postop day 7 between treatment groups. On post operative day 7, groups the rats that received curcumin encapsulated dendrimer demonstrated significantly higher averages of contra-lateralization than curcumin-only, empty dendrimer, and HBSS control groups. The stroke rats within the combined treatment groups (dendrimer and curcumin) demonstrated the greatest use of their contralateral limb, showing statistical significance compared to all control subgroups; with the greatest significance being seen between the combined treatment and the HBSS control. Results suggest relevance of combined dendrimer and curcumin therapy to potentially mitigate stroke sequalae; specifically, the impacts of ischemic stroke on contralateral limbs. Furthermore, due to the increased significance between combined therapeutic and pure HBSS control as compared to other groups, potential evidence exists to suggest role of dendrimer and curcumin in potential therapeutics – whereas in isolation, neither demonstrated the effects seen in combining both agents. These results invite further investigation of the therapeutic potential of nanoparticles in the ischemic patient model.

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Nanosymposium

178. Novel Therapeutic Interventions for Ischemic Stroke

Location: SDCC 11

Time: Sunday, November 13, 2022, 1:00 PM – 3:30 PM

Presentation Number: 178.10

Topic: C.09.Stroke

Title: A Novel Specific Nanoparticle Delivery to Cerebrovascular Endothelial Cells in Ischemic Stroke Brains

Authors: *H. HU[1], T. LEE[1], A. GUSDON[1], R. KITAGAWA[1], A. CHOI[1], **X. REN**[2];
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Abstract: Background: Cerebrovascular endothelial cells (CECs) are key components of the blood-brain barrier (BBB) and are the first cells to respond to ischemic stroke. However, therapeutic strategies targeting CESs are underdeveloped. Vascular cell adhesion molecule-1 (VCAM-1; CD106) is a member of the immunoglobulin-like superfamily (IgSF) that shows increased expression in CECs after stroke. Herein, we test if an RNA aptamer-based nanoparticle targeting VCAM-1 can be specifically demonstrated to target CECs in stroke brains. **Methods:** Anti-VCAM-1 aptamer nanoparticles (conjugated with Cy5.5) and control aptamer nanoparticles (randomized oligonucleotides conjugated with Cy5.5) were chemically synthesized. A transient middle cerebral artery occlusion (tMCAO, 60 min occlusion) stroke model was induced in mice.

Nanoparticles (0.5 nmol per mouse) were injected through their tail vein at 6 hours after stroke. *Ex vivo* whole brain images were acquired by IVIS imaging system. Then, the brain tissues were cryosectioned and stained against CD31 to visualize brain vasculature. Immunofluorescent images were acquired by Nikon confocal fluorescence microscope. **Results:** Higher fluorescence intensity was detected by IVIS images preferentially from the brain of stroke mice treated I anti-VCAM-1aptamer nanoparticles (n=4)- compared to the mice injected with control aptamer nanoparticles (n=4, p<0.05) or PBS (n=4, p<0.01). One mouse died in the PBS treated group but no mice died in nanoparticles treated group, demonstrating no toxicity of RNA nanoparticles. Quantified mean fluorescence intensity (MFI) showed the mice treated with anti-VCAM-1 aptamer nanoparticles had significantly higher MFI in ischemic hemisphere compared with stroke mice. Our confocal miscropy data from immunofluorescence staining against CD31 further confirmed that Cy5.5 signals were overlapped with CD31+ CECs. **Conclusion:** Our data suggest that CECs affected by stroke can be selectively targeted with anti-VCAM-1 aptamer nanoparticles. The VACM-1 specific aptamer nanoparticles demonstrate an efficiency and safety of RNA-based nanoparticle as a useful delivery platform for miRNA-based therapy specifically into CECs after stroke. This method is expected to overcome the challenge from the lack of cell-specific targeting approaches needed to reduce off-target side-effects.

Disclosures: H. Hu: None. **T. Lee:** None. **A. Gusdon:** None. **R. Kitagawa:** None. **A. Choi:** None. **X. Ren:** None.

Nanosymposium

179. Social Vision: Mechanisms of Face and Body Perception

Location: SDCC 24

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 179.01

Topic: D.06. Vision

Title: A computational understanding of zoomorphic perception in the human brain

Authors: S. DUYCK[1], S. BRACCI[2], ***H. P. OP DE BEECK**[1];
[1]KU Leuven, KU Leuven, Leuven, Belgium; [2]Univ. of Trento, Trento, Italy

Abstract: It is common to find objects that resemble animals, often on purpose (e.g., toys). The categorization of such objects as animal-like seems obvious to the human visual system. However, this "Animal Bias" for zoomorphic objects is strikingly absent in the recent benchmark artificial models of vision, deep convolutional neural networks (DCNNs). Here we provide a computational understanding of the human Animal Bias. First, we successfully induced this bias in multiple DCNNs (AlexNet, VGG). These DCNNs were pre-trained on ImageNet followed by

explicit exposure to zoomorphic objects during transfer learning and the instruction to categorize the zoomorphic objects as animals. After this transfer learning, DCNNs showed an Animal Bias with a held-out test set of animals, zoomorphic objects, and regular objects, as well as an increased correspondence with neural representations in human occipitotemporal cortex as measured through functional magnetic resonance imaging. Second, we further considered the possibility that the human Animal Bias could also emerge indirectly from several other well-known characteristics of human object perception, such as its peculiar sensitivity for faces and bodies, its explicit sensitivity for the superordinate distinction between animate and inanimate classes, its bias for relying upon shape rather than texture, and its exposure to a more ecologically valid distribution of image categories. We included DCNNs trained with stimulus sets and tasks specifically designed to test each of these hypotheses, but none of these training regimes induced an Animal Bias or an increased correspondence with neural representations. These findings provide computational support that the Animal Bias for zoomorphic objects observed in the human brain might specifically reflect the importance of such objects during development.

Disclosures: S. Duyck: None. **S. Bracci:** None. **H.P. Op de Beeck:** None.

Nanosymposium

179. Social Vision: Mechanisms of Face and Body Perception

Location: SDCC 24

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 179.02

Topic: D.06. Vision

Title: The neural building blocks of cognition: a new framework for mirror neurons.

Authors: *T. AFLALO[1], S. CHIVUKULA[1], C. ZHANG[1], N. POURATIAN[2], R. A. ANDERSEN[1];
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Abstract: We don't just see the world, we understand it. From a brief video or even a still image of a person in action, we can infer what they are doing, why they are doing it, what they will do next, or what they might have done but didn't. A fundamental question in neuroscience is how neural populations transform sensory inputs into such deep and versatile understandings. Mirror neurons are proposed to be the neural basis for such understanding, at least for how we infer what another person intends or feels. However, the mirror hypothesis does not encompass all aspects of understanding. For example, if understanding comes from activating our own high-level action representations, how can we understand actions we have never performed (e.g.,

jumping a skateboard)? Or could never perform (e.g., flying)? Alternate theoretical frameworks have emerged in parallel based on the concept that human-like learning and thinking is the product of how neural systems build and use internal models of the world, what we will call the "cognition through internal models" framework. These internal models are characterized by features such as generalizability and compositionality – attributes that overcome many of the limitations ascribed to the mirror hypothesis.

We hypothesize that "mirroring" is one manifestation of a more general mechanism by which we create generalizable internal representations of the world. To test this hypothesis, we recorded from populations of neurons in human PPC while a participant experienced actual touch (to the participant) or observed touch (to another individual) while manipulating the location of a touch (cheek, shoulder) and touch type (pinch, press, rub, and tap). The population was not well characterized by individual sensations being mirrored between actual and observed sensations. Instead, basic-level tactile variables related to body location and touch type were encoded as generalizable properties of the neural population, encoded in similar ways across different task conditions. These encodings could be explained using a small number of latent neural subspaces associated with basic aspects of the stimuli, such as the touch type and body location, consistent with compositionality. Our work supports the cognition through internal models framework. We speculate that populations of neurons achieve versatile understanding, not through a process of mirroring but instead by encoding representational building blocks of cognition.

Nanosymposium

179. Social Vision: Mechanisms of Face and Body Perception

Location: SDCC 24

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 179.03

Topic: D.06. Vision

Title: Fine-scale dynamics of functional connectivity in the face processing network during movie watching

Authors: *G. LEVAKOV[1], O. SPORNS[2], G. AVIDAN[3];
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Abstract: Face are naturally dynamic, multimodal and embedded in rich social context. However, mapping the face processing network in the human brain and its relation to behavior is typically done during rest or using isolated, static face images. The use of such contrived stimuli

might result in overlooking widespread cortical interactions obtained in response to naturalistic context and the temporal dynamics of these interactions. Here we examined large-scale cortical connectivity patterns measured in response to a dynamic movie in a sample of typical adults (n=517), to determine how inter-subject functional connectivity (ISFC) relates to face recognition scores. We found a positive correlation with recognition scores in edges connecting the occipital visual and anterior temporal regions and a negative correlation in edges connecting attentional dorsal, frontal default, and occipital visual regions. These ISFC patterns resembled previous findings comparing individuals with congenital prosopagnosia to normal controls and the viewing of inverted compared to upright faces. To further examine these connectivity patterns, we developed a novel method that allows analysis of inter-subject stimulus-evoked node/edge responses at a single TR resolution. Using this method, we demonstrated that co-fluctuations in face-selective edges observed here and in previous work are related to local activity in core face-selective regions. Finally, correlating this temporal decomposition of the observed ISFC patterns to the movie content revealed that they peak during boundaries between movie segments rather than during the presence of faces in the movie. Our novel approach demonstrates how visual processing of faces is linked to fine-scale dynamics in attentional, memory, and perceptual neural circuitry.
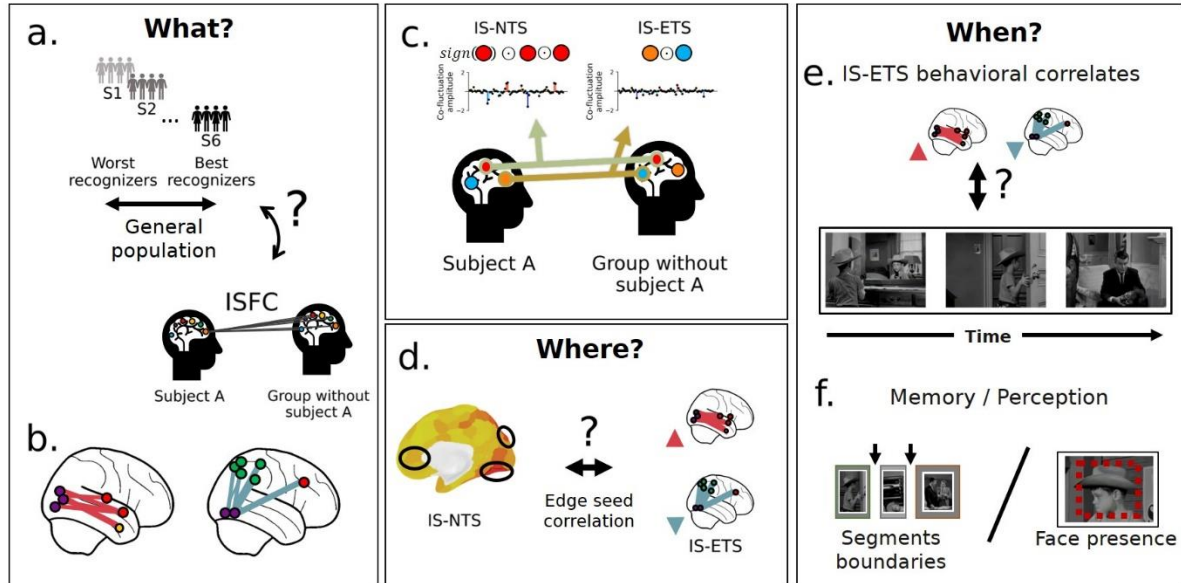
Fig. 1 – Study outline and outstanding questions. What - (a) We tested what are the ISFC correlations of face recognition in the normal population. (b) This test results in a set of edges positively (red) or negatively (blue) correlated with face recognition scores. (c) We present a novel method that decomposes the inter-subject connectivity among homologous (e.g., same voxel or brain region) or different brain regions in time to their edge time series [20]. When applied to homologous brain regions (inter-subject node time-series; IS-NTS) is computed as the element-wise multiplications of the z-scored signals of two nodes across subjects. The time series is then multiplied by the sign of the nodes' signal to differentiate co-activations and co-deactivations. When applied across different brain regions, (inter-subject edge time series; IS-ETS), the same procedure is used without the last multiplication step. (d) In a procedure we term 'IS- edge seed correlation', the two measures are correlated to reveal whether local brain activations (seed) may recapitulate the cortical-wide correlations of successful face recognition. (e) We then examine when, during the movie, these connectivity-behavior correlations appear. (f) We tested whether these neural-behavioral correlates would be related to the visual perception of faces or to their encoding in memory.

Nanosymposium

179. Social Vision: Mechanisms of Face and Body Perception

Location: SDCC 24

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 179.04

Topic: D.06. Vision

Title: Univariate and multivariate analyses of single-cell electrophysiological measurements support homology between the macaque middle face patch and the human fusiform face area

Authors: *F. M. RAMIREZ[1], P. BANDETTINI[1,2];
[1]Section on Functional Imaging Methods, [2]Functional MRI Core, NIMH, Bethesda, MD

Abstract: Freiwald and Tsao (2010) [Functional Compartmentalization and Viewpoint Generalization Within the Macaque Face-Processing System. Science 330:845-851] observed that neurons in the middle-lateral and middle-fundus (ML/MF) face patches—also denoted as middle face patch—were maximally responsive to a single preferred face view. Neurons in the anterior-lateral face patch (AL), the subsequent processing stage, responded maximally and similarly to mirror-symmetric face views—e.g., left and right profile views. Finally, neurons in the anterior medial (AM) face patch, the last processing stage, were virtually viewpoint invariant. Several studies have probed the form of view-tuning of neural populations in human face-selective areas with a combination of fMRI and pattern analysis methods. While some studies concluded the human Fusiform Face Area (FFA) is view-tuned, like ML/MF, others concluded it is mirror-symmetrically tuned, like AL. We proposed a model that accounts for these inconsistent conclusions (Ramírez, 2018). The model suggests that stronger responses to frontal face views in FFA led to the artefactual observation of mirror-symmetry due to analysis choices that skew the results—i.e., data recentering and choice of pattern dissimilarity measure in Representational Similarity Analyses (RSA). Two predictions stemming from the model are that (i) if human FFA and ML/MF are homologues, ML/MF should reveal stronger responses for frontal than lateral face views, as observed in FFA, and (ii) combining neurons in ML/MF to emulate measurements from neuroimaging methods that reflect pooled activity of large numbers of neurons should lead to artefactual observations of mirror-symmetry if data are demeaned prior to RSA or the Euclidean distance used as measure of pattern dissimilarity. We reanalyzed the data from Freiwald and Tsao (2010) and confirmed both predictions. Increased average spike counts for the frontal face-views were observed in ML/MF, but not AL. We also observed a marked increase in the degree of mirror-symmetry inferred with RSA with the Euclidean distance and when the data were demeaned prior to RSA with the cosine distance. Our findings demonstrate that inferences regarding neural coding with RSA are not translation invariant (i.e., data recentering can change the conclusions), and underscore the importance of taking into consideration measurement scale and pattern dissimilarity measure when interpreting RSA observations. Our findings do not support a recently suggested homology between macaque AL and human FFA, but support instead the original homology proposed by Tsao et al. (2003) between the middle face patch (ML/MF) and FFA.

Disclosures: F.M. Ramirez: None. **P. Bandettini:** None.

Nanosymposium

179. Social Vision: Mechanisms of Face and Body Perception

Location: SDCC 24

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 179.05

Topic: D.06. Vision

Title: Macaques recognize features in synthetic images derived from ventral stream neurons

Authors: *K. MUELLER[1], M. BURKEMPER[2], J. KANSUPADA[2], C. R. PONCE[1];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Washington Univ. Sch. Of Med., St Louis, MO

Abstract: Primates can learn to recognize features in virtually all images, a perceptual ability still unparalleled by machine vision. One hypothesis is that cortical neurons encode important patterns from scenes, objects, and textures, then use this information to interpret incoming visual signals (Poggio & Edelman, 1990). To understand the visual patterns encoded by cortical neurons along the ventral stream, we have previously used generative machine learning algorithms to synthesize strongly activating images, called *prototypes* (Rose *et al*., 2021). We implanted two macaques with chronic microelectrode arrays and synthesized prototypes from neurons in primary visual cortex (V1) and inferotemporal cortex (IT). Prototypes from IT neurons often resembled abstracted parts of real-world objects, such as monkey faces and body parts, as determined by pre-trained neural networks and human judgements (Bardon *et al*, 2021). However, it was unknown if monkeys could also perceive these similarities, as would be expected if prototypes represent neuronally encoded abstractions from experience. We investigated this question using a two-alternative forced choice task in which we trained two macaques to saccade to generator-stylized images of conspecifics and not to other objects (e.g., wheels, houses, or plants). We then tested how they spontaneously classified prototypes from IT and V1 in randomly rewarded probe trials (interleaved 15% of the time). Monkeys chose IT prototypes over shuffled prototypes above chance level ($M \pm$ SEM; $73.1 \pm 3.2\%$ right-sided Student t-test, $P = 9.1$ x 10^{-7}) and selected V1 prototypes at chance level ($48.4 \pm 4.0\%$, $P = 0.66$). For each prototype, we calculated a "monkey-similarity" score using activations of the AlexNet *macaque* unit. We found these scores correlated positively with how often each prototype was chosen by the monkeys (Pearson's r = 0.66, $P = 8.0$ x 10^{-6}). In additional experiments, we confirmed monkeys could abstract simple shape features from real-world objects — such as circles and triangles from street signs — and found they could also abstract features common to categories which are less ecologically relevant than conspecifics (e.g., chickens and chairs). Finally, we confirmed human participants also reported similarities between monkeys and IT prototypes in a computerized replication of the first experiment (IT: $77.6 \pm 2.5\%$, $P = 1.2$ x 10^{-6}; V1: $54.8 \pm 3.0\%$, $P = 0.081$). Like macaques, human behavior was positively correlated with monkey-similarity scores (r = 0.82, $P = 5.0$ x 10^{-6}). These results provide evidence that prototypes from cortical neurons arise as abstractions from experienced scenes, objects, and textures.

Disclosures: K. Mueller: None. **M. Burkemper:** None. **J. Kansupada:** None. **C.R. Ponce:** None.

Nanosymposium

179. Social Vision: Mechanisms of Face and Body Perception

Location: SDCC 24

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 179.06

Topic: D.06. Vision

Title: Characterizing social perception through the lens of space, time, and task demands

Authors: *L. T. DOWDLE[1], L. VIZIOLI[2], G. M. GHOSE[1];
[1]Neurosci., [2]Neurosurg., Univ. of Minnesota, Minneapolis, MN

Abstract: We can effortlessly and rapidly classify information based on subtle changes in visual features. Human faces are exemplary in this regard: social categories, such emotive state, are of obvious and immediate behavioral relevance but characterized by modest changes in visual input. While previous research has identified a wide network of face preferential areas, how faces are actually processed to extract social meaning is still unknown. Our previous work with visually degraded faces suggests that the use of stimulus relevant tasks, as opposed to more generic tests of memory such as N-back, may be necessary to characterize these complex networks. Here we extend those findings by combining social perception tasks with neuroimaging data at fine spatial and fast temporal scales.We used a fixation task and two stimulus relevant tasks – perception of gender and perception of expression. We acquired separate high spatial (0.8mm isotropic) and temporal (0.5s) resolution 7 Tesla BOLD images. Participants viewed partially degraded faces (1s on, 2 to 6s ISI), and made 2AFC choices (male/female; happy/neutral, blue/red border) for each stimulus. Only task demands and spatiotemporal scale of the BOLD images differed for each run; images and stimulus timing were identical. We estimated single trials as well as task specific hemodynamic responses. We find substantial heterogeneity between tasks, with the stimulus irrelevant color task associated with significantly ($p<0.05$, multiple comparison corrected) weaker single trial amplitudes in the fusiform face and occipital face areas (FFA; OFA). For social perception tasks, perceiving expression drives larger BOLD responses in the superior temporal sulcus (STS), however in other regions (FFA, OFA) there are few differences. By leveraging the rapidly sampled data we find that estimated hemodynamic responses differ substantially between tasks, particularly in atypical regions (e.g. medial/lateral prefrontal cortices). Similarly, by exploiting fine scale detail, multivariate classification succeeds in task decoding, with an average of 70% accuracy (chance 33%). Through the lens of socially relevant task demands and high spatial and temporal resolution brain imaging we observe signals that 1) may not be resolvable with typical acquisition strategies, 2) have substantial variability 3) are relevant to naturalistic perception and 4) highlight novel areas. Despite broad consistency in 'core' areas, we find variability between individuals that group averaging would obscure. These findings highlight the ability of fMRI to capture dynamic changes in network configuration, based on moment to moment perceptional demands.

Disclosures: L.T. Dowdle: None. **L. Vizioli:** None. **G.M. Ghose:** None.

Nanosymposium

179. Social Vision: Mechanisms of Face and Body Perception

Location: SDCC 24

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Presentation Number: 179.07

Topic: D.06. Vision

Support: Intramural Research Program at NIMH (ZIA-MH-002909)

Title: A cortico-amygdala pathway for foveally and peripherally-presented faces

Authors: *K. D. RANA[1], M. SARGEANT[1], J. TAUBERT[2], L. G. UNGERLEIDER[1], E. P. MERRIAM[1];
[1]Natl. Inst. Of Mental Hlth., Bethesda, MD; [2]Univ. of Queensland, St Lucia, Australia

Abstract: Face processing is typically thought to be foveally biased, which is consistent with the foveal bias of the ventral temporal cortex (VTC), where the cortical face regions are located. However, the amygdala, which also contains face-selective neurons, responds to peripherally-presented faces. Given this discrepancy in visual field sensitivity, how does the amygdala interface with the cortical face regions? We addressed this question by investigating cortico-amygdala activity and connectivity in a passive-viewing task in which human participants viewed three-item arrays consisting of faces, objects, and scenes. Each array had one centrally presented stimulus and two flankers in the periphery. We measured brain activity using magnetoencephalography (MEG). Decoding face location revealed peak decoding accuracy at 170 ms, both in VTC and in the amygdala. Pairwise decoding of location in VTC showed high decoding accuracy for centrally-presented faces, and at each peripheral location, but poor decoding between the two peripheral flanker locations. This is consistent with the foveal bias of VTC. However, all three locations could be decoded using amygdala activity, suggesting the amygdala plays a role in locating faces in the visual field. We performed a searchlight analysis based on the phase-slope index connectivity, which was seeded on the amygdala. This analysis revealed gamma-band connectivity from the amygdala to the anterior superior temporal cortex (aSTS). We then computed phase-slope connectivity between aSTS and early visual cortex (EVC), and areas in lateral occipital cortex (LO) and fusiform gyrus (FG) that were found sensitive to faces in a separate control task. This connectivity analysis revealed gamma-band communication from aSTS to EVC and FG. Our findings suggest that the amygdala communicates information about face location to aSTS, which then sends information back to EVC and FG. Location information in the amygdala may support orienting behavior to face stimuli.

Disclosures: K.D. Rana: None. **M. Sargeant:** None. **J. Taubert:** None. **L.G. Ungerleider:** None. **E.P. Merriam:** None.

Nanosymposium

179. Social Vision: Mechanisms of Face and Body Perception

Location: SDCC 24

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 179.08

Topic: D.06. Vision

Title: Large scale EcoG recording during social scene watching and social behaviors in marmosets

Authors: *H. XU, Y. SU, J. SHARMA, W. MENEGAS, C. TRIMMER, F. LIANG, R. LANDMAN, B. ZHANG, M. SUR, R. SAXE, G. FENG, R. DESIMONE;
MIT, Cambridge, MA

Abstract: Humans can understand social scenes at a glance. Abilities such as these are thought to rely on a network of brain regions often referred to as the social brain. Although people have found cortical patches selective for social content including faces, bodies and social interactions, it is mostly unknown how these domains work collectively dynamically during perception. People with autism spectrum disorders typically have deficits in some aspects of social cognition, but there is uncertainty over which brain regions are responsible. Here we "map" the regions of cortex important for social perception and cognition in wild type marmosets which is a new world monkey that is highly social. In the traditional paradigm we showed images and videos with marmoset faces and marmosets engaged in several forms of social behavior, together with matched control images and videos. We mapped brain regions responsive to the visual stimuli with micro-electrocorticogram (EcoG) recordings over the posterior and middle temporal cortex and lateral prefrontal cortex in animals seated in a primate chair with head fixed and gaze monitored. Stimulus selectivity was evident in the high gamma band. We localized several face/body/object patches in temporal and frontal lobe, which appear to be in similar locations to areas mapped with fMRI scanning. We also identified patches that appear to be selective for social interactions. Timing data suggests a first posterior to anterior feedforward wave of activation, followed by a second feedback wave. Moreover, in the naturalistic paradigm, we also recorded with the same EcoGs but with untethered, wireless recordings in the same animal, during social behaviors in the home cage. A topdown-view camera was used to record and track the marmosets' behaviors. We found the face and social patches showed higher activity when the animal with the electrodes was facing towards the cage-mate, compared with when they were facing apart. With these recordings, we hope to compare patterns of activation during the perception of social stimuli with patterns activated during performance of social behaviors.

Disclosures: H. Xu: None. **Y. Su:** None. **J. Sharma:** None. **W. Menegas:** None. **C. Trimmer:** None. **F. Liang:** None. **R. Landman:** None. **B. Zhang:** None. **M. Sur:** None. **R. Saxe:** None. **G. Feng:** None. **R. Desimone:** None.

Nanosymposium

180. Stress: Molecules, Cells to Behaviors

Location: SDCC 25

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 180.01

Topic: F.03. Stress and the Brain

Title: Molecular Mechanism of Targeting the Dopamine Transporter by Selective Autophagy

Authors: M. SINGH[1], S. SHANMUKHA[1], M. SHISHIKURA[1], ***M. HARRAZ**[2,3];
[2]Neurosci., [1]Johns Hopkins Univ., Baltimore, MD; [3]Departments of Psychiatry and Pharmacol.,
Univ. of Maryland Sch. Of Med., Baltimore, MD

Abstract: The dopamine transporter (DAT) is one of the major determinants of dopamine
signaling. We have previously shown that autophagy, or self-eating, selectively targets DAT for
degradation, suggesting that autophagy regulates dopamine signaling. However, the molecular
mechanism of selective targeting of DAT by autophagy is unknown. Autophagy is a homeostatic
process that targets organelles and proteins for degradation to recycle their basic building
components. Selective autophagy uses various eat-me signals to recognize specific cargo. One of
these signals is the pentapeptide KFERQ motif. We used bioinformatics to identify a KFERQ-
like motif in the cytoplasmic domain of DAT. We hypothesized that "DAT's KFERQ-like motif
mediates its autophagic degradation." Using site-directed mutagenesis, we show that the DAT
selective autophagy motif is functional and mediates its degradation by autophagy. Our findings
uncover a novel mechanism by which autophagy regulates dopamine signaling.

Disclosures: M. Singh: None. **S. Shanmukha:** None. **M. Shishikura:** None. **M. Harraz:**
None.

Nanosymposium

180. Stress: Molecules, Cells to Behaviors

Location: SDCC 25

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 180.02

Topic: F.03. Stress and the Brain

Title: Differential expression of kynurenine pathway and its association with behavioral deficits
under hypobaric hypoxia

Authors: *K. VERMA, E. KOHLI, A. ., D. N. PRASAD;
Dept. of Neurobio., Defence Inst. Of Physiol. And Allied Sciences, DRDO, NEW DELHI, India

**Abstract: Differential expression of kynurenine pathway and its association with
behavioral deficits under hypobaric hypoxia**The Kynurenine Pathway (KP) is a responsible
entity for tryptophan metabolism and produces various metabolites exhibiting neuroactive,
neurotoxic, and immunomodulatory properties. Modulation of KP due to environmental stress

alters its metabolites, further resulting in behavioral dysfunction. To understand the regional expression of KP metabolites in the brain in relation to depression and anxiety-like behavior was studied in animals under hypobaric hypoxia. Male Sprague-Dawley rats weighing 230–250 g were exposed to hypobaric hypoxia (HH) (at 25,000 ft.) for 1, 3, and 7 days. The depression-like behavior of the rats was assessed using the Forced Swim Test (FST), while the anxiety and locomotor activity of the rats were examined using the Open Field Test (OFT). The levels of KP metabolites in the pre-frontal cortex (PFC), neocortex, and hippocampus were measured using high-performance liquid chromatography to elucidate the region-dependent variations. Further, the correlation between behavioral analogues and KP metabolites was evaluated using Pearson's correlation. In the PFC, neocortex, and hippocampus, tryptophan increases on 1-day HH exposure but further decreases on prolonged HH exposure. The kynurenines were found to be increased in all brain regions up to 3-day HH. However, in the PFC and hippocampus, the quinolinic acid branch of KP was found to be activated, while in the neocortex, the kynurenic acid branch of KP was activated. Serotonin was significantly reduced in all three brain regions throughout HH exposure. On behavioural assessment, a significant increase in immobility, indicating the depression-like state was found to be highest on 3-day HH exposure. Similarly, in OFT, the highest immobile time and least total distance travelled were observed on 3-day HH exposure. Further, we have shown the positive correlation between behavioral parameters and kynurenine and quinolinic acid in a time-dependent manner in the PFC and hippocampus. In the case of the neocortex, a positive correlation of behavioral analogues was observed with kynurenine and kynurenic acid. Under HH stress, differential activation of KP in a region-dependent manner was observed. Furthermore, a positive correlation of kynurenine, kynurenic acid, and quinolinic acid with depression and anxiety-like states in rats exposed to HH was found. This study strongly portraits KP as a therapeutic target to treat HH-related psychological and physiological abnormalities.

Disclosures: K. Verma: None. **E. Kohli:** None. **A. .:** None. **D.N. Prasad:** None.

Nanosymposium

180. Stress: Molecules, Cells to Behaviors

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Presentation Number: 180.03

Topic: F.03. Stress and the Brain

Support: NIMH Grant 1R56MH124930-01A1

Title: Stressed out from the gut to the head

Authors: *D. R. KROPP, J. R. RAINVILLE, M. E. GLOVER, T. WHITTLE-HAGE, M. TSYGLAKOVA, S. M. CLINTON, G. E. HODES;
Virginia Tech., Blacksburg, VA

Abstract: Alterations of the gut microbiome are identified as contributing to various inflammatory disorders including major depression, autism spectrum disorder, and anxiety disorders. All of these psychiatric disorders are also linked to changes in inflammation and circulating levels of cytokines in the blood. The 'leaky gut' hypothesis (Maes et al, 2008) suggests that peripheral inflammation results from leakiness of the gut/body barrier, thereby permitting gut microbiota, metabolites, and/or cytokines to infiltrate other parts of the body, including the brain. Chronic stress is a well-known trigger for emotional disorders, and stress has been shown to shift gut microbiota composition, at least in male rodents. As depression and anxiety disorders are 2x more likely to occur in women than men, we hypothesized that there are sex differences in the impact of chronic stress on gut bacteria species and gut microbiome composition over time that relate to patterns of circulating cytokines in blood and brain. We exposed young adult male and female C57/BL6 mice to a variable stress paradigm that is known to disrupt emotional behavior. The stress paradigm consists of three stressors (100 mild foot shocks at 0.45 mA for 1h; restraint for 1 h; tail suspension for 1 h) alternated daily during a set stress period. Our past work with this paradigm revealed sex differences in stress susceptibility, with females showing greater stress susceptibility than males after 6-d of variable stress, but both males and females being negatively impacted by 28-d stress exposure. Fecal samples were collected from all subjects at three time points: baseline, before stress exposure began; then after 6-d, and after 28-d of the variable stress paradigm (or control condition). Fecal samples were prepared for next-generation sequencing; 16S rRNA amplicon sequencing data was collected and analyzed utilizing Rstudio and various 16s amplicon sequencing analytics packages (DADA2, Phyloseq, DESeq2, Corrplot, gplots, and RRHO). Blood was also sampled at each timepoint to measure metabolic conditions and cytokines. These data were then correlated with cytokine levels detected in plasma as well as hippocampus and nucleus accumbens collected from stressed and control mice Preliminary data shows compositional alterations in the Lachnospirales and Lactobacillales orders as well as shifts in single taxa that correlate strongly with specific cytokines. This exploratory analysis of compositional changes in the gut will elucidate convergent and divergent sex differences by which stress impacts immune responses via the microbiome-gut-brain-axis.

Disclosures: D.R. Kropp: None. **J.R. Rainville:** None. **M.E. Glover:** None. **T. Whittle-Hage:** None. **M. Tsyglakova:** None. **S.M. Clinton:** None. **G.E. Hodes:** None.

Nanosymposium

180. Stress: Molecules, Cells to Behaviors

Location: SDCC 25

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 180.04

Topic: F.03. Stress and the Brain

Support: MIUR- PRIN 2015 2015SKN9YT_002
MIUR- PRIN 2017 2017AY8BP4_002
Avvio alla Ricerca 2020 AR120172B78C92B6

Title: Late glucocorticoid receptor antagonism normalizes the programming effects of brief and repeated periods of social isolation stress in adolescent rats

Authors: *G. MANCINI[1,2], B. DI CESARE[2,3], J. BUURSTEDE[1,4], A. PISANESCHI[2,3], O. MEIJER[1,4], P. CAMPOLONGO[2,3];
[1]Leiden Univ. Med. Ctr., Leiden, Netherlands; [2]Sapienza Univ. of Rome, Rome, Italy; [3]Neurobio. Of Behavior Laboratory, Santa Lucia Fndn., Rome, Italy; [4]Einthoven Lab. For Exptl. Vascular Medicine, Leiden Univ. Med. Ctr., Leiden, Netherlands

Abstract: Social isolation stress (SIS) is one of the most commonly used stress paradigms to reproduce psychiatric-like disorders in rodents and it is generally conducted for several weeks from weaning to adulthood. However, the long-term effects of briefer periods of SIS only during early-adolescence, a critical phase for brain development, are less explored. The present study aims at investigating the programming effects induced by brief and repeated SIS at early-adolescence and the potential effectiveness of late glucocorticoid receptor (GR) antagonism in counteracting such SIS-induced alterations. Further, the neurobiological mechanisms underlying these effects were evaluated as well. Male Sprague-Dawley rats were subjected to two hours of SIS per day during early-adolescence from postnatal day (PND) 28 to PND 34. Adult animals stressed in early-adolescence and their relative control groups were intraperitoneally treated with the GR antagonist RU486 (30 mg/kg) or vehicle at PND 83, 85 and 87. Potential reversal of programming effects on behavioral reactivity was evaluated starting 1 week after treatment (PND 90). To investigate the neurobiological mechanisms underlying such effects, transcriptome analysis was performed within ventral and dorsal hippocampus. Our results demonstrated that brief and repeated periods of SIS during early-adolescence induced a reduction of time spent in the open arms, number of entries in the open arms and frequency of head-dippings in the elevated plus maze task, and an enhanced emotional reactivity in the acoustic startle response task, suggesting the development of anxious-like profile later in life. Strikingly, we found that treatment with RU486 at adulthood normalized such SIS-induced programming effects in rats tested 1 week after treatment. However, transcriptome analysis did not reveal significant alterations within ventral and dorsal hippocampus, indicating that gene expression in these two brain areas is not involved in the behavioral effects of SIS and treatment. Our data reveal that glucocorticoid stress hormones due to SIS exposure during early-adolescence induced effects on emotional reactivity which persist later in life and which are counteracted by late GR antagonism. These findings have a groundbreaking potential of introducing a promising therapeutic approach to treat and counteract the development of stress-related disorders long-after trauma. However, additional studies are needed to understand the neurobiological underpinnings of this process.

Disclosures: G. Mancini: None. **B. Di Cesare:** None. **J. Buurstede:** None. **A. Pisaneschi:** None. **O. Meijer:** None. **P. Campolongo:** None.

Nanosymposium

180. Stress: Molecules, Cells to Behaviors

Location: SDCC 25

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 180.05

Topic: F.03. Stress and the Brain

Support: Italian Ministry for University Education and Research (Prin: 2017K2NEF4)

Title: Acute stress recruits endogenous histamine type 2 receptor signaling in medium spiny neurons of the nucleus accumbens

Authors: L. NARDELLA[1], G. ACETO[1], C. COLUSSI[2], C. GRASSI[1], *M. D'ASCENZO[1];
[1]Neurosci., Univ. Cattolica del Sacro Cuore, Roma, Italy; [2]Inst. Di Analisi dei Sistemi ed Informatica Antonio Ruberti, Natl. Res. Council (CNR-IASI), Roma, Italy

Abstract: We recently showed that histaminergic transmission in the nucleus accumbens (Nac) increases medium spiny neurons (MSNs) intrinsic excitability through histamine type 2 receptor (H2R)-dependent modulation of Kv4.2 channels (Aceto et al., 2022). Given that HA-containing hypothalamic tuberomammillary nucleus (TMN) neuron output is increased during acute stress (Ito, 2000; Manz et al., 2021), we asked whether acute immobilization stress (AIS) recruits endogenous histamine signaling in the Nac. We employed an AIS paradigm in which mice were restrained for 120 minutes, followed by a 15-minute recovery period, after which brain slices were prepared for ex vivo electrophysiology. Using whole-cell patch-clamp recordings, we found that pharmacological activation of H2R elevates MSN excitability of control mice. However, in mice that underwent AIS, H2R activation failed to increase MSN excitability. Moreover, H2R activation in AIS mice did not affect: i) subthreshold depolarization in response to current injection; ii) the latency to fire and iii) action potential afterhyperpolarization. Consisting with the hypothesis that AIS engages TMN-to MSN volume transmission, thereby altering the H2R-dependent modulation of Kv4.2 channels, we found that activation of H2R was unable to decrease A-type K+ currents in AIS mice. Furthermore, western blotting analysis revealed increased Kv4.2 channel internalization in Nac tissues from these mice. Interestingly, in mice treated with H2R-antagonist prior to AIS paradigm (intraperitoneal injection of zolantidine dimaleate; 10 mg/Kg body weight), H2R activation, similarly to what observed in control mice, increased evoked firing and decreased A-type K+ currents. Together, our findings indicate that AIS recruits endogenous H2R function in MSNs and suggest a possible involvement of H2R signaling in stress-induced behaviors.

Disclosures: L. Nardella: None. **G. Aceto:** None. **C. Colussi:** None. **C. Grassi:** None. **M. D'Ascenzo:** None.

Nanosymposium

180. Stress: Molecules, Cells to Behaviors

Location: SDCC 25

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 180.06

Topic: F.03. Stress and the Brain

Title: Dexamethasone suppression of LPS-evoked TNFa is associated with symptoms of depression in older adults

Authors: *S. A. NORTON, I. HANSEN, I. SPEARS, T. F. OLTMANNS, R. BOGDAN; Washington Univ. in St. Louis, ST. LOUIS, MO

Abstract: <u>BACKGROUND</u>: Elevated inflammation has been reliably associated with depression, leading to theories that inflammation may induce depression. Research has suggested that increased inflammation in depressed individuals may be due to impaired negative feedback of glucocorticoids on the immune system – in other words, the cells of the immune system in depressed individuals may become *glucocorticoid resistant*, leading to unchecked immune activation. Most studies that have evaluated the association between inflammation and depression have quantified basal levels of circulating cytokines. The present study seeks to expand on this work by evaluating inflammatory markers evoked by the immune stimulus lipopolysaccharide (LPS) in whole blood. In addition, the ability of dexamethasone (DEX), a synthetic glucocorticoid, to suppress this LPS-evoked inflammatory response was assessed. <u>METHODS</u>: Participants (n = 27) were drawn from the St. Louis Personality and Aging (SPAN) study, a community sample of older adults (mean age = 72) residing in the St. Louis area. Participants completed surveys about their physical and mental health, including the Beck Depression Inventory (BDI), as well as a fasting blood draw. A portion of the blood was allowed to clot and serum was collected to assess basal levels of circulating cytokines. In addition, whole blood was stimulated with 1 ng/mL of LPS with and without 1, 10, 100, or 1000 nM of DEX and incubated at 37º C, 5% CO_2 for 6 hours. Both basal and LPS-evoked TNFα levels were quantified via ELISA. <u>RESULTS</u>: As expected, the addition of LPS generated a massive upregulation of TNFα, which was suppressed at all concentrations of DEX. A reduction in DEX suppression of the TNFα response was correlated with higher BDI scores, but only at the two lowest doses of DEX (DEX 1 r= -.50, *p*< .01; DEX 10 r= -.51, *p*< .01; DEX 100 r= -.15, *ns*; DEX 1000 r= -.09, *ns*). In contrast, basal serum levels of TNFα were not associated with BDI scores (r= .19, *ns*). <u>CONCLUSIONS</u>: Depression is associated with reduced dexamethasone suppression of LPS-evoked TNFα. The ability of glucocorticoids to suppress the immune system may be compromised in individuals with elevated depressive symptomology. The lack of correlation between basal TNFα and depression may be attributable to the small sample size of the current study. <u>FUTURE DIRECTIONS</u>: This study is currently enrolling additional participants to increase power. Evaluation of the LPS-evoked IL-6 and IL-1β response are also ongoing.

Disclosures: S.A. Norton: None. **I. Hansen:** None. **I. Spears:** None. **T.F. Oltmanns:** None. **R. Bogdan:** None.

Nanosymposium

180. Stress: Molecules, Cells to Behaviors

Location: SDCC 25

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

**Presentation Number:** 180.07

Topic: F.03. Stress and the Brain

Title: Transcriptional and cell type profiles of cortical brain regions showing ultradian cortisol rhythm dependent responses to emotional face stimulation

Authors: *P. HABETS[1], K. KALAFATAKIS[2], O. DZYUBACHYK[1], S. VAN DER WERFF[1], A. KEO[1], J. THAKRAR[2], A. MAHFOUZ[1], A. PEREIRA[1], G. RUSSELL[2], S. LIGHTMAN[2], O. MEIJER[1];
[1]Leiden Univ. Med. Ctr., Leiden, Netherlands; [2]Henry Wellcome Labs. Of Integrative Neurosci. And Endocrinology, Bristol Med. School, Univ. of Bristol, Bristol, United Kingdom

Abstract: The characteristic endogenous circadian rhythm of plasma glucocorticoid concentrations is made up from an underlying ultradian pulsatile secretory pattern. Recent evidence has indicated that this ultradian cortisol pulsatility is crucial for normal emotional response in man. In this study, we investigate the anatomical transcriptional and cell type signature of brain regions sensitive to a loss of ultradian rhythmicity in the context of emotional processing. We combine human cell type and transcriptomic atlas data of high spatial resolution with functional magnetic resonance imaging (fMRI) data (see attached figure). We show that the loss of cortisol ultradian rhythm alters emotional processing response in cortical brain areas that are characterized by transcriptional and cellular profiles of GABAergic function. We find that two previously identified key components of rapid non-genomic GC signaling – the ANXA1 gene and retrograde endocannabinoid signaling – show top differential expression and the most significant enrichment. Our results further indicate that specific cell types, including a specific NPY-expressing GABAergic neuronal cell type, and specific G protein signaling cascades underly the cerebral effects of a loss of ultradian cortisol rhythm. Moreover, using recent brain studies defining transcriptomic signatures of several psychiatric disorders, we find enrichment of transcriptomic signatures of major depressive disorder, post-traumatic stress disorder, bipolar disorder, schizophrenia, autism spectrum disorder and alcoholic abuse disorder. Our results provide a biological mechanistic underpinning of our fMRI findings, indicating specific cell types and cascades as a target for manipulation in future experimental studies. Our results are relevant for all stress-related (brain) diseases, but also for improving our understanding of the side effects of synthetic glucocorticoid hormones.
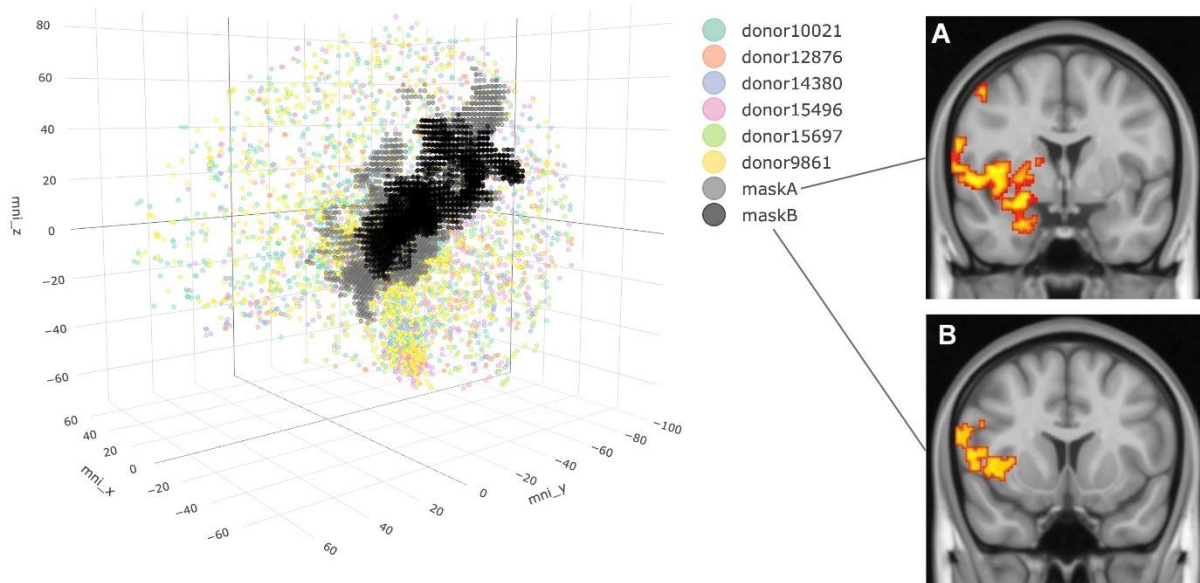
Nanosymposium

180. Stress: Molecules, Cells to Behaviors

Location: SDCC 25

Time: Sunday, November 13, 2022, 1:00 PM – 3:00 PM

Presentation Number: 180.08

Topic: F.03. Stress and the Brain

Title: Molecular signature of war-zone related post-traumatic stress disorder

Authors: *S. MUHIE[1,2], A. GAUTAM[1], R. YANG[1], B. MISGANAW[1], P. CONSORTIUM[3], R. HAMMAMIEH[1], M. JETT[4];
[1]MRSB/WRAIR, Silver Spring, MD; [2]The Geneva Fndn., Silver Spring, MD; [3]PTSD

Consortium, Silver Spring, MD; [4]US Army MRDC, Walter Reed Army Inst. Res., Silver Spring, MD

Abstract: Post-traumatic stress disorder (PTSD), following exposure to serious traumatic events, may progress to a syndromic, multisystem condition affecting cardiovascular, neurologic, metabolic, and immune functions. In this study, we used data-driven integrated multi-omics and clinical analyses to identify multimodal molecular profiles seen in combat-related PTSD. Proteomic, metabolomic, and epigenomic assays were conducted on blood samples of two well-characterized PTSD cases and healthy control cohorts: Systems Biology Consortium (SBC; 340 veterans) and Fort Campbell Cohort (FCC; 180 active-duty soldiers). All participants had been deployed to Iraq and/or Afghanistan. All cases and controls had been exposed to military-service related criterion A trauma, but only cases developed PTSD. The active-duty cohort included blood biomarkers and clinical features assessed longitudinally before and after deployment. Proteomic datasets from an SBC training cohort of 218 veterans (109/109 PTSD+/-) were used to discover altered molecular and pathway signatures. Identified molecular and pathway signatures were initially tested in a newly recruited SBC validation group of 82 veterans (41/39 PTSD+/-) and then tested in the FCC, an independent cohort of 180 active-duty personnel (PTSD+/-). These molecular profiles were computationally integrated with upstream epigenetic regulators (miRNA and methylation marks) and downstream functionally related metabolites. We identified reproducible biological features of PTSD including perturbations in inflammation, oxidative stress, metabolism, and angiogenesis. This tetrad of processes, when perturbed, may play a role in psychiatric and physical comorbidities associated with PTSD, including impaired wound healing, cardiovascular and metabolic disease and may also contribute to co-occurring neuropsychiatric disorders including depression and risk for dementia.

Disclosures: S. Muhie: None. **A. Gautam:** None. **R. Yang:** None. **B. Misganaw:** None. **P. Consortium:** None. **R. Hammamieh:** None. **M. Jett:** None.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.01

Topic: G.04. Emotion

Support: Texas A&M Triads for Transformation (T3) Grant

Title: Corticosterone Administration in Adolescence Enhances Habit Learning in Adulthood: Blockade by Mifepristone

Authors: *T. M. GADBERRY[1], M. G. PACKARD[2];
[1]Texas A&M Univ., [2]Psychological and Brain Sci., Texas A&M Univ., College Station, TX

Abstract: Extensive evidence indicates a link between early life stress (ELS) in humans and a subsequent predisposition to psychopathologies that are characterized in part by maladaptive

habitual behaviors. Stress and anxiety are factors that can influence the relative use of mammalian memory systems implicated in such disorders. Specifically, cognitive memory functions of the hippocampus are typically impaired by stress/anxiety, whereas habit memory functions of the dorsolateral striatum (DLS) are enhanced. However, these effects have been primarily observed in adult animals, and the effects of stress on these memory systems have not been extensively investigated in the context of ELS. We have previously observed that administration of the stress hormone corticosterone (CORT) during adolescence enhances subsequent DLS-dependent habit formation in adulthood. The present study was designed to further elucidate the role of CORT in this phenomenon by examining the effects of concurrent administration of the broad steroidal antagonist Mifepristone (MIFP). Four groups of Long-Evans male adolescent rats (PND 37±4) received two peripheral injections daily over five days: first, either MIFP (30mg/kg) or its vehicle, followed by a 15-min interval, then either CORT (5mg/kg) or its vehicle. Subjects then matured into adulthood undisturbed (~3 weeks) before training in a DLS-dependent water plus maze task. In this task, rats were released from different starting positions (i.e. north or south) and were required to make a consistently reinforced body turn response (i.e. always turn right) at the maze choice point to mount a hidden escape platform. Consistent with our previous findings, the administration of CORT during adolescence facilitated task acquisition in adulthood relative to controls. However, MIFP given prior to CORT in adolescence blocked subsequent enhancement of habit formation. Finally, rats given MIFP alone did not significantly differ from controls. Taken together, these findings provide further evidence that chronic administration of corticosterone during adolescence can facilitate subsequent DLS-dependent habit formation, and highlights glucocorticoid function as a potential underlying mechanism by which ELS may influence habit learning in adulthood.

Disclosures: T.M. Gadberry: None. **M.G. Packard:** None.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.02

Topic: G.04. Emotion

Support: NIH R00MH119320

Title: Fear and safety conditioning differentially scale later responding to threat

Authors: *H. MEYER;
Boston Univ., Boston, MA

Abstract: Stimuli associated with threat (fear cues) are highly salient. It is largely believed that biasing attentional resources to potential threat in the surrounding environment is adaptive for survival, as this can inform appropriate behavioral responding. Yet, when left unchecked, the inability to disengage attention from threat, or attend to alternative indicators of safety, can

contribute to fear-related psychiatric disease such as anxiety or PTSD. Using a mouse model, we have begun investigating how brief exposures to threat in the form of fear conditioning alter later learning and memory related to potential threat. This talk will present evidence of generalization (fear exhibited to non-threatening stimuli that share qualities with a fear cue) on both short (1 day) and long (1 month) timescales, including evidence of greater fear generalization in females. This talk will also show evidence that safety conditioning, a process that engages the ventral hippocampus, provides an avenue to reduce responding to threat cues when presented in parallel with fear cues or alongside fear cues during extinction. Moreover, safety conditioning early in life, during adolescence, may aid in overcoming fear generalization later in life. This work emphasizes the impact that exposure to affective stimuli (both fear and safety) can have on affective learning and memory and suggest both age- and sex-specificity in the promise of safety signal-based treatments for fear-based psychiatric disease.

Disclosures: H. Meyer: None.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.03

Topic: G.04. Emotion

Title: Influences of reward on neural memory mechanisms and representations across development

Authors: *A. O. COHEN[1], C. V. PHANEUF[2], M. GLOVER[4], X. SHEN[2], K. AVALLONE[2], L. DAVACHI[5], C. A. HARTLEY[3];
[1]New York Univ., New York Univ., Brooklyn, NY; [3]New York Univ., [2]New York Univ., New York, NY; [4]Wayne State Univ., Wayne State Univ., Detroit, MI; [5]Columbia Univ., New York, NY

Abstract: Rewards influence behavioral and neural memory processes. In adults, memory for high-reward memoranda is related to increased connectivity involving mesolimbic dopamine systems, centered around the ventral tegmental area (VTA), the anterior hippocampus, and cortical areas both during and after encoding. Additionally, prior work conducted in adults has shown that rewards alter hippocampal activation patterns during encoding and that cortical

pattern similarity between encoding and retrieval is associated with better memory for both neutral and emotional stimuli. However, few studies have examined how rewards influence these memory processes across development. To address these knowledge gaps, we had 89 participants ages 8 to 25 years-old complete a reward-motivated encoding and retrieval fMRI paradigm with baseline and post-encoding active rest periods. Participants then returned 24-hours later for a behavioral memory retrieval test. We find both age-invariant enhancements and nonlinear age-related differences in reward-motivated associative memory after 24 hours. Neuroimaging analyses suggest that reward enhances memory through differential engagement of mesocorticolimbic systems and differential neural memory reinstatement across development. We find evidence for age-related differences in engagement of subcortical and cortical circuits supporting reward-motivated memory processes. We also find evidence that reinstatement of memory representations may differ by both reward level and age across the brain. Taken together, our findings suggest that reward motivation enhances memory somewhat differently across development through neural mechanisms that vary with age.

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Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.04

Topic: G.04. Emotion

Support: Sir Henry Dale Fellowship via Wellcome Trust and the Royal Society

Title: How curiosity affects learning and memory via the dopaminergic circuit

Authors: *M. GRUBER;
Cardiff Univ., Cardiff, United Kingdom

Abstract: Over the last decade, research on curiosity – the desire to seek new information – has been rapidly growing. Several studies have shown that curiosity elicits activity within the dopaminergic circuit and thereby enhances hippocampus-dependent memory. However, given this new field of research, we do not have a good understanding yet of (i) why some people show better learning improvements due to curiosity than others, (ii) how curiosity-based learning changes across the lifespan, and (iii) how curiosity affects exploratory behaviour and thereby potentially influence memory. In this talk, I will present a series of behavioural and neuroimaging studies that address these three questions about curiosity. First, I will present findings on how inter-individual differences in the magnitude of curiosity-based learning depend on the strength of resting-state functional connectivity within the cortico-mesolimbic dopaminergic circuit. Second, I will show data on how curiosity and interest affect memory differently in childhood and adolescence. Third, in a virtual reality paradigm, I will present

findings on how states of curiosity are associated with increases in spatial exploration. Together, our findings help to refine our recently proposed framework – the Prediction, Appraisal, Curiosity, and Exploration (PACE) framework – that attempts to integrate theoretical ideas on the neurocognitive mechanisms of how curiosity is elicited, and how curiosity enhances learning and information seeking. Furthermore, our findings highlight the importance of curiosity research to better understand how curiosity can be harnessed to improve learning and information seeking in real life.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.05

Topic: G.04. Emotion

Title: Neural state dynamics in a shared low-dimensional manifold reflect cognitive and attentional dynamics

Authors: *H. SONG[1], W. SHIM[2], M. D. ROSENBERG[1];
[1]Univ. of Chicago, Univ. of Chicago, Chicago, IL; [2]Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Cognition and attention arise from the adaptive coordination of neural systems in response to external and internal demands. Recent work suggests that the dynamics of large-scale brain states—characterized by distinct patterns of activity and functional interactions—capture performance in cognitive tasks (Shine et al., 2019; Yamashita et al., 2021; Cornblath et al., 2020) or attentional engagement during movies (Van der Meer et al., 2020). However, whether a common set of latent neural states underlies cognitive state dynamics across contexts is unknown. We tested the possibility that neural dynamics in a shared low-dimensional subspace reflect cognitive, attentional, and arousal dynamics in humans. We conducted functional MRI as participants performed gradual-onset continuous performance tasks, watched comedy sitcom episodes and an educational documentary, and rested. Hidden Markov model analyses (Vidaurre et al., 2017) revealed four latent brain states that generalized across participants, datasets, and task and naturalistic contexts. Latent state dynamics traversed canonical gradients of the functional brain connectome (Margulies et al., 2016), with one transitional "hub" state situated at the center of the gradient axes. Global and transient de-synchronization in functional connections (Faskowitz et al., 2020) preceded neural state transitions. Though the same latent states recurred

across fMRI runs and datasets, distinct state-traversal patterns were observed during rest, task, and naturalistic conditions. Neural state dynamics were synchronized across participants during engaging movies (sitcom episodes), though synchrony was lower during a less engaging movie (an educational documentary). Neural state dynamics were temporally aligned to narrative event boundaries and cognitive task blocks. Furthermore, brain state dynamics reflected fluctuations in attention task performance and self-reported engagement (i.e., emotion-laden attentional arousal with consequences for memory; Song et al., 2021) during movies. Whereas different neural states were involved in attentionally engaged states in task and naturalistic contexts, a common neural state indicated inattention in both contexts. The latent neural subspace and functional roles of latent states were extensively replicated with independent datasets (Van Essen et al. 2013; Chen et al., 2017; Rosenberg et al., 2016; Chang et al., 2021). Together, our findings suggest that human cognition and attention arise from neural dynamics that traverse latent states in a shared low-dimensional gradient space.

Disclosures: H. Song: None. **W. Shim:** None. **M.D. Rosenberg:** None.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.06

Topic: G.04. Emotion

Title: The role of memory in counterfactual valuation

Authors: *N. BIDERMAN[1,2], S. J. GERSHMAN[4,5], D. SHOHAMY[1,2,3];
[1]Psychology, [2]Zuckerman Inst., [3]The Kavli Inst. For Brain Sci., Columbia Univ., New York, NY; [4]Psychology, [5]Ctr. For Brain Sci., Harvard Univ., Cambridge, MA

Abstract: Value-based decisions are often guided by memory for past experiences. If a choice led to a good outcome, we are more likely to repeat it. However, it is unclear how memory helps us assign value to options we didn't choose and which we therefore never had the chance to learn about directly. Research on counterfactual reasoning has focused on how people learn from and react to explicit information about what they could have gained if they had chosen the other option, leaving open questions about how people evaluate unchosen options which were never directly experienced. Here, we show that memory for the association between chosen and unchosen options has a lingering effect which manifests in counterfactual valuation. In multiple preregistered experiments, we find that people persistently compare the chosen and unchosen options: if the chosen option proved to be worthwhile, the unchosen option is deemed less

desirable; if the former disappoints, the latter suddenly becomes attractive. A phenomenon we termed the "inverse decision bias". Moreover, we show that memory for the association between choice options determines the extent of the inverse decision bias, and retroactive interference eliminates the inverse decision bias altogether. Finally, we present a new memory-based policy gradient model that predicts both the inverse decision bias and its dependence on memory. Our findings point to the causal role of memory in the valuation of unchosen options and introduces a new perspective on the relationship between memory, decision-making and counterfactual reasoning.

Disclosures: N. Biderman: None. **S.J. Gershman:** None. **D. Shohamy:** None.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.07

Topic: G.04. Emotion

Title: Social isolation during adolescence disrupts adolescent decision-making trajectories and limbic-cortical circuits

Authors: *S. M. GROMAN[1], I. GREEN[2], M. JACK[1], K. LAROCCO[1], P. VILLIAMMA[1];
[1]Univ. of Minnesota, [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Social interactions during adolescence are critical for the development of the brain, including the formation and stabilization of neural circuits that are critical for learning and memory functions. Previous studies have observed decision-making deficits in adult animals who were isolated during adolescence, but the impact of this isolation stress on age-related decision-making functions during adolescence is not known. We hypothesized that social isolation during adolescence would disrupt the formation and stabilization of prefrontal circuits that guide decision-making functions and sought to test this hypothesis using our recently developed protocol to rapidly assess decision-making functions in rats as young as P30. We repeatedly assessed decision-making functions during adolescence and adulthood in rats that were either 1) socially housed throughout adolescence and adulthood, 2) socially housed during adolescence and isolated in adulthood 2) socially isolated during adolescence and adulthood. We found that adolescent decision-making trajectories were attenuated in rats isolated during adolescence compared to socially housed rats and rats who were isolated in adulthood. Computational analyses of trial-by-trial choice data revealed that social isolation during adolescence, but not during adulthood, disrupted value updating following positive outcomes. We hypothesized – based on our previous work demonstrating that amygdala projections to the orbitofrontal cortex (OFC) control positive-outcome updating – that abnormal decision-making

trajectories in socially isolated rats were the result of neurodevelopmental disruptions in the amygdala-to-OFC circuit. To investigate the impact of social isolation on the amygdala-to-OFC circuit, we combined the Cre-lox and Flp-FRT systems to 1) selectively label both amygdala and OFC neurons within this circuit and 2) express ChrimsonR in amygdala neurons that project to the OFC and assess neural activity in OFC neurons. Our preliminary data indicates that isolation during adolescence disrupts amygdala control over OFC neurons. We propose that social isolation during adolescence disrupts amygdala input into the OFC and persistent decision-making problems. Our ongoing work is investigating the protein mechanisms responsible for these neurodevelopmental abnormalities to identifying potential treatments for restoring plasticity within these decision-making circuit in adulthood.

Disclosures: S.M. Groman: None. **M. Jack:** None. **K. LaRocco:** None. **P. Villiamma:** None.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

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Presentation Number: 181.08

Topic: G.04. Emotion

Title: Mnemonic Generalization and Precision: Young and Older Adults Who Lapse More, Learn and Remember Less

Authors: *T. T. TRAN, K. MADORE, H. LLOYD, J. RATHMANN-BLOCH, S. HSU, A. WAGNER;
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Abstract: Although aging is often associated with a decline in memory function, there are some individuals who retain memory function and some who show a decline. What factors explain why some older adults remember better than others? One set of processes that could contribute to episodic memory differences in aging is diminished attention processes that lead to poor memory encoding and diminished remembering. To address these questions, we investigated how individual differences in a sustained attention (assayed via gradCPT) relate to associative retrieval, generalization, and memory precision in younger and older adults. In Experiment 1, we examined how sustained attention relates to associative memory for premise pairs (AB, BC) and associative inference / memory integration pairs (AC). Data were collected from 71 young adults

(M = 26.9 yrs, SD = 4.57) and 68 older adults (M = 66.8 yrs, SD = 4.27). In Experiment 2, we examined how sustained attention relates to object-color and object-location memory precision in an independent dataset of 79 young adults (M = 23.34, SD = 4.21) and 84 older adults (M = 69.75, SD = 3.51). We computed two assays of attention lapsing from the gradCPT – (a) commission error rate on infrequent 'no go' trials and (b) reaction time (RT) variability for responses on frequent 'go' trials using a coefficient of variance metric. On the gradCPT, the younger and older adult groups did not significantly differ in commission errors nor RT variability. Linear regression models indicated that sustained attention metrics predicted memory performance across all metrics of memory performance (e.g., associative memory for premise pairs, mnemonic generalization / inference trial performance, and memory precision) in both young and older adults. These findings suggest that, while attention does not explain age-related differences in hippocampal-dependent memory, individual differences in the propensity to suffer attention lapses partially explain why some older adults remember better than others.

Disclosures: T.T. Tran: None. **K. Madore:** None. **H. Lloyd:** None. **J. Rathmann-Bloch:** None. **S. Hsu:** None. **A. Wagner:** None.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.09

Topic: G.04. Emotion

Title: Dynamic arousal and hippocampal states organize memories of coherent events

Authors: *D. V. CLEWETT[1], R. HUANG[2], L. DAVACHI[3];
[1]UCLA, [2]UCLA, Los Angeles, CA; [3]Psychology, Columbia Univ., New York, NY

Abstract: Episodic memories do not reflect the continuous stream of experience. Rather, they represent the passage of discrete and meaningful events. Recent work shows that pupil-linked arousal measures help signal this transformation of continuous experience into memorable episodes (Clewett et al., 2020). Here, we aimed to determine the neural mechanisms by which arousal shapes the temporal organization of memory. We hypothesized that noradrenergic and dopaminergic activity trigger memory separation under arousal, given their known role in biasing hippocampal processes to encode new memories. To test this hypothesis, we combined high-resolution functional magnetic resonance imaging (fMRI) with pupillometry to measure human brain activity during sequence learning. While in an MRI scanner, healthy young adult males and females (N = 32) encoded lists of object images as they listened to simple auditory tones in their left or right ear. At infrequent yet regular intervals within each sequence, the tone

switched to the participant's other ear to create an 'event boundary' that divided each item sequence into discrete auditory events. Replicating prior work, we found that auditory event boundaries elicited increased pupil dilation, impaired temporal order memory, and led to more exaggerated retrospective estimates of temporal distance between items from recent event sequences. At the local level, boundary-evoked pupil dilations were associated with concomitant increases in ventral tegmental area (VTA) activation. This discrete activation of the VTA was also correlated with greater subjective time dilation effects in memory for boundary-spanning information, suggesting that dopamine elicits the subjective separation of temporally adjacent events in memory. On a larger timescale, we found that prolonged periods of pupil variability, a putative index of noradrenergic activity, were related to worse temporal order memory as well as less stable patterns of hippocampal and lateral occipital cortex (LOC) activity across time. This reduction in hippocampal pattern stability was also associated with the subjective dilation of time in memory. Together, our findings suggest that stability and change in neuromodulatory and hippocampal processes scaffold the formation of episodic representations in long-term memory.

Disclosures: D.V. Clewett: None. **R. Huang:** None. **L. Davachi:** None.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.10

Topic: G.04. Emotion

Title: Neural Circuitry Involved in Conditioned Inhibition via Safety Signal Learning is Sensitive to Trauma Exposure

Authors: *S. KRIBAKARAN[1], P. ODRIOZOLA[2], E. M. COHODES[2], S. MCCAULEY[2], S. J. ZACHAREK[3], H. HODGES[4], J. T. HABERMAN[2], J. C. PIERRE[2], D. G. GEE[2];
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Abstract: Exposure to trauma throughout the lifespan is prevalent and increases the likelihood for the development of mental health conditions such as anxiety and post-traumatic stress disorder (PTSD). Safety signal learning (SSL)–a form of conditioned inhibition that involves reducing fear via conditioned safety–has been shown to effectively attenuate fear responses among individuals with trauma exposure, but the association between trauma exposure and the neural mechanisms of SSL remains unknown. Adults (ages 18-30; n=64) with a range of trauma exposure (assessed using the UCLA PTSD Reaction Index) completed a conditioned inhibition

task during functional MRI scanning and collection of skin conductance response (SCR). The task included stimuli representing threat, safety, and a safety compound (i.e., CS+ and CS- were paired). fMRI data were analyzed using FSL for a priori regions of interest (e.g., amygdala and anterior hippocampus) activation analyses and for exploratory whole-brain activation analyses. Statistical analyses included repeated measures analyses of covariance. Conditioned safety signals reduced psychophysiological reactivity (i.e., SCR) in the overall sample ($t(26) = 3.14$, $p = 0.004$). Although exposure to a higher number of traumatic events was associated with elevated SCR across all task conditions ($F(1,15) = 4.91$, $p = 0.036$), SCR did not differ between threat in the presence of conditioned safety (i.e., SSL) relative to threat alone in a trauma-related manner. At the neural level, however, a significant interaction between trauma exposure and task condition for both amygdala ($F(3,186) = 3.20$, $p = 0.024$) and anterior hippocampal ($F(3,186) = 2.75$, $p = 0.044$) activation emerged. Specifically, higher levels of trauma exposure (i.e., a greater number of total traumatic events), but not lower levels of trauma, were associated with diminished hippocampal and amygdala engagement during SSL. Exploratory whole-brain analyses revealed that exposure to more trauma was associated with lower dorsolateral prefrontal cortical activation during SSL. While conditioned safety signals can reduce fear in the presence of threat even among individuals exposed to higher degrees of trauma, the neural circuitry involved in SSL is in fact sensitive to trauma exposure. Future research investigating neural processes during SSL among individuals with PTSD or anxiety disorders can further elucidate the ways in which SSL and its neural correlates may reduce fear and link trauma exposure with later mental health conditions.

Disclosures: S. Kribakaran: None. **P. Odriozola:** None. **E.M. Cohodes:** None. **S. McCauley:** None. **S.J. Zacharek:** None. **H. Hodges:** None. **J.T. Haberman:** None. **J.C. Pierre:** None. **D.G. Gee:** None.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.11

Topic: G.04. Emotion

Title: Dopamine signaling in the nucleus accumbens core causally mediates latent inhibition

Authors: M. G. KUTLU[1], J. E. ZACHRY[2], P. MELUGIN[3], J. TAT[3], S. CAJIGAS[3], A. ISIKTAS[4], D. PATEL[3], C. SICILIANO[3], G. SCHOENBAUM[5], M. SHARPE[6], **E. S. CALIPARI**[3];

[1]Vanderbilt Univ. Sch. Of Med., SPRING HILL, TN; [2]Vanderbilt Univ., [3]Vanderbilt Univ., Nashville, TN; [4]Yale Univ. Sch. Of Med., New Haven, CT; [5]Natl. Inst. On Drug Abuse, Baltimore, MD; [6]UCLA, Los Angeles, CA

Abstract: Systems neuroscience studies often focus on defining the neural mechanisms by which associations between cues and predicted outcomes control behavior. These studies regularly use associative learning frameworks to understand the neural control of behavior. While powerful, these frameworks do not always account for the full range of effects of novelty on behavior and future associative learning. Here we show that dopamine in the nucleus accumbens (Nac) core is evoked by novel, neutral stimuli in isolation, and that these responses causally influence future learning for valenced stimuli. We used optical approaches to record and manipulate dopamine signals in the Nac core of awake and behaving mice during exposure to neutral stimuli and defined their influence on future learned behavior. Dopamine was evoked by novel neutral stimuli and the trajectory of this response over time tracked habituation. Habituation to novel cues prior to associative learning reduced future associative learning, a psychological construct termed latent inhibition. Critically, trial-by-trial dopamine response patterns tracked this phenomenon. Finally, optically stimulating or inhibiting dopamine responses to the cue during the habituation period bidirectionally influenced future aversive and appetitive associative learning. Our findings highlight the causal role of dopamine signaling in the NAc core in novelty-based learning in a way that cannot be predicted based on purely associative factors.

Disclosures: M.G. Kutlu: None. **J.E. Zachry:** None. **P. Melugin:** None. **J. Tat:** None. **S. Cajigas:** None. **A. Isiktas:** None. **D. Patel:** None. **C. Siciliano:** None. **G. Schoenbaum:** None. **M. Sharpe:** None. **E.S. Calipari:** None.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.12

Topic: G.04. Emotion

Support: NIH DP5 OD023106-01

Title: Neuromodulation of hippocampal memory traces: Implications for stress-related disorders

Authors: *S. L. GRELLA[1], C. W. HARLEY[2], D. F. MARRONE[3], J. H. BLADON[4], S. RAMIREZ[5];
[1]Psychology, Loyola Univ. Chicago, Chicago, IL; [2]Psychology, Mem. Univ. Newfoundland, St John's, NL, Canada; [3]Psychology, Wilfrid Laurier Univ., Waterloo, ON, Canada; [4]Psychology, Brandeis Univ., Waltham, MA; [5]Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: Contextual information is encoded in the hippocampus partially through the recruitment of distinct neuronal ensembles. It is believed that reactivation of these ensembles

underlies memory retrieval processes. The ability to update hippocampal representations through retrieval induced plasticity is considered adaptive, occurring by virtue of the inherent malleability of memories. Given that a wide range of neuromodulators have been found to influence memory, we are investigating the role of norepinephrine (NE) and dopamine (DA) locus coeruleus projections to the hippocampus in assigning new networks to mediate encoding that reflects environmental change. We hypothesize that NE / DA may provide a mnemonic switch signaling hippocampal systems to move from a state of retrieval to encoding in the presence of novelty, potentially constituting an important pathway in memory updating. Stress induced dysregulation of these neuromodulatory arousal systems may contribute to maladaptive memory processes such as impairments in memory updating. To mitigate dysregulated signalling and maladaptive cognition and behavior often observed in stress related memory disorders such as post-traumatic stress disorder, we aim to develop novel memory modulation strategies. To that end, we recently showed that optical stimulation of a positive memory (self-stimulation of the ventral tegmental area) during recall of a negative (fear) memory, acutely and enduringly reduced the expression of fear and permanently altered the fear memory ensemble. We are currently exploring how this modulation strategy affects hippocampal NE / DA neurotransmission toward a better understanding of the neuromodulatory mechanisms involved in memory updating, and how they are impacted by stress.

Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

Location: SDCC 33

Time: Sunday, November 13, 2022, 1:00 PM – 4:30 PM

Presentation Number: 181.13

Topic: G.04. Emotion

Title: Hydrocortisone alters neural mechanisms supporting emotional episodic memory

Authors: B. HARRIS[1], B. SHERMAN[2], N. B. TURK-BROWNE[2], R. SINHA[3], *****E. V. GOLDFARB**[4];
[2]Psychology, [1]Yale Univ., New Haven, CT; [3]Yale Univ. Sch. Med., New Haven, CT; [4]Yale Sch. Of Med., Yale Sch. Of Med., New Haven, CT

Abstract: Stress can profoundly influence encoding of individual experiences, particularly for emotionally salient information. Across species, these effects are frequently linked to glucocorticoid responses, which can enhance and impair encoding of individual items. Yet even within a single experience, different types of information can be remembered, including item-level, relational, and affective representations, and we previously showed that these are

differentially modulated by acute stress. Here we investigated the cognitive and neural mechanisms by which glucocorticoids influence multiple components of emotional episodic memories using a double-blind, placebo-controlled, within-subjects design. Participants (N = 27 social drinkers) encoded associations between 80 photographs of objects and neutral scenes and rated their emotional responses to each object/scene pair during an fMRI scan. Of these 80 objects, 50% were neutral (handheld household objects) and 50% were emotionally salient (alcoholic beverages), previously validated to be comparable in perceptual features. Twenty-four hours after encoding, participants retrieved memories for different components of these episodes, including item-level (object recognition), relational (recognition of scene associated with object), and affective (vividness of memory for feelings) representations. They completed this process twice, once receiving 20 mg hydrocortisone ~1 hour prior to encoding and once receiving a placebo. We found that glucocorticoid administration prior to encoding amplified the perceived emotional salience of object/scene pairs. Furthermore, glucocorticoid administration interacted with emotional arousal to shape later memories. Following hydrocortisone, higher emotional salience enhanced memory for items and relations; however, this association was flipped following placebo, with higher emotional salience impairing both forms of memory. In contrast, whereas higher emotional salience enhanced affective memory following placebo, cortisol blocked this enhancement. Preliminary evidence indicates that cortisol may have modulated memory by altering hippocampal circuitry: cortisol enhanced background functional connectivity between hippocampal subfields and changed the association between hippocampal connectivity and subsequent relational memory behavior. Together, these findings highlight novel mechanisms by which stress-related hormones regulate the formation of emotional memories.

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Nanosymposium

181. Emotional and Motivational Influences on Learning and Memory

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Presentation Number: 181.14

Topic: G.04. Emotion

Support: F32 MH129136 (SEC)
             R01 MH122387 (JED)

Title: Latent associative structures facilitate the transfer of learned threat

Authors: *S. E. COOPER, A. C. HENNINGS, S. A. BIBB, J. E. DUNSMOOR;
Dept. of Psychiatry and Behavioral Sci., Univ. of Texas at Austin, Austin, TX

Abstract: Exposure therapy is the gold-standard treatment for PTSD, but its effectiveness is limited by reliance on techniques addressing only a circumscribed set of stimuli and experiences that have clear associative links (e.g., fear of driving after a vehicle accident). Here, we

investigated how latent associations between stimuli can facilitate the transfer of threat from one to the other (i.e., generalization). In a two-day fMRI experiment drawing on Pavlovian preconditioning and episodic memory designs, 37 psychiatrically healthy participants first learned the association between one of two categories (e.g., animals or tools) and a specific shape (e.g., circle or square) on day 1. Next, one of the previously encountered shapes was paired with a mild shock (CS+) and the other shape was a safe control stimulus (CS-) – this resulted in a latent associative structure, in which shock was indirectly related to one of the previously encountered categories (PS+) via its previous CS+ association, while the other category (PS-) remained unassociated with shock. We tested these associations in a subsequent generalization test, in which participants encountered novel PS+ and PS- exemplars. Day 2 involved a second generalization test and an item recognition test for day 1 PS+ and PS- stimuli. We predicted that neural and behavioral indices of threat would show generalization to the PS+ (relative to PS-), despite never directly pairing the PS+ with the shock. Univariate fMRI analyses revealed hyperactivation in the insula for the PS+ relative to PS- on day 1; this effect was attenuated at the day 2 test. Insula activity also predicted retrospective self-report valence ratings of the PS+, but not PS-. These results accord with a growing body of work implicating the insula as a key region underlying generalized threat learning. Additionally, the item recognition test found that PS+ items viewed before aversive conditioning were better remembered than PS- items, suggesting a retroactive enhancement effect. MVPA was also used to assess integration and similarity of the preconditioning and conditioning memories. Results are discussed in the context of current theories of abstract threat generalization and its relevance to improving exposure therapy for PTSD.

Disclosures: S.E. Cooper: None. **A.C. Hennings:** None. **S.A. Bibb:** None. **J.E. Dunsmoor:** None.

Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.01

Topic: H.05. Working Memory

Support: NSF 2050833

Title: Transient and sustained visuomotor selection during working-memory guided behavior

Authors: R. WEESE[1], *E. F. ESTER[2];
[1]Psychology, Univ. of Nevada, Reno, Reno, NV; [2]Psychology, Univ. of Nevada, Reno Integrative Neurosci. Grad. Program, Reno, NV

Abstract: Working memory (WM) is a capacity- and duration-limited system that forms a temporal bridge between fleeting sensory phenomena and possible actions. Many everyday behaviors require that WM-guided actions be timed to a future event or executed in a specific

sequence. Once a future goal state becomes obvious, what happens to the mnemonic representations of visual and motor information needed to reach it? One possibility is that task-relevant visuomotor representations stored in WM are selected and prioritized until actions are completed, enabling precise and continuous control of action by the contents of visual WM. A second possibility is that task-relevant visuomotor representations are selected and transformed from retrospective stimulus- and rule-based representations into prospective action-based representations, rendering the persistent selection of visual WM information during action planning and execution unnecessary. We tested these alternatives by recording EEG while participants performed a retrospectively cued orientation recall task. A one-second gap separated the retrocue display and a subsequent probe display; thus, participants were free to select task-appropriate visual and motor WM representations following the retrocue but could not execute a response until 1.0 sec later. Following earlier work, we independently manipulated the physical location of the to-be-recalled orientation (i.e., left vs. right visual hemifield) and the affordance used to generate a response (i.e., left vs. right hand); this allowed us to track the selection of visual and motor signals in trial-averaged EEG data independent of one another and nuisance effects (e.g., volume conduction). Comparisons of EEG signals associated with visual and motor selection revealed transient selection of the task-relevant visual information that began and ended before the onset of the probe display, accompanied by sustained selection of the task-relevant motor response that began concurrently with the selection of visual information but that persisted throughout the delay and subsequent response period. A complementary decoding analysis revealed strong and sustained above-chance decoding of planned motor-responses over occipitoparietal and frontocentral clusters but weak and transient decoding of visual information over the same sites. These observations are difficult to reconcile with models of WM-guided behavior that require the continuous selection of visual information but can be explained by a hybrid selection model where visual WM representations are selected and transformed from a stimulus-specific to a response-specific format.

Disclosures: R. Weese: None. **E.F. Ester:** None.

Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.02

Topic: H.05. Working Memory

Support: NIH Grant 1R01EY030854

Title: Neural representations of targets and distractors in visual working memory

Authors: *Y. XU;
Yale Univ., New Haven, CT

Abstract: Previous studies have shown that the content of visual working memory (VWM) can be decoded in both human early visual cortex (EVC) and posterior parietal cortex (PPC). At the same time, task-irrelevant distractors shown during the VWM delay period can also be decoded in these brain regions. This study examines how target and distractor objects may be represented together in a VWM task in EVC and PPC, as well as in lateral and ventral occipito-temporal cortex (LOT/VOT) involved in visual object processing. In each trial of the study, participants retained a target object while viewing either a blank screen or a stream of distractor objects during the VWM delay period. The target and distractor objects came from the same four object categories, allowing exemplars from the same object category to serve as targets or distractors in different trials. Results showed that while the overall object representational structure during VWM delay was dominated by distractors in EVC and LOT/VOT, it was shaped by both targets and distractors in PPC. To examine how target representations may or may not be affected by distractor presence in VWM, a classifier was trained to decode a pair of targets when distractors were present to then cross-decode the same pair of targets when distractors were absent and vice versa. Target representation in VWM was found to differ between when distractors were present and when they were absent in all three sets of brain regions. Using the same cross-decoding approach, target representation in VWM is further shown to depend on the type of distractors present. Lastly, by training a classifier to decode a pair of objects when they were distractors to then cross decode the same pair of objects when they were targets, it was found that target representation in VWM differed from distractor representations in all three sets of brain regions. Overall, these results show that targets in VWM are retained differently with and without distractors, and target representation in VWM partially dependent on the type of distractors present. Moreover, targets are also retained differently in VWM compared to the distractors. In both ventral and dorsal regions, VWM code is thus transformed with distraction, such that targets retained in VWM are not coded independently of distractor representations.

Disclosures: Y. Xu: None.

Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.03

Topic: H.05. Working Memory

Support: NIMH Grant R01MH115042

Title: Geometry of Transformations in Working Memory

Authors: *A. ARDALAN, J. YE, P. KOLLIAS, T. BUSCHMAN;
Princeton Neurosci. Inst., Princeton, NJ

Abstract: Working memory acts as a flexible workspace for cognition, allowing us to maintain the stimulus inputs, goals, and motor actions needed for behavior. Recent work has suggested

that each working memory item is represented in an independent 'subspace' within the high-dimensional pattern of neural activity (Bouchacourt and Buschman, 2019; Panichello and Buschman, 2021). By representing information in orthogonal subspaces, the brain can avoid interference between memories (Libby and Buschman, 2021). Cognitive control can then act on the representations within working memory. For example, by transforming a representation in a way that it aligns with the output-potent subspace of a downstream region, cognitive control can route task-relevant information to particular downstream region(s). However, the details of how information is embedded in a subspace and how it moves between subspaces, either to be exposed to or hidden from downstream areas, are still largely unknown. To address this, we analyzed the dynamics of neural activity when monkeys and computational models solve a set of working memory tasks that require the animal/model to maintain and manipulate information in working memory. By identifying, quantifying, and comparing the transformations of neural representations during the task, we characterized the building blocks of how to use subspaces to perform neural computations. In particular, we investigated the specific class of rotational transforms, identifying their contribution to mapping information in a task-relevant manner. Altogether, our results begin to quantify the dynamics of representations in working memory and understand how these dynamics facilitate cognition.

Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.04

Topic: H.05. Working Memory

Title: Characterizing the spatial organization of population codes in prefrontal cortex

Authors: *J. D. XIANG, M. ROUSSY, B. CORRIGAN, R. LUNA, M. MOFRAD, L. MULLER, J. MARTINEZ-TRUJILLO, M. MUR;
Univ. of Western Ontario, London, ON, Canada

Abstract: The lateral prefrontal cortex (LPFC) plays a key role in higher-order cognition. Electrophysiology studies in behaving animals show that patterns of activity across neural populations in LPFC code task-relevant information. However, fMRI studies in humans report weak decoding of task-relevant information from LPFC activity. We hypothesize that the contradiction arises because the spatial topography of prefrontal population codes is too fine-grained to be effectively resolved by standard fMRI ($2\times2\times2$ mm^3).
We analyzed microelectrode array data (Utah array, 4×4 mm^2, 10×10 channels spaced 0.4 mm

apart) recorded from LPFC areas 8A and 9/46 of two macaques performing three cognitive tasks: a visuospatial working memory task, an associative learning task, and an oculomotor delayed response task. For each monkey, task, and measurement session, we estimated each channel's tuning profile by computing its mean firing rate for each condition. To assess the spatial scale of the population codes, we estimated tuning profile similarity between channel pairs as a function of distance, using global Moran's I. To inspect the spatial topography of the population codes, we visualized channel tuning similarity on the arrays.

We find that tuning profiles are spatially autocorrelated up to a distance of 2.5 mm across all tasks in most sessions for both monkeys (p<.05 corrected, channel permutation test). The array map visualizations suggest moderate spatial clustering of channels with similar tuning and a somewhat irregular topography. The observed topography and fine-grained spatial scale of the prefrontal population codes, which falls above the Nyquist frequency of standard fMRI sampling, may limit the sensitivity of standard fMRI to prefrontal population codes.

The spatial resolution of standard fMRI appears insufficient to robustly detect prefrontal population codes. High-field fMRI, with increased spatial resolution, may be able to measure the fine-grained population codes from the prefrontal cortex, and bridge the gap between monkey electrophysiology and human fMRI studies.
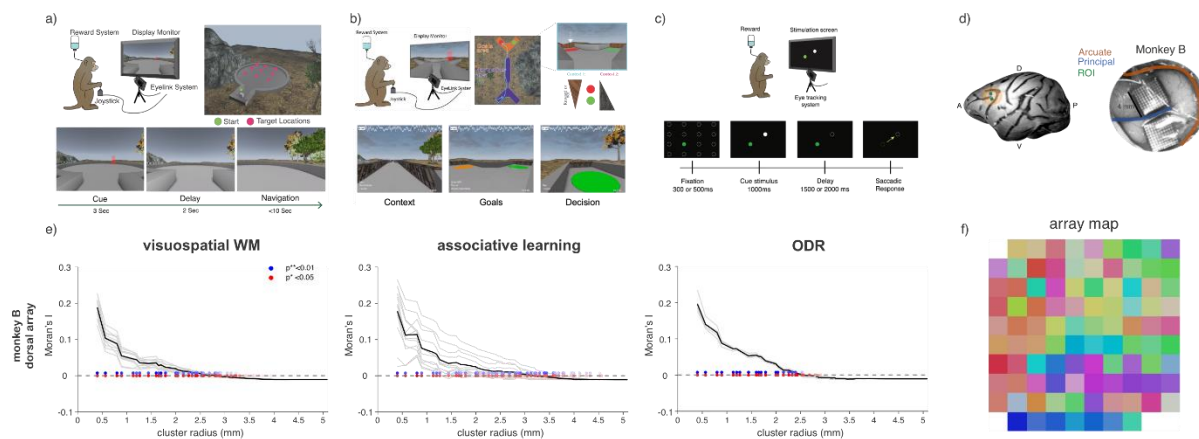

Figure 1 | Task paradigms and results

a) Visuospatial working memory task.
b) Associative learning task.
c) Oculomotor delayed response (ODR) task.
d) Array implants.
e) Spatial autocorrelation functions for one monkey, dorsal array. Grey lines represent individual measurement sessions. Black lines represent session-averaged results. Red and blue dots indicate significance under permutation tests (p<.05 and p<.01 respectively). Saturation of the dots indicates the proportion of sessions with significant results.
f) Topographic map for one monkey and measurement session, dorsal array, visuospatial WM task. Multidimensional scaling (MDS) was conducted on the pairwise channel tuning correlation distance matrix to reduce the dimensionality of each channel to two. Colors were assigned to channels based on their location in the 2D MDS space. Similar colors indicate similar tuning profiles.

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Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.05

Topic: H.05. Working Memory

Title: Univariate and multivariate load-dependent signals in human cortex

Authors: *K. C. S. ADAM[1], E. AWH[3], J. SERENCES[2];
[1]UCSD, [2]Psychology, UCSD, La Jolla, CA; [3]Psychology, Univ. of Chicago Dept. of Psychology, Chicago, IL

Abstract: Early fMRI investigations of visual working memory identified a spatially localized, load-dependent increase in BOLD activity in the intraparietal sulcus, and this region has been hypothesized to play a particularly important role in working memory maintenance. However, emerging evidence suggests that working memory codes are widely distributed across cortex. For example, the identity of a single remembered item can also be decoded from activity patterns in early visual cortex, and working memory load can be decoded from a spatially global EEG signal. Here, we looked for evidence of working memory load signals across the visual stream, while ruling out potential sensory confounds that had not been previously controlled. In a pre-registered fMRI experiment, n = 12 human observers (t = 12 scanner hours per observer) performed a visual working memory task with set sizes 0, 1, 2, and 4. On each trial, observers encoded 4 items (colored squares). After a short interval (.8 sec), observers were given a retro-cue indicating which items should be prioritized, remembered, or dropped from memory. We replicated a classic load-dependent increase in BOLD activity with set size in the intraparietal sulcus. However, we also observed diverse univariate and multivariate load signals across the entire visual stream. For example, we were able to decode working memory load from multivariate patterns of activity in V1-IPS in a sustained fashion, and we also observed diverse patterns of load-dependent univariate modulations. Our results highlight the supporting role of many different regions in working memory maintenance and point to future avenues for understanding the distributed nature of working memory codes.

Disclosures: K.C.S. Adam: None. **E. Awh:** None. **J. Serences:** None.

Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.06

Topic: H.05. Working Memory

Title: Neural population dynamics of human working memory

Authors: *H.-H. LI[1], C. E. CURTIS[2];
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Abstract: Working memory (WM) depends on the activity of neural populations to maintain internal representations of information that are no longer available in the environment. The neural population dynamics that support WM in the human cortex remain unexplored. We studied neural representations of WM in the human cortex when human participants performed a memory-guided saccade task in an MRI scanner (n=14, 7 females): A target dot (12° eccentricity) appeared at a randomly chosen location (polar angle) in each trial. After a delay, observers reported remembered locations by a saccadic eye movement. Utilizing fMRI and time-resolved multivariate analyses, we found evidence of both stable and dynamic WM representations during a classic memory-guided saccade task. Using dimensionality reduction techniques, we identified robust neural subspaces in early visual, parietal and even frontal cortex that were stable throughout the delay period with memorized locations organized within a two-dimensional representation of visual space. The dynamic subspace, which was largely restricted to early visual cortex, had both an early and late delay component during which the WM representation changed. Leveraging models of the receptive fields of voxels comprising each of the visual field maps, we visualized how the dynamic population code changed over time. Early in the trial, the population neural response in V1 was dominated by a narrowly tuned activation among voxels with receptive fields centered on the location of the peripheral memory target. Late in the trial, the population neural response was driven by diffuse activation among voxels with receptive fields along a line between the fovea and the peripheral location of the target. Together, the timing, shape, and spatial distribution of the WM dynamics in early visual cortex suggest that the early component encodes feedforward visual inputs, while the late component encodes abstracted task-relevant mnemonic representations resulting from feedback signals from higher-level brain areas.

Disclosures: H. Li: None. **C.E. Curtis:** None.

Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.07

Topic: H.05. Working Memory

Title: Long-term experience shapes short-term memory codes

Authors: *A. KIYONAGA[1], J. MILLER[2], A. TAMBINI[3], M. D'ESPOSITO[2];
[1]Univ. of California San Diego, UCSD, La Jolla, CA; [2]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA; [3]Nathan Kline Inst. For Psychiatric Res., Orangeburg, NY

Abstract: Working memory (WM) keeps information in mind for short-term use, but immediate goals may build off long-term experience. Successful WM holds information that varies in familiarity, over multiple time scales, and with fluctuating competition from concurrent demands. This range of usages may underlie inconsistencies in how and where the brain stores WM content. Namely, monkey electrophysiology vs. human neuroimaging advance distinct conclusions about whether WM is stored in prefrontal or sensory cortices, respectively. However, monkeys typically undergo months of task training and thousands of trials before recordings take place. Expertise and long-term memory are increasingly recognized as contributors to human WM. Therefore, we hypothesized that extensive stimulus experience and learned associations might shift the neural activity that supports WM. Here, we used a longitudinal 'precision fMRI' approach to test this prediction. Across 4 months, 3 human subjects completed regular at-home behavioral testing and were scanned repeatedly (~24 scans apiece) during task performance. Subjects trained on (1) a serial reaction time (SRT) task, wherein complex kaleidoscope stimuli were embedded in probabilistic sequences, and (2) a delayed recognition task probing WM for trained or novel kaleidoscope stimuli. Across training, WM delay activity became more distributed across the PFC and more stimulus-selective – fMRI responses shifted to resemble typical non-human primate findings and to reflect stimulus associations from the SRT task. This changing distribution of WM activity was also accompanied by changes in behavioral metrics of WM decay and inter-stimulus interference. As stimuli became well-learned, WM recognition was less susceptible to impairment from longer delay times, and more robust to competition from familiar lure stimuli. Hippocampal activity also increased progressively during the WM probe across training, consistent with the idea that the task came to engage long-term retrieval. The same WM stimulus can therefore engage distinct activity patterns depending on its level of familiarity and its position in higher order knowledge structures. While laboratory task stimuli often comprise simple arbitrary features, real-world WM maintains complex stimuli that have individual meaning and learned associations. By densely sampling evolving behavior and fMRI activity across learning, we show how this prior knowledge may scaffold WM and support maintenance via robust distributed codes.

Disclosures: A. Kiyonaga: None. **J. Miller:** None. **A. Tambini:** None. **M. D'Esposito:** None.

Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.08

Topic: H.05. Working Memory

Title: Bodily orienting responses in virtual reality reveal the spatial codes supporting immersive working memory

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Abstract: How we use working memory as we move through our environment remains poorly understood. This is in part because most laboratory tasks rely on participants remaining still – often positioned in a chin rest, gazing at one spot, and responding to experimental elements with simple button presses. However, bodily effectors such as head and eye movements become critically linked to behavior when studying working memory during more unconstrained tasks. In virtual reality, co-registration of head and eye movements is possible and can provide an extremely rich output metric. In a series of virtual reality experiments, participants remembered two visual items. After a working memory delay, a central colour cue indicated which item needed to be reproduced from memory. We investigated how head-direction and gaze track internally directed selective attention in working memory after the cue. We show that when participants can freely move their head, focusing internal attention to locations in memorized space is associated with a head-direction and a gaze bias in the same direction. The involvement of both effectors reveals that common neural circuitry is engaged during external and internal orienting of attention. We further used the discovered gaze bias to investigate the spatial codes supporting working memory following self-movement, that is when visual material is rendered out of sight because the spatial relations between observer and environment change. Directional biases in gaze revealed that multiple representations of our spatial environment can support different stages of working memory within the context of free movement: maintaining and selecting information. Overall, our findings show that there is a general bodily orienting response during internal selective attention and that multiple spatial codes support working memory during unconstrained behavior.

Disclosures: D. Draschkow: None. **F. van Ede:** None. **K. Nobre:** None.

Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.09

Topic: H.05. Working Memory

Title: A gradual transition from veridical to categorical representations along the visual hierarchy for memory but not perception.

Authors: C. CHUNHARAS[1,2], **M. HETTWER**[3], **R. L. RADEMAKER**[3];
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Abstract: The ability to stably maintain visual information over brief delays is central to many cognitive tasks. A potential neural mechanism to achieve visual working memory stability is to maintain multiple concurrent representations at various levels of abstraction and cortical loci. Recent work has shown "sensory-like" mnemonic representations in early visual cortex, while the same mnemonic information is represented in a transformed format in the intraparietal sulcus. As an explicit test of mnemonic code transformations along the visual hierarchy, we quantitatively modeled the progression of veridical-to-categorical orientation representation via a reanalysis of an existing fMRI dataset. Six participants performed both a visual perception (rare target detection) and a visual working memory task (delayed estimation). fMRI activation patterns in different retinotopic regions of interest were sorted into bins based on the orientation shown or remembered. For each task and retinotopic area, the representational similarity of activation patterns in each orientation bin was determined based on Euclidean distances. We compared the resulting confusion matrices with two explicit models: The veridical model assumes that each orientation is most similar to adjacent orientations, and increasingly dissimilar to more distant orientations. The categorical model assumes that orientations are coded in quadrants relative to cardinal axes, so between either "twelve-to-three" or "three-to-six" o'clock. For the perceptual task, the veridical model explained the data well in all retinotopic areas, while the categorical model did not. While the veridical model also did well in the working memory task, the categorical model gradually gained explanatory strength for increasingly anterior retinotopically defined areas. These findings suggest that once visual representations are no longer tethered to sensory inputs, there is a gradual progression from veridical to more categorical mnemonic formats along the visual hierarchy.

Disclosures: C. Chunharas: None. **M. Hettwer:** None. **R.L. Rademaker:** None.

Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.10

Topic: H.05. Working Memory

Title: Interhemispheric serial dependence in working memory in prefrontal cortex

Authors: *M. TSCHIERSCH[1], J. BARBOSA[2], A. UMAKANTHA[3], R. WILLIAMSON[3], M. A. SMITH[3], A. COMPTE[1];
[1]IDIBAPS, Barcelona, Spain; [2]École normale supérieure, Paris, France; [3]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Working memory (WM) content is mostly stored in neurons preferring contralateral items in bilateral prefrontal cortex (PFC) [1], but representations can travel between hemispheres [2]. This is thought to support full-field spatial WM continuity. Recently, temporal continuity in WM has been linked to activity-silent mechanisms in PFC supporting behavioral serial dependence (SD) between successive trials [3]. Indeed, SD increases when prefrontal activity-silent traces of previous memories are reactivated in the fixation period by either internal (e.g. attention) or external (e.g. transcranial magnetic stimulation, TMS) inputs [3]. However, how memory traces and reactivations interact with anatomical lateralization to ensure both WM spatial and temporal continuity is currently unknown. Here, we asked if SD is lateralized and whether it propagates between hemispheres.
We tested the lateralization of SD using human and monkey behavioral responses and TMS experiments in humans. Further, we analyzed simultaneous bilateral PFC multiunit recordings in three monkeys performing a spatial WM task to assess interhemispheric transfer of reactivations during fixation periods. We found that SD is reduced when items are presented in opposite hemifields as compared to within the same hemifield, providing behavioral evidence for some spatial discontinuity in SD. However, we also found trial-averaged bilateral reactivations of the previous trial's stimulus representation in the fixation period, a neural correlate of SD, suggesting spatial continuity of SD. To understand how these seemingly inconsistent findings could be reconciled, we performed canonical correlation analysis on the activity of neurons between hemispheres. We found strong single-trial canonical noise correlations during the delay, hinting towards the coordination of trial-by-trial memory representations across hemispheres, but not during the time of reactivations. This suggests that trial-by-trial memory reactivations are private to each hemisphere, which may explain the spatial discontinuity of SD in working memory. TMS experiments in humans were also consistent with private reactivations, as the SD increase through single-pulse TMS was lateralized.
[1] Funahashi, S. et al., J Neurophysiol 61:2, 331-349 (1989) [2] Brincat, S. L. et al., Neuron 109:6, 1055-1066.e4 (2021)

Disclosures: M. Tschiersch: None. **J. Barbosa:** None. **A. Umakantha:** None. **R. Williamson:** None. **M.A. Smith:** None. **A. Compte:** None.

Nanosymposium

182. Cognitive Neuroscience of Working Memory: Decoding and Networks

Location: SDCC 7

Time: Sunday, November 13, 2022, 1:00 PM – 3:45 PM

Presentation Number: 182.11

Topic: H.05. Working Memory

Support: NIH Grant R01 EY017077

Title: Heterogeneity of Population Low-dimensional dynamics in working memory

Authors: W. DANG[1], S. PU[2], S. LI[5], X.-L. QI[6], C. CONSTANTINIDIS[1,3,4];
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Abstract: The manifold and trajectory of neuronal population responses can reveal neural computations underlying cognitive functions. Previous studies have shown that population geometry undergoes various transformations during the execution of cognitive tasks. It is unknown, however, if such transformations are unique for specific brain areas or common across areas during execution of the same task. It is also often assumed that transformations are task-dependent, yet this assumption has not been tested rigorously. We therefore analyzed neural spiking data of six macaque monkeys from different prefrontal subdivisions before and after the animals were trained in a spatial (n=2195 neurons) and a feature (n=1640) delayed match-nonmatch working memory task. We found that in the anterior portion of the prefrontal cortex, a low dimensional population manifold rotation between different task epochs already exists in naïve animals. The results suggest that some transformations observed in prefrontal cortex may be an inherent property of the circuit rather than the reflection of certain cognitive operations required by a trained task. We also compared the population geometry of prefrontal (n=591) and posterior parietal (n=835) populations from two macaque monkeys in a delayed match-nonmatch working memory task. In this task, trial difficulty was determined by how far a memorized stimulus was located relative to a reference location, which changed daily. Thus subjects gradually inferred the reference location within each session. We found that the geometry of stimulus representation was more stable in prefrontal cortex across various task epochs. At the same time, both prefrontal and parietal areas showed plasticity during a single session, which correlated with behavior change. In summary, our results indicate that heterogeneity exists in the geometry of neuronal representation across different high-order brain regions. Specifically, we found that population transformations, which are often assumed to represent trained cognitive operations, emerge automatically even in subjects naïve to training. Moreover, systematical rotation dynamics exist for training time scales spanning a few minutes to a few months.

Disclosures: W. Dang: None. **S. Pu:** None. **S. Li:** None. **X. Qi:** None. **C. Constantinidis:** None.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.01

Topic: I.08. Methods to Modulate Neural Activity

Support: NARSARD Young Investigator Award
              NSF ERC PATHS-UP

Title: Ultra-low power multi-channel brain implants for wireless optogenetics

Authors: M. JEONG[1], B. LIM[2], ***S. PARK**[3];
[1]Univ. of California, San Diego, TX; [2]Biol. Sci., UCSD, La Jolla, CA; [3]NuFund Venture Group, San Diego, CA

Abstract: For many optogenetic experiments, it would be ideal to stimulate multiple brain regions simultaneously and independently. Although implantation of two optical fibers into target brain regions could enable similar level of functionality, its application to more than three different brain regions would be challenging due to their rigid neural interface (tissue damage) and management of wired-fiber cables (constraints on movement and physiological/psychological stresses imposed on animals). It would be also perfect to selectively control multiple animals within a social group for behavior experiments using optogenetics (e.g., study of interactions by individuals and their effects on mental health). Currently, it is impossible with optical fiber methods due to entanglement of fibers. While wireless techniques (NFC or Bluetooth) for multichannel operation have been described, they suffer from major limitations in their ability to identify and select a channel(s) in a single platform device due to high power consumption (>30 mW). Such high power requirements for operation would render a wireless device energy-hungry and make it impossible to operate while an animal with a device implanted freely behaves in a cage. Here, we introduce a miniaturized, ultra-low power multichannel wireless optogenetic brain implant that can selectively modulate <u>multiple brain regions (<5) of a freely behaving animal</u> or <u>distributed neural circuits (<3) of freely behaving animals (<2)</u>.To validate the functionality of the dual-channel wireless optogenetic system in vivo, we chose to manipulate the mesolimbic dopaminergic (DA) system and tested whether we can selectively deliver light to stimulate distinct VTA DA axonal fibers in Nac with two μLEDs (470 nm & 650 nm) controlled by different channels.We performed 3-chamber conditioned place preference test with this manipulation. Here, one μLED on a probe of a wireless dual-channel brain implant directly interfaces with dorsal-Nac (d-Nac) while the other μLED does ventral-Nac (v-Nac) (they are separated by a distance of 1mm). Indeed, consistent with previous findings, we found that the stimulation of DA axonal fibers with μLEDs targeting d-Nac can induce strong preference in conditioned place preference (CPP) test. Then, we found that this preference was reversed by stimulating DA axonal fibers with μLEDs targeting v-Nac. This strongly suggests that we can selectively and simultaneously modulate the different neural circuitry in same animals with newly developed wireless optogenetic systems.

Disclosures: M. Jeong: None. **B. Lim:** None. **S. Park:** None.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.02

Topic: I.08. Methods to Modulate Neural Activity

Title: Demonstration of an optimized large-scale optogenetic cortical interface for non-human primates

Authors: *D. J. GRIGGS[1], J. BLOCH[1], S. FISHER[1], W. OJEMANN[2], K. COUBROUGH[1], K. KHATEEB[1], M. CHU[1], A. YAZDAN-SHAHMORAD[1];
[1]Univ. of Washington, Seattle, WA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: High spatial and temporal precision and cell type specificity make optogenetics a powerful tool for studying fundamental neural mechanisms and developing neurorehabilitation techniques. Here we demonstrate an optimized large-scale optogenetic cortical interface for non-human primates (NHPs) [1, 2]. This interface consists of a custom built multi-modal artificial dura (MMAD) which is an advancement in comparison to artificial duras used for optical imaging in that platinum particles are printed into a biocompatible and transparent polymer enabling electrical stimulation and recording in combination with optical access to the cortex [3]. We have surgically implanted the MMAD in two rhesus macaques which provides both optical access to about 2.7 cm^2 and recording capability from about 1 cm^2 of the posterior parietal cortex (PPC). We also developed a custom LED array compatible with our MMAD for optical stimulation. To achieve large-scale optogenetic expression, we delivered AAV-hsyn-Jaws-GFP into PPC using convection enhanced delivery (CED), an efficient pressure-based viral delivery approach [2, 4-5]. This interface improves upon the stability and scale of our previous interface iterations [4]. We confirmed expression across large cortical areas (>2 cm^2) with both neurophysiology and epifluorescent imaging. Furthermore, using this interface we observed that the243eactivationn of PPC using optogenetics leads to longer reaches in a center-out reach task. Our optimized design supports 3+ months of optical access in comparison to 2-4 weeks in our previous version and uses up to 16 LEDs for simultaneous optical stimulation which is an order of magnitude improvement over our previous setup. Lastly, our custom designed MMAD enables our printed circuit boards to be clamped on the MMAD polymer only for the duration of stimulation and recording [3] making the entire setup more stable. This work will set the stage for the development of stable, large-scale, multi-modal neural modulation protocols for the purpose of studying cortical organization and plasticity, and developing neurorehabilitation techniques.
References:[1] DJ Griggs et. Al., *SPIE,* 2019.[2] DJ Griggs et. Al., *EMBC,* 2022, (accepted).[3] DJ Griggs et. Al., *J. Neural Eng.,* 2021.[4] A Yazdan-Shahmorad et. Al., *Neuron*, 2016.[5] K Khateeb et. Al., *J. Vis. Exp.,* 2019.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.03

Topic: I.08. Methods to Modulate Neural Activity

Title: Microcoil-based magnetic stimulation of the CNS

Authors: *S. FRIED[1,3], S. RYU[1], S. LEE[2], J.-I. LEE[2], A. J. WHALEN[2];
[1]Massachusetts Gen. Hosp., [2]Neurosurg., Massachusetts Gen. Hosp., Boston, MA; [3]Res., Boston VA Healthcare Syst., Boston, MA

Abstract: Our group has been investigating the use of magnetic stimulation from tiny, implantable coils (referred to as microcoils) as a way to improve the efficacy of artificial stimulation of the CNS. While there have been some remarkable successes with electrode-based prostheses, e.g., cochlear implants (Cis) and deep brain stimulation (DBS) for the treatment of Parkinson's disease and other motor disorders, there are some intrinsic limitations associated with the implantation of electrodes that have limited progress for other applications. For example, it is difficult to control the spread of activation with electrodes; inadvertent activation of non-targeted neurons can lead to a myriad of side effects and also limits the resolution, of larger concern for devices that target sensory systems. Electrodes are also limited by the amount of charge they can deliver, their long-term biocompatibility, foreign body responses, etc. Magnetic stimulation from microcoils is an attractive alternative to conventional electrodes because the asymmetry in the fields they produce allows improved selectivity in targeting neurons. In addition, the high permeability of biological materials to magnetic fields allows passage through bone and other materials that impede the passage of electric fields. Thus, time-varying magnetic fields can be used to 'carry' an electric field across a high-impedance boundary that is difficult to target with conventional electrodes. This same permeability also may confer improved stability of coil performance in cases where the electrical properties of the surrounding tissue change over the course of implantation. To date, we have tested microcoil-based magnetic stimulation in several regions of cortex, deep brain regions, the retina, and the cochlea. In the cortex, the spatially asymmetric fields from coils preferentially activate vertically-oriented pyramidal neurons while avoiding horizontally-oriented passing axons, thus better confining activation to focal regions around the coil. In the cochlea, the magnetic fields from coils positioned within the scala tympani pass readily through the surrounding bony walls to activate

peripheral processes of spiral ganglion fibers. Coupled with the narrower spread of fields in perilymph, coils result in narrower spectral spread (than electrodes) and thus offer the potential for an increased number of independent spectral channels. Despite these intriguing results, coils have some important limitations including the need for high amplitude currents and the potential for excessive heating. Magnetic cores and enhanced insulation offer potential solutions to these limitations.

Disclosures: S. Fried: None. **S. Ryu:** None. **S. Lee:** None. **J. Lee:** None. **A.J. Whalen:** None.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.04

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS098231

Title: Decision making and fMRI responses modulated by remote magnetogenetics stimulation in rats

Authors: *R. R. ISRAELI[1], A. W. JOHNSON[2,3], Y. CHEN[4,7], C. QIAN[4], S. A. PAKRAY[5], A. A. GILAD[3,4,6], G. PELLED[3,4,5];
[1]Biomed. Engin., [2]Psychology, [3]Neurosci., [4]Radiology, [5]Mechanical Engin., [6]Chem. Engin. And Materials Sci., Michigan State Univ., East Lansing, MI; [7]High-field Magnetic Resonance, Max Planck Inst. For Biol. Cybernetics, Tuebingen, Germany

Abstract: Neuromodulation is critical in studying and treating many neuronal diseases and disorders; however, it relies on techniques that are often either invasive or nonspecific. We are developing a technology to noninvasively activate specific neurons using electromagnetic fields and an electromagnetic perceptive gene (EPG). Glass catfish *(Kyptopterus vitreolus)* has a gene that encodes a protein responsive to magnetic fields. This gene (EPG) has been isolated, cloned, and expressed in mammalian cells. EPG can be used through viral injection to target specific neurons which can then be noninvasively stimulated using electromagnetic fields (Krishnan et al., 2018). Our research focuses on the modulating neurons in the visual cortex since it is widely studied and can be researched by behavioral experiments and through functional imaging. Long Evans rats were genetically engineered to express EPG in excitatory neurons of the primary visual cortex via stereotaxic injection of adeno-associated virus containing EPG under CaMKII promoter. These rats were then tested for behavioral responses to magnetic stimulus using an operant conditioning chamber. While in the chamber, an electromagnetic coil attached to the rat's head delivered 50 mT of magnetic field for 2 seconds every minute, repeated for 25 trials. The sham stimulus included the same electromagnetic coil attached to the rat's head with the same electric current flowing, but in opposing direction, eliminating the magnetic field. In response to magnetic stimulus, rats pressed a lever and received a sucrose solution reward. Once

the rats learned to associate the stimulus with pressing the lever, the same experiment was conducted using the sham stimulus. Preliminary data has shown significantly (p<0.05) faster responses when receiving an electromagnetic stimulation as compared to sham stimulation. Additionally, we used a 7T Bruker MRI to perform fMRI on rats expressing EPG in their visual cortex. Using fiber optic cables, we stimulated both eyes with a 5Hz flashing light for 20 seconds. While both control and EPG rats showed significant activation in the superior colliculus, only EPG rats had significant activation in the both hemispheres of the visual cortex (p<0.01), corresponding to the region of neurons containing the EPG. This may suggest that EPG increases activity and connectivity between neurons when under the strong magnetic fields of the MRI. Overall, these results suggest that EPG may be a new method to noninvasively activate specific neurons.

Disclosures: R.R. Israeli: None. **A.W. Johnson:** None. **Y. Chen:** None. **C. Qian:** None. **S.A. Pakray:** None. **A.A. Gilad:** None. **G. Pelled:** None.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.05

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS098231

Title: Magnetogenetic stimulation of CA3 inhibitory interneurons reduces seizure activity in a kainic acid model of acute epilepsy

Authors: *A. C. METTO[1], A. A. GILAD[2,3,4], G. PELLED[3,4,5];
[1]Biomed. Engin., [2]Chem. Engin. And Materials Sci., [3]Neurosci., [4]Radiology, [5]Mechanical Engin., Michigan State Univ., East Lansing, MI

Abstract: Eighty percent of temporal lobe seizures originate in or near the hippocampus. Deep brain stimulation and optogenetics have shown success in some cases of intractable epilepsy. Nevertheless, there remains a need to identify more novel targets and biomarkers for pharmacoresistant seizures. We propose a novel minimally invasive gene-based intervention, the electromagnetic perceptive gene (EPG), which encodes a protein that responds to magnetic fields (Krishnan et al, 2018). The premise for our work rests on the fact that during a seizure, electric currents arise from abnormal firing of neuronal networks, and these currents generate a magnetic field, as evidenced by magnetoencephalography (MEG) studies. We hypothesize that EPG expressed in interneurons will sense the magnetic fields generated during a seizure, which will in turn activate the neurons that will shut down or disrupt the circuit, interrupting seizure activity and progression in a closed-loop and cell-specific fashion. Our approach utilizes an acute kainic acid (KA) model of temporal lobe epilepsy in adult Long Evans rats. In the experimental group (n=9), EPG was stereotaxically injected in the CA3 region of the right hippocampus using a viral

vector under hDlx promoter, which is a promoter that is specific to inhibitory interneurons (AAV9-hDlx-EPG-IRES-EGFP). Control rats received an injection of a control virus (AAV9-hDlx-GFP) (n=9). Two to three weeks following stereotaxic procedure, intrahippocampal injection of KA (0.2 µg/0.2 µl) was administered to the right hippocampus. Tungsten microelectrodes were used to obtain hippocampal local field potential recordings starting at 10 minutes after KA injection. Seizure detection was performed in NeuroScore. Signals were high pass filtered at 0.1Hz and seizure detection performed using a dynamic threshold. Seizure events were defined as having at least 4 spikes, minimum interspike interval of 0.05s, maximum interspike interval of 0.6s, minimum train duration of 7.5s, and a train join interval of 1s. GraphPad Prism was used to perform an independent samples t-test for seizure onset, duration, and number of seizures between the hDlx EPG and hDlx GFP group. We found that hDlx EPG rats presented a significant delay in the onset of first seizure (1142.72 ± 186.35s) compared to hDlx GFP rats (644.03 ± 15.06s), *$P = 0.028$. They also experienced significantly less seizures (4.11 ± 1.03) compared to hDlx GFP rats (8.33 ± 1.58), *$P = 0.042$. These preliminary findings suggest that magnetogenetics via EPG may be an effective strategy to alleviate seizure severity in a minimally invasive, closed loop and cell specific fashion.

Disclosures: A.C. Metto: None. **A.A. Gilad:** None. **G. Pelled:** None.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.06

Topic: I.08. Methods to Modulate Neural Activity

Title: Freeform Stimulator and a Path to More Versatile Neural Implants

Authors: *G. FRIDMAN[1], C. STEINHARDT[2], A. CHENG[1], P. ADKISSON[1], R. BAID[1], S. RAGHUNATH[1], A. WANG[1];
[1]Johns Hopkins Univ., BALTIMORE, MD; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: In our laboratory we have been developing the Freeform Stimulator (FS). This neural implant can deliver ultra-low frequency electric fields to neurons safely. Conventional neural implants use charge balanced biphasic pulses to evoke action potentials (Aps) in target neurons. They are constrained to delivering short pulses to avoid bubbles due to electrolysis, pH changes, and corrosion. However, it has been shown in many previous experiments that delivery of long duration waveforms, including direct current, can significantly expand the range of neural control. It can cause neurons to not only excite their activity in a natural stochastic fashion in contrast to phased-locked pulse-evoked Aps, but also reduce or inhibit spontaneous firing rate, sensitize neurons to input, and modulate LTP. FS works by rectifying biphasic charge balanced

pulses delivered to the electrodes embedded within the device into direct ionic current (iDC) at the output of the device. The output is delivered via electrolytic gel through microcatheters to the target neurons rather than through wires and electrodes. This newest FS design is uses hydrogel and hydrogel switches to conduct and control ionic current and is therefore not susceptible to the same reliability problems as the previous generation of the technology, which relied of electrolytic fluid and microvalves. We previously showed the strong impact of FS on peripheral nerve modulation. In our recent experiments, we explored using the FS implant to modulate the activity of cortical networks. We implanted two rats with recording electrode array and microcatheter tubes that delivered iDC to the motor cortex. We delivered 10s-long bipolar iDC stimuli at 0-100uA through micropipettes to the motor cortex of an anesthetized rat while also recording the responses of cortical neurons to this stimulation. The neurons showed strong excitatory and inhibitory responses for both animals, with 12 excitatory, 1, inhibitory, and 3 neurons with no effect for one animal, and correspondingly 6 excitatory, 6 inhibitory and 2 no effect for the second animal.

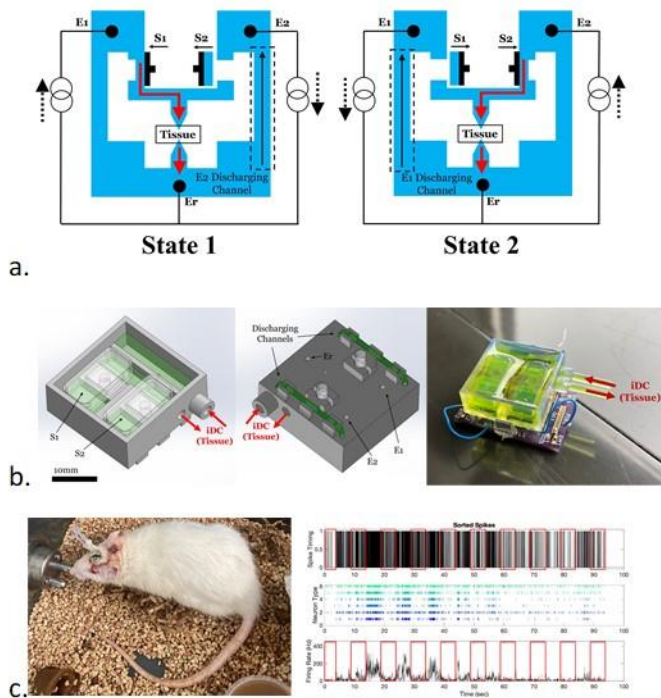$$MISSING OR BAD IMAGE SPECIFICATION {9ED0915B-F9E2-498B-A456-40CDE39B04DB}$$

Figure 1: The mechanical design of the FS chip and cortical stimulation. a.) Construction of FS showing the two states of operation. b.) Chip design and completed chip. The agar gel reservoirs are indicated in green. c.) Rat implanted with Neuronexus electrodes and tubes to deliver iDC from the FS (left). Sample of neural recordings when biphasic stimuli were delivered between tubes.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.07

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF GRFP

Title: Transparent Nanopillar Electrode Arrays for Characterizing Neuronal Networks In Vitro

Authors: *S. SHUKLA[1], D. MEGANATHAN[2], Z. JAHED[2];
[1]Bioengineering, [2]Nanoengineering, Univ. of California San Diego, La Jolla, CA

Abstract: Patch clamp, the gold standard for neuronal electrophysiology, cannot keep up with the demand for high throughput intracellular recordings. These recordings are imperative for studying network activity in healthy vs diseased neuronal populations, for correlating ion channel activity to biological cascades, and for rapid screening of CNS-targeted, personalized therapeutics. Here, we present a transparent, higher throughput, robust, and tunable platform for manipulating and probing single neuronal electrical function during simultaneous fluorescence microscopy. Our platform consists of arrays of transparent nanopillar (NP) electrodes (2-5 um tall, < 500 nm diameter), shown in Figure C, made using a maskless photolithography and dry etching technique, followed by pillar shrinkage using wet etching. After seeding neurons on our NP electrode array, electrical stimuli provided to the NPs induces nano-sized pores across the overlying plasma membrane (electroporation), providing the NPs with access to the cell interior including the nuclear member (Figure A). Our nanopillar electrode arrays are compatible with various cellular constructs including 2D primary and iPSC-derived cultures (Figure D), brain slices, and 3D organoids. Using cell micropatterning, we aim to design simple neuron circuits to study network activity in various neuronal architectures while performing fluorescence imaging to visualize subcellular structures. Finally, we fabricated devices which can obtain simultaneous intracellular action potential and extracellular (EC) recordings, depicted in Figure B, from neuronal networks in 2D monolayers to compare single-cell activity to neuronal circuit behavior. Comparing IC and EC signals will help relate single-cell and subthreshold events to local field potentials and culture-wide behavior. These experiments reveal a large corpus of knowledge which can be gleaned from electronic devices at the cell-nanopillar interface.
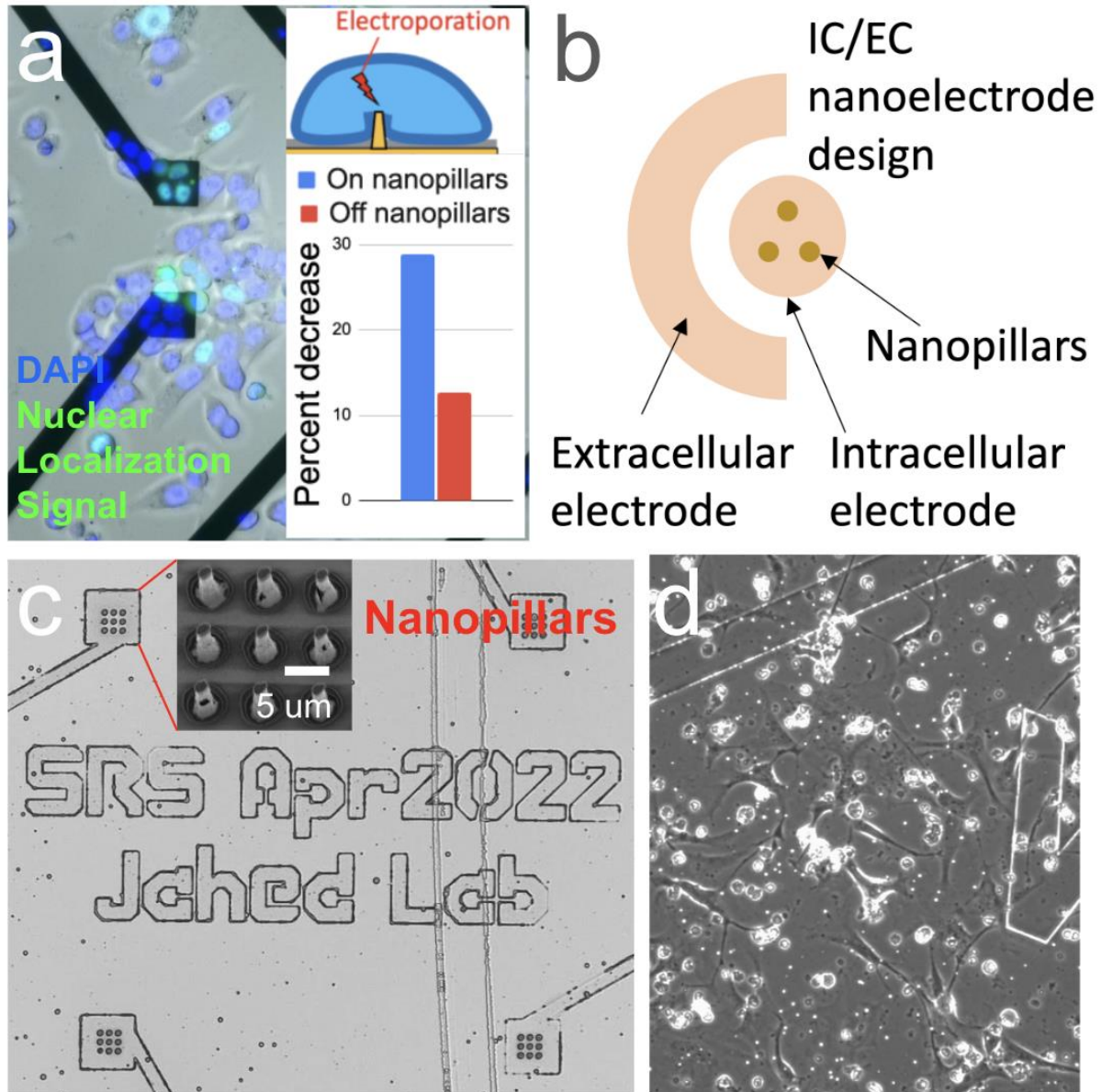
Figure 1. Versatile NP electrode arrays.

Disclosures: **S. Shukla:** None. **D. Meganathan:** None. **Z. Jahed:** None.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.08

Topic: I.08. Methods to Modulate Neural Activity

Title: Interactive, Easy-to-Use, Online-Accessible Planning Tool for Classic and Temporal Interference Brain Stimulation

Authors: *A. M. CASSARA[1], M. STEINER[2], K. ZHUANG[2], M. GUIDON[2], O. MEITZ[2], N. KUSTER[3], S. J. REGEL[4], E. NEUFELD[2];
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Abstract: Temporal interference stimulation (TIS) [Grossman *et al.*, Cell. 2017;169(6):1029-1041.e16] is considered a promising approach for non-invasive targeted modulation of deep brain region activity. Compared to classic transcranial electric stimulation (tES) approaches (e.g., tDCS, tACS), it strongly reduces stimulation of overlaying structures and is more customizable. However, to facilitate TIS research, precise hardware and a reliable planning tool are required. We have developed a cloud-based, online-accessible tES/TIS planning tool to: (i) allow exploration and identification of optimized stimulation conditions (electrode montages, current magnitudes) to maximize stimulation selectivity and TI exposure strength at the user-selected target, while minimizing off-target exposure; (ii) quantify and visualize TI dose and electric field distributions along with key metrics for target and off-target regions to interactively assess performance and safety.
The highly interactive planning tool is based on the o2S2PARC platform [Neufeld *et al.* The FASEB Journal 2020; 34.(S1):1-1] for open and collaborative computational (neuro-)sciences. Electromagnetic simulations were performed using a low-frequency electro-quasistatic simulator from Sim4Life (ZMT Zurich MedTech AG, Zurich, Switzerland) in combination with detailed anatomical human, mouse and monkey head models from the IT'IS Virtual Population. The use of precomputed field distributions enables efficient multi-goal optimization (high focality, minimal off-target TI delivery, minimal E-field deposition at skin), after which conflicting goals can be weighted and the exposure can be further fine-tuned interactively before exporting a detailed report for the identified configurations of interest.The novel planning tool has already been used to facilitate several preclinical studies worldwide. work is currently underway to further support study design by (i) enabling highly automated, personalized (in terms of anatomy as well as heterogeneous and anisotropic brain properties), image-based exposure assessment and optimization using innovative artificial intelligence-based algorithms; (ii) performing extensive modeling uncertainty assessment and providing a larger range of models in view of inter-subject variability; (iii) supporting modeling of multi-polar (i.e., more than two channels) TIS; (iv) creating a curated, user-extensible database of target-specific standard treatment configurations. A user-friendly highly interactive planning tool was developed that offers optimization and improved analyses of tES and, in particular, TIS.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.09

Topic: I.08. Methods to Modulate Neural Activity

Support: VLAIO Grant HBC.2021.0187

Title: Microelectrode arrays for calcium imaging of advanced intracortical stimulation

Authors: *M. SCHELLES[1], R. FIÁTH[2,3], F. CEYSSENS[1,4], K. WIERDA[5], I. ULBERT[2,6], M. KRAFT[1,4];
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Abstract: An increasing amount of neural interfaces is directly targeting the cortex for electrical neurostimulation, and it has been hypothesized that the stimulation resolution could benefit from using more advanced stimulation patterns. However, behavioural experiments offer too little information on such fine-scale differences, while electrophysiological measurements are often limited to a too small part of the brain. Calcium imaging can be a solution to study the effect of microstimulation on the mesoscale, but it is non-trivial to use extracellular, penetrating electrode arrays designed for electrophysiology, in a calcium imaging environment, due to different shape requirements and the necessary compatibility of the materials with 2-photon imaging. We present a pilot study where we designed, optimized, and tested a set-up that combines 2-photon calcium imaging with intracortical microstimulation, using multiple extracellular electrodes at the same time, both with mouse brain slices, as well as *in vivo* in the mouse cortex, showing the applicability to different brain models.
Our electrode arrays consisted of flexible, polyimide shanks of 20x70 µm², a length of 400 to 1200 µm (depending on the brain model), and located at a fixed distance of 150 to 200 µm from each other. Iridium oxide electrodes of size 20x30 µm² were spread along the width and length of each shank, creating a grid of intracortical electrodes, with distances varying from 15 µm to several 100 µm, allowing to study the effect on the neural activity at different length scales. All materials used in the electrode arrays were tested on their fluorescence and were shown to be compatible with 2-photon imaging.
During the experiments in brain slices, the electrode arrays were positioned with the shanks pointing upwards, and a coronal brain slice with a thickness of 400 µm of a P30 GcaMP6f mouse was put on top, such that the shanks penetrated the slice from the bottom. For the *in vivo* experiments, the shanks were inserted into the cortex at a very steep angle (~90° perpendicular to cortical layers). A 2-photon set-up, with a wavelength of 960 nm, was then used to image the neural activity in cortical layer 2/3. The shank size and the electrode distances were optimized in order to reach a good fit between the neurons and the electrodes.
Our setup allows 2-photon calcium imaging of the neural tissue, using multiple intracortical

stimulation electrodes. This setup will be used to investigate several stimulation paradigms and their effect on neural activation, comparing conventional microstimulation with one electrode with a situation where multiple electrodes are used simultaneously.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.10

Topic: I.08. Methods to Modulate Neural Activity

Title: Non-linear responses of neurons to pulsatile stimulation provide evidence of average population firing rate-based encoding schemas in sensory systems

Authors: C. R. STEINHARDT[1], G. Y. FRIDMAN[2];
[1]Dept. of Biomed. Engin., [2]Dept. of Otolaryngology, Johns Hopkins Univ., Baltimore, MD

Abstract: Pulsatile electrical stimulation is the standard paradigm used in neural implants to restore function, but how it interacts with the direct targets of stimulation is not well understood. Recent findings showed that about half of pulses delivered to vestibular afferents produce action potentials (Mitchell *et al.* 2016). We investigated the effects of pulses on firing rate first through single-unit recordings of macaque vestibular afferents in response to pulse trains of fixed pulse rates from 25 to 300 pulses/s and fixed amplitudes from 25 to 100% of the safe range (13 combinations). In spontaneously firing vestibular afferents, the slope of pulse rate versus firing rate varied from -0.2 to 0.5 spike/s/pulses/s. We then used a biophysical model of the vestibular afferent to simulate pulsatile stimulation of a macaque vestibular afferent with fine sampling of pulse rate and pulse amplitude combinations (625 conditions). We found that pulses interacted with themselves at the axon, producing non-linear additive and blocking effects. Pulses also interacted with spontaneous EPSCs producing an approximately separable set of non-linear additive and blocking effects. Because pulse trains are given with fixed inter-pulse intervals, we found that the average probability of each of these interactions could be estimated without a dependency on time. Using that concept, we produced an equation that predicted the induced firing rate during stimulation as a function of the pulse amplitude and pulse rate delivered and the spontaneous firing rate of the neuron being stimulated. We parameterized this equation to the simulation then tested whether it could predict macaque vestibular afferent firing responses. We found that it could predict the data across conditions with rms error of 6.30±1.13 spikes/s(N=13). An important finding was that afferents experiencing the same pulse amplitude and pulse rate of

stimulation would have different firing rates and changes in firing rate dependent on the spontaneous rate of the neuron. At most pulse amplitudes, neurons with high spontaneous rates (i.e. 130 spikes/s) would be inhibited while neurons with low spontaneous rates (i.e. 0-20 spikes/s) were strongly excited. However, a simulation of the response of a population of 200 afferents with natural variance in spontaneous rates showed that the average population firing rate increased with pulse amplitudes in the same range. These findings suggest that neural implants, such as vestibular implants, affect different portions of the population as pulse amplitude increases but proportionally modulate the average population firing rate, leading to coherent changes in behavioral responses.

Disclosures: C.R. Steinhardt: None. **G.Y. Fridman:** None.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.11

Topic: I.08. Methods to Modulate Neural Activity

Title: Confined and persisting site-specific offline target engagement of pericentral cortex following TDCS of both primary motor hand areas

Authors: *M. LIU[1], R. B. LANGE[1], F. GREGERSEN[1,2], R. POHMANN[4], K. H. MADSEN[1,3], A. THIELSCHER[1,2], H. R. SIEBNER[1,5,6];
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Abstract: Background: Transcranial Direct Current Stimulation (TDCS) of the primary motor hand area (M1-HAND) has been shown to modulate corticomotor excitability, with aftereffects lasting up to several hours. Specifically, bi-polar TDCS targeting the right and left M1-HAND has been used to shift the interhemispheric balance. Pseudo-continuous Arterial Spin Labeling (pc-ASL) maps regional cerebral blood flow (rCBF) and can capture the rCBF changes that are caused by a change in regional neuronal activity in M1-HAND and connected areas during and after TDCS.
Objective: To elucidate how TDCS with a bi-hemispheric M1-HAND montage engages the bi-hemispheric pericentral cortex and shifts in interhemispheric balance.
Methods: In separate sessions, 20 healthy right-handed participants received 10 minutes of active

TDCS (30 sec ramp-up and down) or sham TDCS (30 sec ramp-up, immediate ramp-down) at 2.0 mA with the anode placed over left M1-HAND and the cathode placed over right M1-HAND. We used 7 x 5 cm rubber electrodes with conductive gel. Real and sham TDCS were applied at two different days in counterbalanced order with subjects being blinded to the TDCS condition. We performed pc-ASL fMRI at 3 T concurrently with TDCS, to measure effects on rCBF both during and for 24 minutes after stimulation. We focused on the post-stimulation period of TDCS (offline effects) and used FSL's FMRI Expert Analysis Tool (FEAT) to model rCBF changes after TDCS.

Results: Compared to sham TDCS, real TDCS induced a relative decrease in rCBF in the deep part of left M1-HAND underlying the anode. This effect persisted during the entire post-TDCS period, reaching the highest effect size 9-16 minutes after the end of TDCS (peak voxel: -30, -30, 52, z-score = 4.67). In contrast, there were no outlasting rCBF changes in the right M1-HAND underlying the cathode.

Conclusion: Our preliminary results show that bi-hemispheric TDCS with standard non-focal electrodes targeting left M1-HAND (anode) and right M1-HAND (cathode) induces lasting site-specific offline effects of TDCS. Aftereffects are regionally confined to the deep area of left M1-HAND underlying the anode. This unilateral aftereffect might underpin the previously reported shift in bi-hemispheric balance between the two M1-HAND.

Disclosures: M. Liu: None. **R.B. Lange:** None. **F. Gregersen:** None. **R. Pohmann:** None. **K.H. Madsen:** None. **A. Thielscher:** None. **H.R. Siebner:** F. Consulting Fees (e.g., advisory boards); Sanofi Genzyme, Denmark, Lophora, Denmark, Lundbeck AS, Denmark. Other; Editor for Elsevier Publishers, Amsterdam, The Netherlands, Editor for Springer Publishers, Stuttgart, Germany, Editor for Gyldendal Publishers, Copenhagen Denmark.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.12

Topic: I.08. Methods to Modulate Neural Activity

Title: Neuronal effects of intracranial direct current stimulation in macaque auditory cortex

Authors: *T. VIGHNESHVEL, M. BROSCH;
Comparative Neurosci. Group, Leibniz Inst. For Neurobio., Magdeburg, Germany

Abstract: In our day-to-day lives, we are constantly inundated with temporally fragmented auditory information which we retain and associate over time to attain a goal-directed behavior e.g. memorizing and noting a person's phone number while it is being recited. The auditory cortex has long been known to be involved in sensory processing but its implication for cognitive processes are not fully explored. To decipher its role in the behavior, external intervention is essential in order to establish a causal-link relationship. Electrical stimulation remains to be the simplest of all reversible activation techniques for causal research in systems neuroscience. Yet it

is a powerful tool to induce a transient effect on neurons, which is crucial to delineate and study the effects on the course of an explicit cognitive function during a complex behavioral task. There has been a tremendous interest in transcranial direct current stimulation i.e. tDCS since its rediscovery a decade ago, which involves localized macrostimulation of a cortical region using low-magnitude currents of either polarity presumably causing shifts in neurons' resting membrane potential. Although it has been shown that transcranial anodal i.e. positive stimulation improves and cathodal i.e. negative stimulation deteriorates the behavioral performance of human subjects on auditory tasks respectively, its neuronal mechanisms have not been thoroughly investigated. Thus, we set out to bridge this connection by assessing the neural effects of intracranial direct current stimulation i.e. iDCS in the auditory cortex of macaque subjects (n = 3). The current study aims to address the following: 1) the quantitative relationship between current intensity and its electrically-evoked neuronal activity; 2) the instantaneous and prolonged stimulation effects on the temporal dynamics of neural activity; 3) the spatial extent of stimulation effects on neural activity; 4) the effects on neural firing rate due to various stimulation types and 5) the effects of different current intensities on sensory encoding in auditory cortex. To this end, we determined the efficacy of iDCS on spontaneous activity and on sensory encoding in auditory neurons with the ultimate goal to manipulate cellular and network-level processes.

Disclosures: T. Vighneshvel: None. **M. Brosch:** None.

Nanosymposium

183. Electrical Stimulation, Magnetogenetics, and Optogenetics

Location: SDCC 5

Time: Sunday, November 13, 2022, 1:00 PM – 4:15 PM

Presentation Number: 183.13

Topic: I.08. Methods to Modulate Neural Activity

Title: Imaging versus electrographic connectivity in human mood-related fronto-temporal networks

Authors: *J. ADKINSON[1], E. TSOLAKI[2], S. SHETH[1], B. METZGER[1], M. ROBINSON[1], D. OSWALT[3], C. MCINTYRE[4], R. MATHURA[1], A. WATERS[5], A. ALLAWALA[6], A. M. NOECKER[8], M. MALEKMOHAMMADI[9], K. CHIU[10], R. MUSTAKOS[9], W. GOODMAN[1], D. A. BORTON[7], N. POURATIAN[11], K. BIJANKI[1];

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Abstract: The efficacy of psychiatric DBS is thought to be driven by the connectivity of stimulation targets with mood-relevant fronto-temporal networks, which are typically evaluated using diffusion-weighted tractography. Leveraging intracranial electrophysiology recordings to better predict the circuit-wide effects of neuromodulation to white matter targets, we hypothesized strong convergence between tractography-predicted structural connectivity and stimulation-induced electrophysiological responses. We utilized detection of evoked potentials elicited by single-pulse stimulation to two common DBS targets for treatment-resistant depression (the subcallosal cingulate (SCC) and ventral capsule/ventral striatum (VCVS)) in two patients undergoing DBS with stereo-electroencephalographic (sEEG) monitoring. These evoked potentials were compared with predicted structural connectivity between DBS leads and sEEG contacts using probabilistic, patient-specific diffusion-weighted tractography. We found that detected evoked potentials and tractography showed strong convergence in both patients in orbito-frontal, ventromedial prefrontal, and lateral prefrontal cortices for both SCC and VCVS stimulation targets, but low convergence was found in anterior cingulate cortex (ACC), where tractography predicted structural connectivity from SCC targets but produced no evoked potentials during SCC stimulation. Further, tractography predicted no connectivity to ACC from VCVS targets, but VCVS stimulation produced robust evoked potentials within the ACC. The two connectivity methods showed significant convergence, but important differences emerged with respect to the ability of tractography to predict electrophysiological connectivity between SCC and VCVS to regions of the mood-related network. This multimodal approach raises intriguing implications for the use of tractography in surgical targeting and provides new data to enhance our understanding of the network-wide effects of neuromodulation.

Nanosymposium

258. Mechanisms and Novel Therapeutic Approaches in Epilepsy

Location: SDCC 33

Time: Monday, November 14, 2022, 8:00 AM – 9:45 AM

Presentation Number: 258.01

Topic: B.08. Epilepsy

Support: R01NS106957

Title: Assessing MRI segmentation methods for the healthy and epileptic human hippocampus

Authors: *H. ZHENG[1], S. I. THOMOPOULOS[1], E. HADDAD[1], C. OWENS-WALTON[1], Y. CHAI[2], M. SHAMAS[3], N. JAHANSHAD[1], M. N. BRASKIE[1], P. M. THOMPSON[1], R. STABA[4];
[1]Imaging Genet. Center, Stevens Neuroimaging & Informatics Inst., Keck Sch. Of Med. Of USC, Marina del Rey, CA; [2]Stevens Neuroimaging & Informatics Inst., Keck Sch. Of Med. Of USC, LA, CA; [3]Neurol., David Geffen Sch. Of Med. At UCLA, LA, CA; [4]Neurol., David Geffen Sch. Of Med. At UCLA, La, CA

Abstract: Hippocampal volume (HV) measures are widely used to assess outcomes of epilepsy surgery. Subtle hippocampal volume changes are associated with aging and with cognitive deficits, and different types of hippocampal sclerosis occur in epilepsy, especially TLE. Here, we compare 3 MRI-based HV segmentation methods: a) Hippodeep (HD), b) FreeSurfer v7.1 gross hippocampal segmentation (Fsvol), and c) FreeSurfer v7.0 "sum of subfields" (Fssum). We hypothesized that Hippodeep, a deep learning model, might fail less frequently and be more sensitive to effects of age, sex, and disease, relative to the other methods.T1-weighted MRI data from 275 participants (159 with epilepsy, 116 controls (CN),151F/124M, mean age of patients: 40.98y (range 20-72 yrs), mean age of CN: 44.96y (range 16-84 yrs), p=0.0069) were analyzed. Epilepsy participants had seizure onset on the left (n=58), right (n=51), or both (n=47) hemispheres; n=3 lacked information. HV were estimated for all participants using Hippodeep, Fsvol and Fssum; all outputs underwent visual quality check (QC). The agreement in HV across methods was evaluated in CN by calculating the Pearson's correlation coefficient in the left (L) and right I hippocampi separately. Dependent samples t-tests were run on L and R HV in the full sample. HV from all three methods were strongly correlated in CN and epilepsy groups. Of the 3 methods, FSvol failed 13/550; FSsum failed 15/550; HD failed 10/550; QC survival rate was higher in HD (Fig. 1A). Comparing L and R HV in CN, HD yielded the lowest mean HV, followed by Fssum and then Fsvol. The mean of L HV was consistently lower than the R HV. We adjusted for ICV, age, and sex effects in CN and patients separately (Fig.1B). L and R HV were significantly lower in patients than CN when using HD (Fig.1B).
Consistent with prior studies, Fsvol and Fssum were sensitive to the known HV changes with age. HD had less QC failure, and greater sensitivity to the hippocampal differences between patients and CN. Detection of age, sex, and disease effects on HV depended on the method used, motivating larger multisite studies.
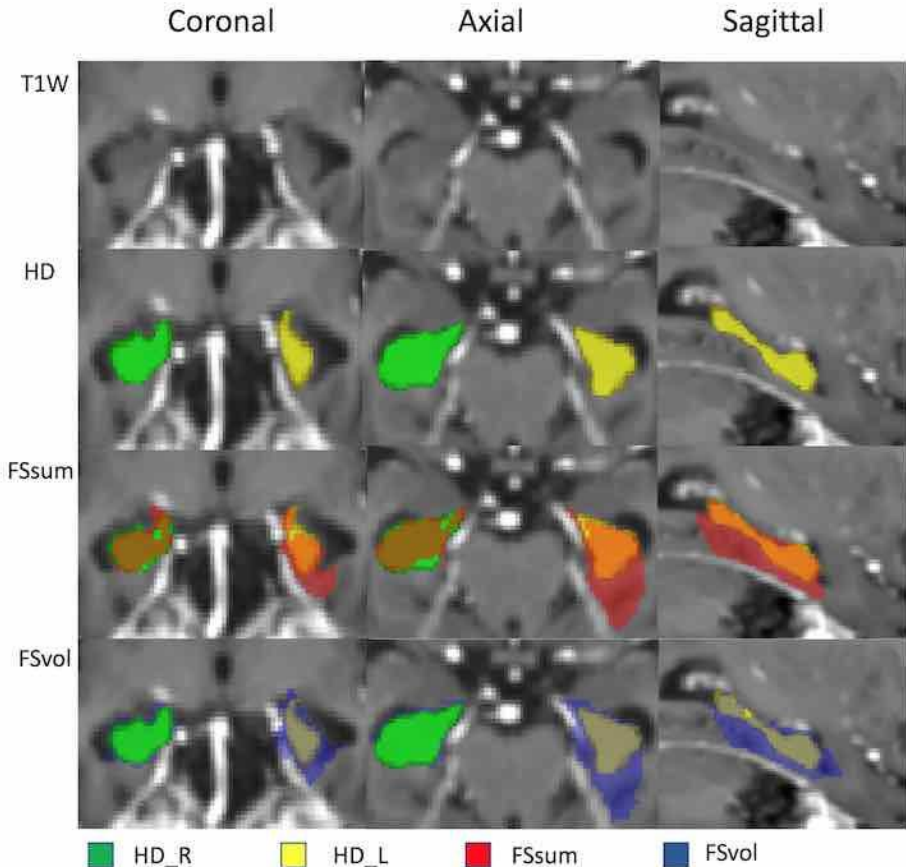
FIGURE 1A: Each segmentation is overlaid on MRI T1WI from an example participant. Each row shows the results of a different automated segmentation method. The left column shows a coronal view of the body of bilateral hippocampi, the middle column shows an axial view of the body of bilateral hippocampi, and the right column shows a sagittal view of the left hippocampus. From the images, the segmentation of left hippocampus is severely overestimated on FSsum and FSvol.

Methods		FSvol (failed 13/550)		FSsum (failed 15/550)		HD (failed 10/550)		AVERAGE (of HV from all 3 methods)	
Subjects		L	R	L	R	L	R	L	R
Control (CN) N=116 Mean age=44.96y Range: 16-84yrs	ICV	0.0033	0.0044	0.0111	0.0027	0.0057	0.0044	0.0001	0.0293
	Age	0.0652	0.0284	0.0370	0.1418	0.2781	0.0808	0.2308	0.0053
	Sex	0.0016	0.0014	0.0003	0.0006	0.1284	0.0355	0.0061	0.0013
	L vs. R	<0.0001		<0.0001		<0.0001		<0.0001	
Patient (Pt) N=159 Mean age=40.98y Range: 20-72yrs	ICV	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
	Age	0.0097	0.0971	0.0621	0.0951	0.0400	0.5444	0.0219	0.2762
	Sex	0.6529	0.9834	0.8805	0.6749	0.4109	0.8523	0.7777	0.6532
	L vs. R	<0.0001		<0.0001		<0.0001		<0.0001	
CN vs. Pt		0.1076	0.2608	0.0572	0.4314	0.0066	0.0380	0.0123	0.2261

Figure 1B: The p values were calculated to compare segmentations from each method in the epilepsy and the CN groups. The p values of the average of the three methods were calculated in the AVERAGE column. The "ICV", "age", and "sex" rows reported the correlation with the left and the right HV using FSvol, FSsum, and HD in the epilepsy and the CN groups separately. The "L vs. R" rows reported the p values of comparing the left HV to the right HV in the epilepsy and the CN groups using the three methods. The "CN vs. Pt" row reported the p values of comparing the left and the right HV of the control group to the epilepsy group using the three methods separately. From the table, we concluded that HV was strongly correlated with ICV for all three methods; detection of age, sex, and disease effects on HV depended on the method used.

Nanosymposium

258. Mechanisms and Novel Therapeutic Approaches in Epilepsy

Location: SDCC 33

Time: Monday, November 14, 2022, 8:00 AM – 9:45 AM

Presentation Number: 258.02

Topic: B.08. Epilepsy

Title: Dynamic analysis of spatial-temporal brain activation linked to inter-ictal discharges in epilepsy patients: Inter-Trial Temporal Synchronisation Analysis

Authors: *P. DHEER[1,2,3], R. GARG[2], D. REUTENS[3];
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Abstract: Introduction: Inter-ictal spikes are thought to occur at random and it may include not only epileptic foci but also networks of distant brain regions. During a single or series of random spikes, a complex sequence of several brain area activations may occur. These sequences of brain activations cannot be explained by standard EEG-FMRI analyses, known as General Linear Model (GLM), which assumes a standard Hemodynamic Response Function (HRF) with predetermined peak timing and shape. The objective of this study is to uncover the spatiotemporal dynamics of Interictal Epileptiform Discharges (IED's) related Blood Oxygenation Level Dependent (BOLD) alterations by temporally and spatially resolving these activations. HRF's exact form and peak timing in epilepsy patients, however, are unknown. Methods: We analysed spatial temporal information using inter-trial temporal 260lzheimer260ation analysis (IT-TSA) and compared it to standard GLM in seven focal epilepsy patients who underwent simultaneous EEG-fMRI resting state acquisition. In contrast to GLM, the Inter Trial Temporal Synchronisation Analysis (IT-TSA) does not assume any canonical HRF for statistical analysis. The null hypothesis is that percent signal changes across trials are normally distributed, with a mean of zero. Synchronization across trials (IED's blocks) is tested by computing a two-sided t-test of percent signal change across trials at each time point 't'. This reveals the spatial temporal sequence of common brain activations during the inter-ictal trial. Results: IT-TSA showed that three of seven individuals had scattered brain areas before the start of trial (spike onset). Later, the seizure focus has appeared and engages other areas of the brain over the course of the trial. It also increased sensitivity and identified additional brain regions not seen in traditional GLM analysis in all patients. Cortical activity revealed extensive symmetric bilateral activations and deactivations across the brain. Interestingly, our study quantitatively confirms that the late negative responses could not have been the undershoot of an earlier positive response. Subcortical activity included thalamus deactivation in four patients, activation in one, and caudate, putamen, and pallidum deactivation in three which is missed by GLM analysis. Conclusion: This study demonstrates that IT-TSA model-free analysis can uncover the large-scale network of brain activations and deactivations spatially and

temporally in epilepsy patients. It has the potential to assist in understanding epileptic networks and interpret fMRI activation maps during treatment.

Disclosures: **P. Dheer:** None. **R. Garg:** None. **D. Reutens:** None.

Nanosymposium

258. Mechanisms and Novel Therapeutic Approaches in Epilepsy

Location: SDCC 33

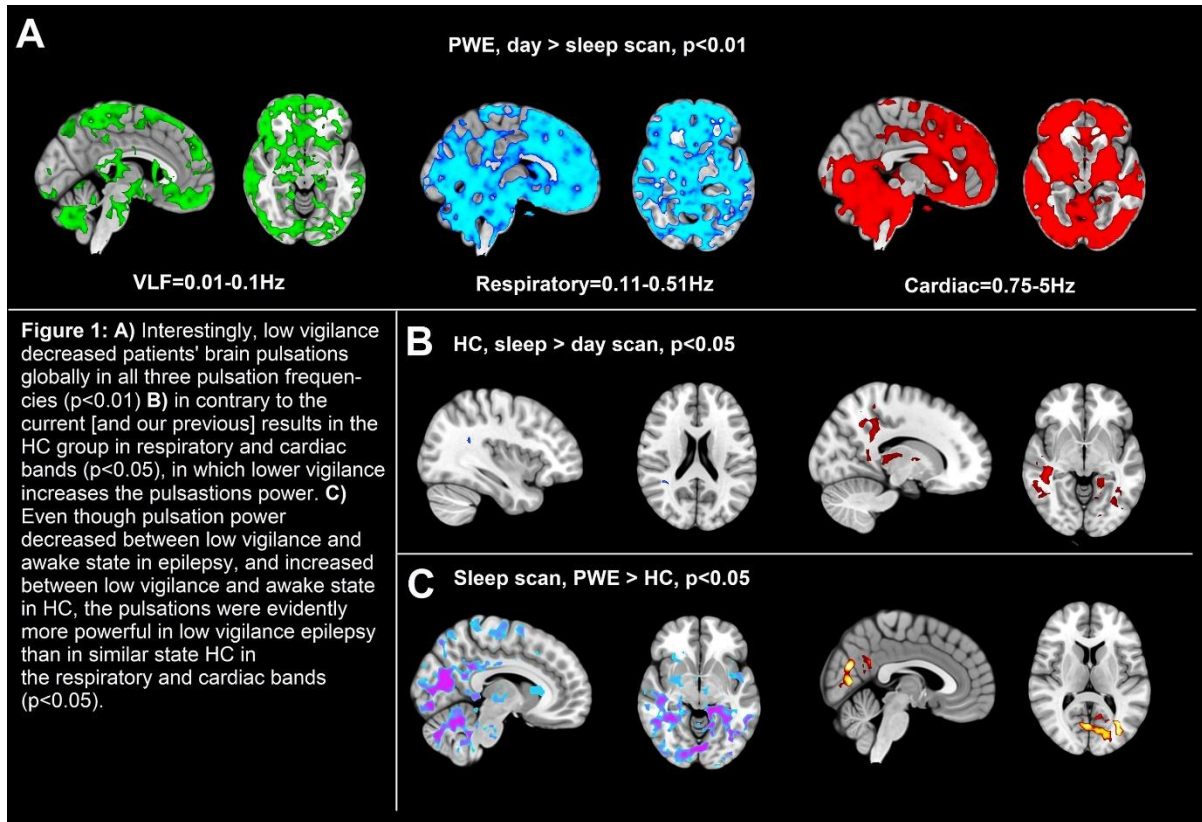**Time:** Monday, November 14, 2022, 8:00 AM – 9:45 AM

Presentation Number: 258.03

Topic: B.08. Epilepsy

Title: Decreased vigilance markedly impacts physiological brain pulsations in focal epilepsy and in contrast with healthy controls

Authors: *J. KANANEN, M. SUHONEN, V. KORHONEN, M. JÄRVELÄ, V. KIVINIEMI;
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Abstract: Sleep is known to enhance glymphatic activity and thus physiological brain pulsations, while in epilepsy sleep lowers seizure threshold. Previously, we have shown how these pulsations are increased in focal epilepsy. Additionally, we have shown how physiological brain pulsations change in low vigilance state and EEG verified sleep in healthy controls (HC). These earlier findings suggest that patients with epilepsy (PWE) may have altered pulsation characteristics between awake and low vigilance states and, furthermore, compared with sleeping HC. Thus, we sought to investigate how decreased vigilance affects brain pulsations in focal epilepsy. Our study group consisted of 12 HC (age:28.6+-5.4, 9 females) and 14 PWE (age:33.9+-14.6, 6 females). Subjects were scanned with 10 min resting-state fast fMRI sequence (TR=100ms) during the day with normal vigilance, and additionally during late evening (10-12pm) with permission to sleep. To maximize the vigilance difference, we used the first 5 min (2861 frames) of the awake scan and the last 5 min of the sleep scan. We calculated the pulsation power in three different frequency bands (c.f. Fig. 1) likewise as in our previous studies. We conducted statistical paired analysis with FSL 261lzheimer between day vs. late evening scans in both groups. Additionally, we performed statistical comparison between patients' and controls' evening scans. As expected, our results inside HC group and between normal vigilance HC and PWE were comparable to our previously published results. However, low vigilance effect was intriguingly in opposing directions inside the groups (Fig. 1). Our new results suggest that the physiological brain pulsations in focal epilepsy, and thus glymphatic clearance, are even more disturbed in epilepsy than previously thought. Additionally, our results give a glimpse into why seizure balance is usually worse during sleep. Thus, we postulate that in epilepsy, the low vigilance state predisposes epilepsy patients to nocturnal seizures as the electrolyte homeostasis governed by glymphatic clearance may be altered.

Figure 1: A) Interestingly, low vigilance decreased patients' brain pulsations globally in all three pulsation frequencies (p<0.01) **B)** in contrary to the current [and our previous] results in the HC group in respiratory and cardiac bands (p<0.05), in which lower vigilance increases the pulsations power. **C)** Even though pulsation power decreased between low vigilance and awake state in epilepsy, and increased between low vigilance and awake state in HC, the pulsations were evidently more powerful in low vigilance epilepsy than in similar state HC in the respiratory and cardiac bands (p<0.05).

Disclosures: J. Kananen: None. **M. Suhonen:** None. **V. Korhonen:** None. **M. Järvelä:** None. **V. Kiviniemi:** None.

Nanosymposium

258. Mechanisms and Novel Therapeutic Approaches in Epilepsy

Location: SDCC 33

Time: Monday, November 14, 2022, 8:00 AM – 9:45 AM

Presentation Number: 258.04

Topic: B.08. Epilepsy

Title: Senescent Cell Ablation Ameliorates Seizure Burden and Spatial Memory Deficits in a Mouse Model of Temporal Lobe Epilepsy

Authors: *T. KHAN, T. CASILLI, M. STECK, J. SEPULVEDA, S. VICINI, P. FORCELLI; Georgetown Univ. Med. Ctr. Interdisciplinary Program In Neurosci., Washington, DC

Abstract: Antiepileptogenic therapies remain limited, even in preclinical models. Thus, identifying targets to prevent the development of epilepsy (i.e., epileptogenesis) is a large unmet

need. In animal models, a common epileptogenic insult is prolonged seizure activity (status epilepticus [SE]) which leads to significant pathological changes. These changes include neuronal apoptosis, DNA damage, oxidative stress, and inflammation are thought to contribute to the emergence of spontaneous recurrent seizures [SRS] in the days-weeks following SE. These resemble the hallmarks of cellular senescence, a conserved program which halts cell proliferation and triggers an inflammatory senescence associated secretory phenotype (SASP) in response to damaging stimuli. Senescent Cells (SCs) canonically upregulate cell-cycle inhibition genes such as p16. Cellular senescence is of growing interest in neurodegeneration. Ablating SCs rescues disease associated impairments. Senescence has not been examined in the context of epilepsy. Methods: We induced SE in mice, and examined the senescence phenotype, which was predominantly in microglia. We examined the microglial motility in live slices of a senescence (p16) and microglia reporter line following SE. In a separate cohort of mice, we ablated SCs immediately following SE and until euthanasia. SC ablated mice and their vehicle-treated (SC remaining) counterparts were monitored for seizure burden with EEG and behavioral analysis. Results: Our data suggest that p16+ SC accumulate in hippocampifollowing epileptogenesis. Interestingly, of the p16+ SCs, approximately 85% colocalize with microglia at all time points. Further, microglial process motility is increased in the SE p16+ microglia compared to SE p16- microglia. There is a significant reduction in seizure burden, and improvement in spatial and context-recognition memory with SC removal. Conclusions: These findings suggest that cellular senescence is induced, predominantly in microglia, following epileptogenesis. Senescent cell ablation reduces seizure burden and improves spatial and context-recognition memory.

Disclosures: T. Khan: None. **T. Casilli:** None. **M. Steck:** None. **J. Sepulveda:** None. **S. Vicini:** None. **P. Forcelli:** None.

Nanosymposium

258. Mechanisms and Novel Therapeutic Approaches in Epilepsy

Location: SDCC 33

Time: Monday, November 14, 2022, 8:00 AM – 9:45 AM

Presentation Number: 258.05

Topic: B.08. Epilepsy

Support: Novo Nordisk Foundation (NNF14CC0001)
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Copenhagen University Hospital, Rigshospitalet
Copenhagen University College

Title: Cacnb3: a lead target in drug-resistant mesial temporal lobe epilepsy?

Authors: *C. KJAER[1], O. PALASCA[2], G. BARZAGHI[3], L. PEDERSEN[1], L. K. BAK[3], R. K. J. DURHUUS[3], E. JAKOBSEN[3], D. WOLDBYE[4], L. H. PINBORG[5], E. D. BARTELS[6], L. J.

JENSEN[2];
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Abstract: Introduction: Identification of differentially expressed genes (DEGs) in mesial temporal lobe epilepsy (mTLE) by transcriptome analysis of hippocampal and temporal lobe neocortical tissue can identify pathophysiological molecular mechanisms leading to mTLE and putative new drug targets. However, such studies need validation in tissue from non-epilepsy subjects to confirm results. Method: Here we reduced a list of 3,040 mTLE significant DEGs to < 120 DEGs using an unbiased bioinformatics approach. A short list (< 20 DEGs) was created using systematic bioinformatics selection criteria, and lead targets among the shortlisted were identified by an overall evaluation including target druggability. Subsequently, lead targets were attempted validated by qPCR and Western blot using mRNA isolated from paired hippocampal and temporal lobe neocortical tissue samples from 17 mTLE patients ((mean age at surgery (MAS): ~ 42 years; sex distribution (SD): 10 female (F) and 7 men (M)) and 16 non-epilepsy subjects (mean age at death (MAD): ~ 39 years; SD: 7 F and 9 M), respectively, and by immunohistochemistry (IHC) using paraffin embedded hippocampal and temporal lobe neocortical tissue from 14 mTLE patients (MAS: ~ 44 years; SD: 8 F and 6 M) and 12 non-epilepsy subjects (MAD: 60,5 years; SD: 6 F and 6 M), respectively. Results: We show that CACNB3 — a voltage gated Ca^{2+} channel subunit with no known link to epilepsy — is significantly regulated in mTLE at both mRNA and protein level. In addition, we suggest a putative role of CACNB3 in seizure generation. Conclusion/Significance: Results from mTLE patients and non-epilepsy subjects support our previous transcriptome finding that *CACNB3* down-regulation in mTLE hippocampus relative to temporal lobe neocortex is caused by disease, although the result is not supported at protein level. However, protein level results consistently find higher CACNB3 expression levels in mTLE, despite comparing to different groups of non-epilepsy subjects. This may imply that increased CACNB3 expression levels in mTLE temporal lobe neocortex is one underlying pathological event in mTLE, indicating that CACNB3 modulation with a drug is likely to have a positive therapeutic effect.

Disclosures: C. Kjaer: None. **O. Palasca:** None. **G. Barzaghi:** None. **L. Pedersen:** None. **L.K. Bak:** None. **R.K.J. Durhuus:** None. **E. Jakobsen:** None. **D. Woldbye:** None. **L.H. Pinborg:** None. **E.D. Bartels:** None. **L.J. Jensen:** None.

Nanosymposium

258. Mechanisms and Novel Therapeutic Approaches in Epilepsy

Location: SDCC 33

Time: Monday, November 14, 2022, 8:00 AM – 9:45 AM

Presentation Number: 258.06

Topic: B.08. Epilepsy

Title: Screening cascade for the evaluation of new anti-epileptic drug candidates

Authors: *E. ESNEAULT, L. H. IVAZZA, M. MARTINEAU, C. M. ROUX;
Porsolt, Le Genest St Isle, France

Abstract: There are a number of anti-epileptic drugs (AEDs) on the market, however, up to 30% of patients are still resistant to these available therapies. Drug-resistant epilepsy mostly concerns mesial temporal lobe epilepsy (mTLE), the most common form of focal epilepsy (80%) and involves structures of the limbic system such as the hippocampus and the amygdala. This reinforces the need for the discovery of new AEDs with improved efficacy and tolerance. Epileptic-like activity can be induced chemically or electrically in various animal models to facilitate the screening of potential new anti-epileptic drug candidates. With this in mind, *ex-vivo* hippocampal slices provide a valuable first line screening tool for new drug candidates allowing higher throughput testing of several compounds, while limiting the number of animals used in testing. At a more advanced stage, acute *in vivo* models provide proof of concept in a more integrated setting by reproducing generalized tonic-clonic seizures, as is seen with the maximal electroshock (MES) test or partial seizure observed in the 6Hz test. While the electrical amygdala kindling test remains the gold standard, as it closely mimics various aspects of mTLE with the induction of partial seizures with secondary generalization. The aim of our experiment was to evaluate the effects of a well-known AED, Retigabine (RET), a Kv7 potassium channel opener, in both *ex vivo* and *in vivo* animal models. For *ex-vivo* experiments, epileptiform activity was induced by the convulsive agent 4-Aminopyridine (4-AP) – a voltage-dependent potassium channel blocker – on mouse hippocampal slices using a multi electrode array (MEA). Bath perfusion with RET at 10 µM did not affect 4-AP- induced epileptiform activity, reflected by isolated spike discharges and bursts, while it fully abolished epileptic-like activity at 100 µM. For *in-vivo* experiments, RET at 50-100 mg/kg, i.p. decreased the number of mice showing tonic convulsions in the MES model but was not effective in the 6 Hz model. In the rat kindling test, RET at 5-10 mg/kg decreased the seizure score and reduced after-discharge duration on the amygdala and cortical signals, recorded by telemetry. These results demonstrate clear anticonvulsant activity of RET in both 4-AP- induced epileptiform activity on mouse hippocampal slices, as well as, in two *in vivo* models. Lower doses of RET can antagonize partial seizures with secondary generalization, as compared with the acute MES model of generalized seizures, RET was devoid of activity in the 6 Hz test. This data confirms the importance of testing a compound across a variety of models to assess anti-convulsant efficacy.

Disclosures: E. Esneault: None. **L.H. Ivazza:** None. **M. Martineau:** None. **C.M. Roux:** None.

Nanosymposium

258. Mechanisms and Novel Therapeutic Approaches in Epilepsy

Location: SDCC 33

Time: Monday, November 14, 2022, 8:00 AM – 9:45 AM

Presentation Number: 258.07

Topic: B.08. Epilepsy

Title: Brite^{Tm}: a cns drug discovery engine integrating human neuronal biology, multi-omics, and artificial intelligence / machine learning for phenotype discovery and therapeutic optimization

Authors: *J. J. FINK, K. HARIKRISHNAN, A. DHANDAPANI, R. H. SAKTHI, C. AMBROSI, V. BARU, J. MEIER, C. L. LEWARCH, V. JOSHI, N. M. DELANEY-BUSCH, S. J. RYAN, G. B. BORJA, L. A. WILLIAMS, O. B. MCMANUS, D. J. GERBER, G. T. DEMPSEY;
Q-State Biosci., Cambridge, MA

Abstract: The human Central Nervous System (CNS) is complex. Synaptic changes occur across billions of neurons connected in complex networks and circuits and these neurons can each have unique electrophysiological profiles. Due in large part to this complexity, existing development methods and information outputs have regularly been proven inadequate to successfully inform decision making in CNS drug development. The full realization of next generation CNS therapeutics will therefore require input of vast, high quality data sets directly linking fundamental human biology with a deeper understanding of phenotypes resulting from pathological perturbations at the level of individual neurons and synapses. To address this need, we have developed BRITE^{TM} (Bioengineered Neuronal Insight-driven Therapeutic Engine), a CNS drug discovery engine that integrates human neuronal disease models, multi-omics measurements and AI/ML-based analytics to generate deep cellular and synaptic insights into CNS disease states for optimizing therapeutic rescue across diverse therapeutic modalities. We have applied BRITE^{TM} to distinct monogenic epilepsies, including Dup15q syndrome and Developmental and Epileptic Encephalopathies (DEE). Using a combination of patient/control induced pluripotent stem cell (iPSC)-derived neuronal lines and CRISPR-edited cell lines, we have used our system to establish unique neuronal phenotypes spanning intrinsic excitability, synaptic transmission, cellular morphology and transcriptomics. Using the identified phenotypes, we are leveraging BRITE^{TM} to optimize ASO therapeutics to directly address the underlying disease pathology. In the case of Dup15q syndrome, we have identified ASOs that knockdown UBE3A, a ubiquitin ligase and key pathogenic driver of the disease. For DEE13, resulting from gain-of-function mutations in SCN8A, we have identified ASOs that selectively knockdown Nav1.6 relative to other Nav channels expressed in the CNS. For DEE4, resulting from haploinsufficiency associated with mutations in STXBP1, a syntaxin binding protein critical to synaptic vesicle release, BRITE^{TM} has identified ASOs that can boost expression of the target gene in neurons. In all cases, we are using our established phenotypes to demonstrate therapeutic rescue in a human neuronal context. Results indicate that our BRITE^{TM} system has generated efficacious, de-risked, and well-tolerated ASOs that engage our disease targets that will move forward into further therapeutic development.

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Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM – 11:30 AM

Presentation Number: 259.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Transcriptomic changes preceding neurodegeneration in a C. elegans model of mixed tau and TDP-43 pathology in Alzheimer's disease

Authors: V. S. JADHAV[1], **R. J. ECK**[1], **S. N. SMUKOWSKI**[1], **L. GARCIA TOSCANO**[1], **C. LATIMER**[1], **B. C. KRAEMER**[2], **P. N. VALDMANIS**[1], ***N. F. LIACHKO**[2];
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Abstract: Alzheimer's disease (AD) is the most common aging-associated neurodegenerative dementia disorder. AD is defined by the presence of amyloid beta (Aβ) and tau aggregates in the brain, but more than 50% of patients also exhibit aggregates of the protein TDP-43 as a secondary pathology. Clinically, AD patients with secondary TDP-43 pathology have more rapid cognitive decline, more severe cognitive impairment, worse brain atrophy, and a shorter disease course. TDP-43 is already implicated in neurodegenerative disease as the major pathological protein aggregate in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD-TDP), two other devastating neurodegenerative diseases. Mutations in the gene coding for TDP-43 cause some cases of familial-inherited ALS, demonstrating that TDP-43 dysfunction is sufficient to cause disease. In patients with mixed Aβ, tau and TDP-43 pathology, TDP-43 dysfunction may synergize with neurodegenerative processes in AD, worsening disease. Using *C. elegans* models of mixed pathology in AD, we have shown that TDP-43 specifically synergizes with tau but not Aβ, resulting in enhanced neuronal dysfunction, worsened neurodegeneration, and increased accumulation of pathological tau. To identify early cellular responses to mixed tau and TDP-43, we have evaluated transcriptomic changes at time-points preceding frank neuronal loss. We demonstrate significant expression and splicing changes in numerous genes including those implicated in immune function, RNA metabolism, synaptic integrity, and lipid catabolism. Characterizing transcriptomic changes resulting from mixed tau and TDP-43 pathology and determining their underlying contributions to disease processes is critical for understanding mixed pathology AD.

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Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM – 11:30 AM

Presentation Number: 259.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Factors regulating tau pathogenesis in seeded models

Authors: *P. CHAKRABARTY[1], T. L. WILLIAMS[2], G. XU[4], B. MOORE[1], Q. VO[2], A. J. RUIZ[1], B. I. GIASSON[5], D. R. BORCHELT[3];
[2]Neurosci., [3]Dept Neurosci, [1]Univ. of Florida, Gainesville, FL; [4]Neuroscience/CTRND, Univ. Florida, Gainesville, FL; [5]Neurosci., Univ. of Florida Dept. of Neurol., Gainesville, FL

Abstract: Alzheimer's disease (AD) is characterized by progressive transmission of aggregated tau and Aβ along neuroanatomically connected brain regions. Our aim is to broadly investigate how tau spreading is regulated in AD. We created a series of bigenic models incorporating the P301S mutant human tau (Line PS19). First, we rederived PS19 mice to express human APOE3 or human APOE4 from the murine Apoe locus, followed by hippocampal seeding with K18-tau aggregates. This allowed us to generate insights into how APOE4, a major AD risk factor, regulates intracerebral propagation of tau. In PS19 mice that were seeded with K18-tau, APOE3 homozygosity resulted in higher phosphorylated tau burden and microgliosis relative to APOE4 (E3>E4). Mice that were heterozygous for APOE3 showed similar results, albeit to a lesser degree. Second, we investigated how Aβ seeds or mixed proteinaceous seeds from human brains would affect tau and Aβ pathology in PS19 mice that co-express human 'swedish' mutant APP (PS19xMHsi). We examined these bigenic mice at 9 months of age when no Aβ deposits were evident while tau pathology was nascently emerging but highly variable. At this age, only 2/7 bigenic mice showed MC1 pre-tangle pathology. Neonatal injection with cored Aβ-enriched Line 107 (APPswe/ind) mouse brain homogenates showed robust induction of Aβ deposits and reproducible presence of MC1-positive tau in all PS19xMHsi mice (n=6). Similar observations were evident in bigenic PS19xMHSi mice injected with secondarily passaged Aβ seeds originally derived from human AD brain (n=6). Gallyas staining was also correlated with the increased MC1 immunostaining in both these seeded cohorts compared to naïve age-matched bigenic mice. In matched siblings that lacked tau transgene (PS19xMHSi), we observed identical acceleration of Aβ deposition but no pathological tau inclusions that were MC1 or Gallyas positive were observed. In conclusion, we demonstrate that accumulation of phosphorylated tau can be accelerated in PS19 mice by the administration of the truncated form of human tau containing the 4 microtubule binding repeats (K18-tau). Presence of APOE3 exacerbated the burden of K18-tau induced emergence of phosphorylated tau, though this did not reproducibly accelerate formation of tau tangles. In the combined presence of tau and APP overexpression, injecting Aβ seeds derived from either transgenic mouse or human origins induced both Aβ deposits and tau tangles. Overall, this shows that accumulation of phosphorylated tau could be

triggered by seed-competent tau while maturation of phosphorylated tau into argentophilic inclusions could be exacerbated through cooperation with APP/Aβ.

Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM – 11:30 AM

Presentation Number: 259.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Superoxide dismutase 1 links redox regulation to nutrient-induced mitochondrial functioning and its dysregulation in 269lzheimer's disease

Authors: *A. NORAMBUENA[1], E. PARDO[2], G. S. BLOOM[3];
[1]Univ. of Virgina, Charlottesville, VA; [3]Dept. of Biol., [2]Univ. of Virginia, Charlottesville, VA

Abstract: Mitochondrial dysfunction, oxidative stress and mTOR dysregulation occur in neurodegenerative disorders like Alzheimer's disease (AD); how these processes mechanistically promote neurodegeneration is poorly understood. We recently discovered 'Nutrient-induced Mitochondrial Activity' (NiMA), an inter-organelle signaling pathway whereby nutrient-mediated stimulation of lysosomal mTORC1 regulates oxidative phosphorylation and DNA synthesis in perikaryal-located mitochondria in neurons (Norambuena, et al. 2018. *EMBO J* 37: e100241.) In a later study, we also reported NiMA being regulated by Tau and sensitive to extracellular amyloid-β oligomers (xcAβOs) (Norambuena, et al. 2022. Neurobiol Dis. Doi: 10.1016/j.nbd.2022.105737.) We now report details of a mechanism by which Tau and mTORC1 function coordinately to regulate mtDNA replication. A lentiviral-mediated shRNA screen targeting 96 mTOR substrates was performed to seek for regulators of the NiMA pathway in iPSC-derived human neurons. Seventeen mTOR substrates were found to regulate NiMA, including SOD1, a major cytosolic redox regulator and key factor in ALS pathogenesis. Mechanistically, we found that Tau upregulates insulin-mediated activity of lysosomal mTORC1, which in turn inhibits SOD1 by phosphorylating it at T40. Pharmacological inhibition of SOD1 in WT mouse neurons inhibited mtDNA synthesis, mimicking the effects of nutrient stimulation in such cells, as well as in Tau knockout mouse neurons expressing hTau. In contrast, xcAβO-mediated inhibition of lysosomal mTORC1 in WT mouse neurons (Norambuena, et al. 2017. *Alzheimers Dement* 13: 152-167) increased SOD1 activity and upregulated mtDNA synthesis. Expressing a constitutively active SOD1-T40E construct in mouse neurons blocked xcAβO upregulation of mtDNA synthesis. These observations suggest that mTORC1-mediated

phosphorylation of SOD1 not only regulates its activity but may also affect SOD1 ability to interact with cytosolic regulators. Proximity-dependent Biotin Identification (BioID) assays followed by mass spectrometry are currently being performed to identify SOD1 phosphoT40-dependent interacting partners. Thus, we are unveiling a fundamental mechanism connecting nutrient sensing, mTORC1 kinase activity and cytosolic redox regulation to perikaryal mitochondrial functioning in mammalian neurons. Also, we provide evidence on how this mechanism may constitute a seminal step in AD pathogenesis. Perhaps agents other than xcAβOs similarly lead to Tau-dependent SOD1 dysregulation in non-Alzheimer's tauopathies, including ALS.

Disclosures: A. Norambuena: None. **E. Pardo:** None. **G.S. Bloom:** None.

Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM – 11:30 AM

Presentation Number: 259.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Synergistic tau pathogenesis due to P301L and S320F mutations is modulated by chaperones

Authors: *M. R. BRYAN, III[1], J. MCGILLION-MOORE[2], T. COHEN[2];
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Abstract: Microtubule-associated tau (*MAPT)* mutations can be causative for dementia. Incorporating two of these mutations, P301L and S320F, is thought to drive synergistic tau pathology resulting in aberrant tau aggregation and cleavage. Here, we sought use this finding to create a new rapid neuronal model that mimics mature Alzheimer's-like pathology, thus facilitating mechanistic discoveries of the factors that drive or suppress tau pathology. Prior to this, it was challenging to recreate tau pathology, which typically requires long timeframes or interventions such as introduction of tau seeds. We can now assess which co-factors are essential for tau aggregation with minimal artificial manipulations. In lentiviral transduced mouse cortical primary neurons, we found that PL-SF elicited conformational (MC1) and aggregated tau species which were not present in neurons transduced with wild-type, PL, or SF tau alone. We also observed increased HSP70, HSC70, and HSP90 expression in PL-SF neurons. We can now generate aggregate-prone tau constructs and evaluate the role of chaperone (HSP) binding using chaperone over-expression and chaperone binding-deficient mutants. Indeed, we overexpressed a panel of chaperones including HSP70, HSC70, and HSP90 and found that HSP90, but not HSP70 or HSC70, exacerbated tau aggregation, suggesting HSP90 specifically plays an integral role in tau aggregation. Lastly, we examined neuronal activity and connectivity by daily multi-

electrode array recordings of spontaneous neuronal activity in lentiviral infected primary neurons. Aggregate-bearing PL-SF neurons displayed changes in neuronal activity, network bursting, and functional synchrony that was consistent with our observation that aggregated tau accumulates at the synapse. Assessing similar parameters of tau species that are chaperone-binding deficient is expected to restore normal network properties. Overall, our new neuronal PL-SF model of progressive tau aggregation and network dysfunction allows us to recapitulate many hallmarks of the progression of human AD pathology on a rapid and reliable timescale, offering numerous benefits including the identification of mechanisms driving tau pathology and therapeutics that alleviate tau pathology.

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Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM – 11:30 AM

Presentation Number: 259.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Big tau isoform resists pathological changes

Authors: D. C. CHUNG[1,3], **J.-P. REVELLI**[1,3], **R. RICHMAN**[1,3], **A. HAN**[1,3,4], **B. TADROS**[1,3], **M. DIAS**[1,3], **H. YALAMANCHILI**[2,3], **H. Y. ZOGHBI**[1,3,5];
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Abstract: In Alzheimer's disease (AD), hyperphosphorylated tau aggregates in selective brain regions such as the cortex and hippocampus. However, the cerebellum remains largely intact even at the most advanced stage of AD. Given this observation of regional brain vulnerability, we questioned whether the various tau isoforms are differentially expressed in the cerebellum and the forebrain. We found that the protein level of the "big tau" isoform is highly elevated in the mouse cerebellum compared to other brain regions using both total tau antibody and our newly developed big tau-specific antibody. Similarly, the transcript level of big tau was also found to be significantly elevated in the mouse cerebellum. Moreover, from the human brain RNA sequencing database analysis, we confirmed that the level of big tau transcript is significantly higher in the human cerebellum than the cortex. These findings were particularly intriguing as previous studies have described big tau mostly in the context of the peripheral nervous system. Of note, big tau has an extremely elongated N-terminal region due to inclusion of exons 4a and 6 of the MAPT gene. Given its unique structure, we speculated that big tau possesses properties distinct from those of other tau isoforms. To assess aggregation propensity

of big tau, we performed detergent fractionation using cells expressing mutant big tau or tau441 (the longest one of six major tau isoforms). This cellular experiment revealed that big tau protein is significantly less likely to aggregate compared to tau441. To further test this in an animal model, we injected neonatal wild-type mice with adeno-associated viruses (AAVs) that express either mutant big tau or tau441. After aging these mice for 6 to 9 months, we examined their brains to analyze pathological changes associated with either of these two tau isoforms. Consistent with results from the cellular model, big tau did not become aggregated or phosphorylated unlike tau441 in the brains of AAV-injected mice at 6 months of age. Big tau remains to be not pathologically altered even after aging these mice for 9 months. Taken together, our data from multiple models suggest that big tau has a significantly lower tendency to undergo pathological changes compared to tau441. The higher level of big tau in the cerebellum may potentially protect this brain region from developing tau pathology in AD.

Disclosures: D.C. Chung: None. **J. Revelli:** None. **R. Richman:** None. **A. Han:** None. **B. Tadros:** None. **M. Dias:** None. **H. Yalamanchili:** None. **H.Y. Zoghbi:** None.

Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM – 11:30 AM

Presentation Number: 259.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Proximity-labelling identifies targets of tau in glutamate receptor trafficking and memory formation

Authors: E. PRIKAS[1], E. PARIC[2], P. R. ASIH[1], K. STEFANOSKA[1], H. STEFEN[2], T. FATH[2], A. POLJAK[3], *A. ITTNER[1];
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Abstract: The microtubule-associated protein tau is central to the development of Alzheimer's disease (AD) and to ~50% of frontotemporal dementias (FTD). However, understanding of molecular tau functions in physiological conditions is quite limited. We used proximity labelling proteomics to gain insight into functional tau interactomes in primary neurons and mouse brain in vivo. Identified tau interactors mapped onto pathways of cytoskeletal, synaptic vesicle, and post-synaptic receptor regulation and showed significant enrichment in proteins linked to Parkinson's, Alzheimer's, and prion diseases. Using colocalization analysis, immunoprecipitation, and microscale thermophoresis with recombinant protein, we confirmed interactions of tau with vesicular proteins and identify a link between tau and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor (AMPAR) trafficking. Tau dose-dependently regulates enzymatic activity of synaptic N-ethylmaleimide sensitive fusion protein (NSF), required for normal AMPAR trafficking. Using chemically induced long-term potentiation, *tau*-deficient (*tau*^{-/-}) neurons undergoing synaptic plasticity showed aberrant localization of this enzyme and of dendritic AMPARs, which was reversed by expression of human tau or NSF inhibition. Further, enhanced AMPAR-mediated associative learning and object recognition memory in *tau*^{-/-} mice was suppressed by both hippocampal tau and infusion of a TAT-fusion peptide inhibitor of NSF. Lastly, both physiologic and pathologic tau - yet not amyloid-β - significantly inhibited the enzymatic activity NSF in AD and FTD mouse model environments, suggesting a direct and independent functional effect of tau. Our results map processive neuronal tau interactomes and delineate a physiologic- and disease-related link between tau and plasticity-associated AMPAR shuttling and memory in task-performing mice.

Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM - 11:30 AM

Presentation Number: 259.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Nsmase2 inhibition reduces tau propagation in alzheimer's disease mouse models

Authors: *C. TALLON[1], X. ZHU[3], B. J. BELL[4], M. MALVANKAR[2], A. G. THOMAS[5], S.-W. YOO[1], A. PAL[1], K. HOLLINGER[1], Y. WU[1], K. COLEMAN[6], A. SHARMA[1], E. EREN[7], R. M. KANNAN[1], D. KAPOGIANNIS[8], N. J. HAUGHEY[9], R. RAIS[1], B. S. SLUSHER[10];
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Abstract: The most common form of dementia worldwide is Alzheimer's disease which is characterized by cognitive decline and progressive neurodegeneration with an accumulation of pathological amyloid-β and hyperphosphorylated tau (pTau). Efforts focused on reducing amyloid-β accumulation have had limited therapeutic benefit, leading to a renewed focus on tau. Misfolded pTau spreads throughout the brain in a stereotypical manner and is able to seed the misfolding of naïve tau in a "prion-like" manner. Recent evidence has identified extracellular vesicles (EVs) as potential carriers of pTau seeds, with several studies demonstrating that genetic knockdown or pharmacological inhibition of nSMase2, an enzyme involved in EV biogenesis, reduces pathology in murine models of AD. Unfortunately, current nSMase2 inhibitors are unsuitable for clinical development given their weak potency and poor physiochemical and pharmacokinetic properties. Given this, we performed a high-throughput screening campaign of >300K compounds followed by extensive chemistry and identified two new nSMase2 inhibitors. The first, PDDC, has nM potency, excellent oral bioavailability and brain penetration. The second, DPTIP, has 10-fold enhanced potency vs PDDC, but has poor oral pharmacokinetics and modest brain penetration. Using brain-targeting hydroxyl-dendrimers conjugated to DPTIP (D-DPTIP) we were able to circumvent this latter limitation and demonstrated D-DPTIP target engagement in the brain following oral dosing. We then administered PDDC and D-DPTIP (100mg/kg oral) to PS19 transgenic mice and our novel rapid tau propagation model where WT mice are unilaterally seeded with an AAV-hTau(P301L) vector stereotaxically injected into the CA1 hippocampal region. After chronic dosing, we quantified the levels of pTau Thr181 in the hippocampus in the PS19 mice and in the contralateral dentate gyrus in the mice with AAV-hTau seeding. PS19 mice treated with PDDC had significantly reduced total and single cell pTau staining, reduced Iba1+ microglial staining, and increased neuronal counts. We also found that PDDC reduced the number and increased the average size of neuron-derived EVs (NEVs) in plasma, and reduced NEV pTau levels compared with vehicle-treated PS19 mice. In the AAV-hTau model, mice treated with either PDDC or D-DPTIP exhibited reduced tau staining intensity in the contralateral dentate gyrus. Importantly, neither compound caused toxicity. Our efficacy data in two AD models using two distinct nSMase2 inhibitors provides strong preclinical support for the use of nSMase2 inhibition as a therapeutic strategy to slow tau propagation and AD disease progression.

Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patent. **B.S. Slusher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patent.

Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM - 11:30 AM

Presentation Number: 259.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Accelerated tau pathology and neuroinflammation in a rhesus monkey model of Alzheimer's disease

Authors: *D. BECKMAN[1], G. B. DINIZ[1], A. BONILLAS[1], S. OTT[1], P. CHAKRABARTY[2], J. H. KORDOWER[3], J. MORRISON[1];
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Abstract: Alzheimer's disease (AD), the most common form of dementia in elderly people, affects around 24 million people worldwide. Our understanding of the pathological events underlying AD have advanced vastly in recent years but successful translation from rodent models into efficient therapies for humans has been extremely limited. We recently described the development of a non-human primate model of AD presenting extensive tau pathology, mainly affecting the hippocampus and connected areas. Here we report the development of extensive neuroinflammatory response in treated monkeys, associated with hippocampal atrophy, detected by MRI, Tau PET imaging, and stereological 3D microscopy. Adult rhesus monkeys were injected with adeno-associated viruses expressing mutant tau (P301L/S320F) in the left entorhinal cortex (ERC) and kept during a 3- or 6-month period to evaluate fluid biomarkers, imaging, and brain microscopy. We detected as soon as 3 months after injections, extensive prion-like tau propagation with full fibrillary tangles in the hippocampus, as well as pre-tangles and phospho-tau in projection areas such as the contralateral ERC and in the retrosplenial and visual cortices. Importantly, neuroinflammation is extensive in the affected areas, with a major role for TREM2+ microglia to drive synaptic and neuronal loss. Finally, we observed that the mutant Tau 4R injected coaptates monkey 3R tau generating more tau aggregation and spreading. These results highlight the first stages of tau pathology and propagation and support the importance of a non-human model of AD, with natural full expression of tau protein, that is highly translational to humans.

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Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM - 11:30 AM

Presentation Number: 259.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH GRANT GR126663

Title: Cytosolic ENC1 increases pathological tau accumulation by inhibiting autophagy flux

Authors: *D. ACOSTA[1], L. LI[1], S. CHEN[1], J. LIANG[1], Y.-K. JUNG[3], G. V. W. JOHNSON[4], J. KURET[2], H. FU[1];
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Abstract: Excitatory neurons are preferentially vulnerable to neurodegeneration and pathological tau accumulation in early Alzheimer's disease (AD). Previous research has identified novel subproteome gene signatures in excitatory neurons, such as ectodermal-neural cortex 1 (ENC1), that may serve as master regulators of selective neuronal and regional vulnerability to tau pathology in early AD. We hypothesize that the interaction between ENC1 and pathological tau may aggravate the propagation of tau pathology, possibly by impairing the autophagy-lysosome pathway (ALP), thereby contributing to the selective neuronal vulnerability to tau pathology in early AD. We aim to further investigate the role of ENC1 in early AD by measuring the protein expression level of ENC1, total tau and p-tau in AD-like tau mouse models and human AD cases at different Braak stages. We further probe the relationship between ENC1 and tau protein by (1) measuring their interaction using the DuoLink proximity ligation assay in human AD and control cases; (2) assessing the effect of ENC1 overexpression and knockdown on DS9 tau seeding activity in SH-SY5Y cells harboring P301S mutant tau; and (3) by using the FUW mCherry-GFP-LC3 lentivirus reporter to visualize free autophagosomes and autolyosomes to test the effect of ENC1 overexpression on autophagy flux. We found that neurons which accumulate pathological tau species in human entorhinal cortex, have decreased nuclear ENC1 levels and increased cytoplasmic ENC1 levels. We identified protein interactions between cytoplasmic ENC1 and tau, which also correlate directly with levels of pathological tau. We also found that overexpression of ENC1 significantly increased DS9 tau seeding activity, while knockdown of ENC1 decreased tau seeding activity in the SH-SY5Y seeding model. Interestingly, overexpression of ENC1 increased the number of autophagosomes and reduced the number of autolysosomes, indicating ENC1 may function to inhibit autophagic flux, thereby disrupting the ALP and promoting the accumulation of tau aggregates.

Disclosures: D. Acosta: None. **L. Li:** None. **S. Chen:** None. **J. Liang:** None. **Y. Jung:** None. **G.V.W. Johnson:** None. **J. Kuret:** None. **H. Fu:** None.

Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM - 11:30 AM

Presentation Number: 259.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Science and Engineering Research Board CRG/2018/004960

Title: Charge Neutralizing Post Translational Modifications as a potential trigger of protein aggregation in Neurodegenerative Disorders

Authors: *S. GUPTA[1], J. D. GADHAVI[1], S. SHAH[1], A. JAIN[2];
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Abstract: Charge-neutralizing post-translational modifications (PTMs) such as acetylation (direct) or phosphorylation (indirect) can significantly alter the physicochemical and structural properties as well as the functionality of a given protein. Naturally, they could also modulate the aggregation propensities of proteins implicated in Neurodegenerative Disorders (NDs). In addition, such proteins (say tau in Alzheimer's and α-synuclein in Parkinson's) are heavily modified in healthy and disease states. As the number of potential proteoforms is enormous, it is challenging to isolate ones responsible for aggregation or simply toxic. Nevertheless, recent studies have demonstrated that proteoforms may indeed differ in their aggregation and seeding behavior. Carbamylation is one such charge-neutralizing non-enzymatic age-dependent PTM that has been demonstrated as a major modifier of aggregation potential. This is due to a very strong intermolecular hydrogen bond formation capacity which is further accentuated in the fibrillar arrangement. Using a series of short tau-derived model peptides, we have demonstrated that tau protein contains at least five hidden aggregation hot-spots far beyond the fibrillar core region, which become activated when carbamylated. These model peptides readily formed amyloid fibrils when carbamylated. Similarly, in α-synuclein KTKEGV, repeat motifs represent such concentration of aggregation hot-spots (4 out of 7) which readily form amyloid fibrils. When aggregated, full-length carbamylated α-syn can act as a seed and trigger aggregation in WT α-syn. Using MD simulation and experiments, we have further demonstrated that carbamylation is a much stronger driver of aggregation than acetylation. We have additionally created a series of mixed peptides libraries with varying modifications. Using modern mass spectrometric, ThT kinetics-based assays, and biophysical characterization, we have further demonstrated that several such modified peptides carry the ability to recruit unmodified peptides while forming aggregates. However, whether such site-specific aggregation modulation behavior could be observed at the scale of full-length protein still needs to be demonstrated.

Disclosures: S. Gupta: None. **J.D. Gadhavi:** None. **S. Shah:** None. **A. Jain:** None.

Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM - 11:30 AM

Presentation Number: 259.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant RF1AG059789-01

Title: Alzheimer-brain derived tau oligomers and sarkosyl-insoluble tau fibrils have similar seeding activities in vivo, but have different microglial activation and spreading properties

Authors: *A. MATE DE GERANDO[1,2], **L. WELIKOVITCH**[1,2], **R. C. PERBET**[1,2], **A. KHASNAVIS**[1], **B. T. HYMAN**[1,2];
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Abstract: Aim: Alzheimer's disease (AD) progression has been associated with the propagation of fibrillar Tau species, but it remains unclear which Tau species are involved. Both soluble Tau oligomers and sarkosyl insoluble fibrils have been implicated in Tau seeding yet their respective contribution to the pathology's dynamics is poorly understood. We now directly compare *in vivo* the spatiotemporal bioactivity of soluble Tau oligomers (hereby referred to as high molecular weight Tau or HMW) and sarkosyl insoluble Tau fibrils (SARK) derived from the same AD brain. **Method:** HMW Tau, prepared from a soluble fraction and isolated over size exclusion column, and SARK Tau were both extracted from the frontal cortex of a Braak VI AD subject. We monitored the bioactivity of these Tau species in 3-month-old hTau mice expressing the 6 human Tau isoforms on a mouse Tau null background and Tau KO littermates which do not express any Tau. Mice were bilaterally injected into the dorsal hippocampus and sacrificed 1 day, 3 days, 1 week or 1 month after injection with one hemisphere kept for histology and the other used for FRET-based seeding assay *in vitro*. **Results**: Histology shows that AD-derived HMW and SARK Tau can both induce AT8-positive Tau pathology starting at 1 month after injection in hTau animals only. While AT8 pathology is similarly triggered in the hippocampus, HMW Tau-injected animals present AT8-positive cells in brain regions distal to the injection site including the somatosensory cortex and the perirhinal cortex. Glial reactivity also differs with the appearance of rod-like microglia solely in the HMW group. Preliminary data of seeding assay on hippocampal homogenates reveals the presence of seed-competent Tau in both HMW- and SARK Tau-injected hTau and Tau KO mice 1 day after injection and its persistence through 3 days post-injection. **Conclusion:** HMW and SARK Tau isolated from the same brain have similar seeding activities, but different spreading properties and trigger differential microglial response *in vivo*. Our data confirm that both fractions contain analogous conformations to promote seeding yet reveal differential cellular response to each of these Tau fractions. Studies of these mechanisms could further unravel the respective contribution of these Tau species to AD pathology.

Disclosures: A. Mate de Gerando: None. **L. Welikovitch:** None. **R.C. Perbet:** None. **A. Khasnavis:** None. **B.T. Hyman:** 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.; Abbvie. F. Consulting Fees (e.g., advisory boards); Dewpoint therapeutics.

Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM - 11:30 AM

Presentation Number: 259.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Longitudinal multiphoton imaging reveals that tau tangle-bearing neurons survive in rTg4510 mice

Authors: T. ZWANG, B. WOOST, B. HYMAN, *R. BENNETT;
Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Neuron loss is a key irreversible event in Alzheimer's disease that drives cognitive decline, but why neurons die is still a mystery. Decades of neuropathology and neuroimaging studies in human disease implicate the aggregation of tau protein into large "tangles" in neurons as an important contributor. Direct evidence that neurons with tangles undergo cell death is lacking, however, challenging our underlying assumptions about disease. Recent advances in cell culture studies show that smaller tau aggregates are rapidly transported within and between cells and that neurons that take up these tau aggregates have a reduced probability of survival. To span the knowledge gap between cell culture systems (which lack tangles), and human neuroimaging studies (that lack the ability to see individual neurons), we employed longitudinal two photon imaging in a mouse model that accumulates progressive tangle pathology and neurodegeneration with age (rTg4510 strain). Tangles were labeled using an intravenous injection of a luminescence-conjugated oligothiophene dye and neurons were tracked over a month of weekly imaging sessions. Neurons without tangles were identified concurrently using an adeno-associated virus expressing cyan fluorescent protein under the synapsin promoter. In total, we identified 820 neurons and 234 tangles in three tau mice at 4-6 months of age. During the period of longitudinal imaging, 91 tangles were observed to form and 64 neurons were lost. Nearly all the neurons that disappeared did not contain tau tangles (61/64). By comparison, 2 out of 285 neurons were lost in a wild-type control mouse. Thus, neuronal loss in the cortex was ten times greater in rTg4510 mice than in a control mouse over a few weeks of observation, and the vast majority of neurons that die (>95%) did not contain a detectable tangle prior to death. Interestingly, tangle-bearing neurons were more likely than chance to form near microvessels. To test this observation using an alternative imaging strategy, we prepared 500-micron thick sections from another group of mice which we cleared and labeled with antibodies to blood vessels (Glut1), neurons (NeuN), and tau (AT8). We identified over 80,000 neurons and 30,000 tau tangles and measured the distances between these features, which confirm that tangle-bearing

neurons are closer to blood vessels (4.50 microns) than the average distance of neurons to blood vessels (5.58 microns). While the cellular mechanisms underlying these observations remain to be explored, these data suggest that tangle formation in neurons favors survival compared to neurons without tangles

Disclosures: T. Zwang: None. **B. Woost:** None. **B. Hyman:** None. **R. Bennett:** None.

Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM - 11:30 AM

Presentation Number: 259.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Depletion of ChromograninA (CgA) Ameliorates Tau Pathology and Neurodegeneration in vivo

Authors: *S. JATI, G. GHOSH, X. CHEN, S. K. MAHATA;
Univ. of California San Diego, San Diego, CA

Abstract: Title: Depletion of ChromograninA (CgA) Ameliorates Tau Pathology and Neurodegeneration in vivo Abstract:Alzheimer's disease (AD) and Frontotemporal dementia (FTD) are the most common neurodegenerative disorders that are primarily characterized by the formation of neurofibrillary tangles, and senile plaques composed of hyperphosphorylated Tau and Aβ42, respectively. CgA is a prohormone known to primarily regulate the neuroendocrine secretion system, vesicle trafficking, insulin sensitivity and inflammation. Although CgA has been implicated in pathogenesis of amyloid β, its role in tau-mediated neurodegeneration has remained elusive. The coexistence of CgA in the senile neurodegenerative plaques intrigued us to undertake both genetic and biochemical approaches to decipher the role of CgA in the progression of AD and FTD.We genetically depleted CgA (CgA$^{-/-}$) in PS19 mice which overexpress human Tau with P301S mutation; hTau$^{+/-}$. We examined the cortexes of CgA$^{+/+}$hTau$^{+/-}$ and CgA$^{-/-}$hTau$^{+/-}$ mice at 6-and 9-month-old male and female for evaluating the change in Tau hyperphosphorylation, neuroinflammation and catecholamine levels. *Ex vivo* organotypic slice culture (OTSC) was used to examine tau fibril (K18/PL) induced seeding and spreading in CgA$^{-/-}$ and CgA$^{+/+}$ hippocampal slices. Ultrastructural evaluation of cholinergic vesicles were made in the CA1 sector of the hippocampus. We also performed behavioral tests (nesting, rotarod, open field, novel object recognition and Morris water maze,) in blinding fashion in a 7-month-old cohort of hTau$^{+/-}$ and non-transgenic (hTau$^{-/-}$) mice with and without CgA deletion.We observed a significant increase of epinephrine both in the plasma and cortex of PS19 mice compared to control. Depletion of CgA substantially reduced Tau hyperphosphorylation in both *in vivo* and *ex vivo* models. Besides, depletion of CgA normalized

epinephrine levels in PS19 (hTau$^{+/-}$) mice. In addition, CgA$^{-/-}$hTau$^{+/-}$ mice showed noticeable improvement of the lifespan and cognitive function.Our results suggest that increased CgA contributes to tau-mediated neurotoxicity in vivo. Depletion of CgA reduces levels of pathological Tau, ameliorate the metabolic phenotype, and improves cognitive function and lifespan. Therefore, CgA might be a therapeutic target against AD and FTD.

Disclosures: S. Jati: None. **G. Ghosh:** None. **X. Chen:** None. **S.K. Mahata:** None.

Nanosymposium

259. Tau Seeding and Pathology *In Vivo* Models

Location: SDCC 1

Time: Monday, November 14, 2022, 8:00 AM - 11:30 AM

Presentation Number: 259.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Grant A2022022F

Title: Master sites of tau phosphorylation as drivers of causative processes in tau-mediated dementia

Authors: *K. STEFANOSKA[1], M. GAJWANI[2], A. R. P. TAN[1], H. AHEI[3], P. R. ASIH[1], A. VOLKERLING[1], A. POLJAK[4], A. ITTNER[1];
[1]Flinders Univ., Bedford Park, Australia; [2]Monash Univ., Melbourne, Australia; [3]Macquarie Univ., Sydney, Australia; [4]Univ. of New South Wales, Sydney, Australia

Abstract: Alzheimer's disease is the most prevalent form of dementia and is characterized by extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs). Hyperphosphorylated tau (= substrate containing multiple moieties) is the main constituent of NFTs and causes neuronal death. Tau is a substrate for several protein kinases, many of which target serine/threonine followed by proline (SP/TP), the most commonly modified phospho-epitope in pathological tau. Despite an association between phosphorylated tau and disease, phosphorylated tau occurs in physiologic states, where site-specific modification can mediate functions other than disease progression. Yet a key question in AD remains - Why does the neuronal tau protein - a central disease factor get progressively hyperphosphorylated? Here, we show that tau phosphorylation at different sites is modulated by complex interdependence - a mechanistic link between an initial site-specific event and subsequent hyperphosphorylation. We define master sites at the centre of this hierarchy, which are essential in shaping modification across the entire tau molecule. These master sites were interrogated for their impact in neurons using CRISPR point mutation and Alzheimer's mice, which revealed site interdependence is an intrinsic physiologic mechanism but may also promote hyperphosphorylation and gain of pathologic function, respectively. Furthermore, we identified the kinases that have the strongest impact at master sites, providing a complex network of phosphorylation events. We show combined targeting of master site and master kinase synergistically ablated tau hyper-phosphorylation. Our work delineates how complex tau phosphorylation arises and provides a

new concept for synergism in post-translational modifications with specific master sites controlling global changes in multi-site substrates. These findings may inform future therapeutic intervention to target specific sub-species of tau.

Disclosures: K. Stefanoska: None. **M. Gajwani:** None. **A.R.P. Tan:** None. **H. AheI:** None. **P.R. Asih:** None. **A. Volkerling:** None. **A. Poljak:** None. **A. Ittner:** None.

Nanosymposium

260. Vision in Action: Sensory Motor Interactions in Gaze Control

Location: SDCC 7

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 260.01

Topic: E.01. Eye Movements

Title: The role of slow eye movements for mechanical stopping of virtual projectiles

Authors: O. SINHA, S. MADARSHAHIAN, M. L. PAINE, *T. SINGH;
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Abstract: During many activities of daily living, humans interact with the environment during motion (e.g., operating machinery, pushing a lawnmower) or interact with moving objects (e.g., recreational sports). The sensorimotor mechanisms of how humans interact with moving objects or the environment during self-motion are not well understood. Defining these mechanisms is important to better understand how the central nervous system processes dynamic sensory information for execution of complex motor skills. Here, we looked at how humans modulate limb force in anticipation of interaction with virtual projectiles that were presented on a KINARM robot. The purpose of this study was to explore the contribution of smooth pursuit eye movements (used to track moving objects) in modulation of limb force prior to impact between the projectile and the arm. We used an experimental paradigm where the participants held a robotic manipulandum and mechanically stopped a virtual projectile moving in the horizontal plane. Participants were asked to apply an impulse that mirrored the projectile momentum to bring it to rest and they received explicit feedback on their performance. Limb kinetics, kinematics and eye movement data were recorded. We varied the momentum of the projectile by either varying the mass or the velocity of the projectile. We measured two gaze variables gaze gain (ratio of gaze velocity/target velocity), and percentage of pursuit (percentage of time the eye was engaged in smooth pursuit) prior to impact between the projectile and the hand. We also measured four limb motor variables - success rate, time of force onset (TOFO), rate of force (RF) increase, peak force (PF) prior to impact. Our analyses show that increasing projectile velocity increased gaze gain (P<0.001) and rate of limb force (P<0.001) but decreased TOFO (P<0.001). In contrast, when the mass of the virtual projectile was increased, we found a higher RF increase (P<0.001) and larger PF, but TLFC and gaze gain did not change. These results suggest that when humans process visually track moving objects, the smooth pursuit eye movements selectively and in a task-specific fashion modulate parameters of limb force control. These

findings have implications for augmenting our understanding of how slow eye movements contribute to limb motor control.

Disclosures: O. Sinha: None. **S. Madarshahian:** None. **M.L. Paine:** None. **T. Singh:** None.

Nanosymposium

260. Vision in Action: Sensory Motor Interactions in Gaze Control

Location: SDCC 7

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 260.02

Topic: E.01. Eye Movements

Title: Spatiotemporal mapping and decoding of oculomotor control in human frontal eye fields

Authors: *S. J. CHANG, M. S. MASHAYEKHI, H. JOSHI, G. REDEKOP, A. SINGHAL, M. TAMBER;
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Abstract: Background: The frontal eye fields (FEFs) are linked to oculomotor control and hypothesized to reside in the prefrontal cortex, where electrical stimulation reportedly evokes contraversive eye movements. The exact location and function of the FEFs in humans is controversial with studies implicating multiple putative regions, including the posterior middle frontal gyrus and the inferior precentral gyrus. Stereo-electroencephalography (SEEG) is a minimally invasive technique used to guide epilepsy surgery. It provides a unique opportunity to collect human neurophysiological data outside of the operating room and has been used by other groups to advance our understanding of specific brain functions.Methods: Four pediatric and four adult subjects undergoing non-lesional epilepsy workup were enrolled into this prospective, IRB-approved study, and received brain MRI prior to SEEG implantation, followed by post-operative CT head for electrode localization. Post-operative stimulation testing and SEEG recordings were performed along with time-aligned video of the subjects' eyes while performing gaze-related tasks.
Results: Stimulation testing elicited contraversive head turning with or without eye deviation, depending on the site of stimulation. Low-threshold sites eliciting these stereotyped movements were located just deep to the inferior precentral gyrus. Stimulation of sites in the posterior middle frontal gyrus did not elicit eye deviation movements in our subjects in the post-operative setting. Analysis of SEEG from electrodes in the inferior precentral gyrus revealed high correlations to eye movements.
Conclusions: Our findings suggest that the human FEFs are located more posteriorly than widely held, involving the motor cortex. Further testing in pediatric and adult subjects is warranted to confirm this hypothesis and test for differences in these populations
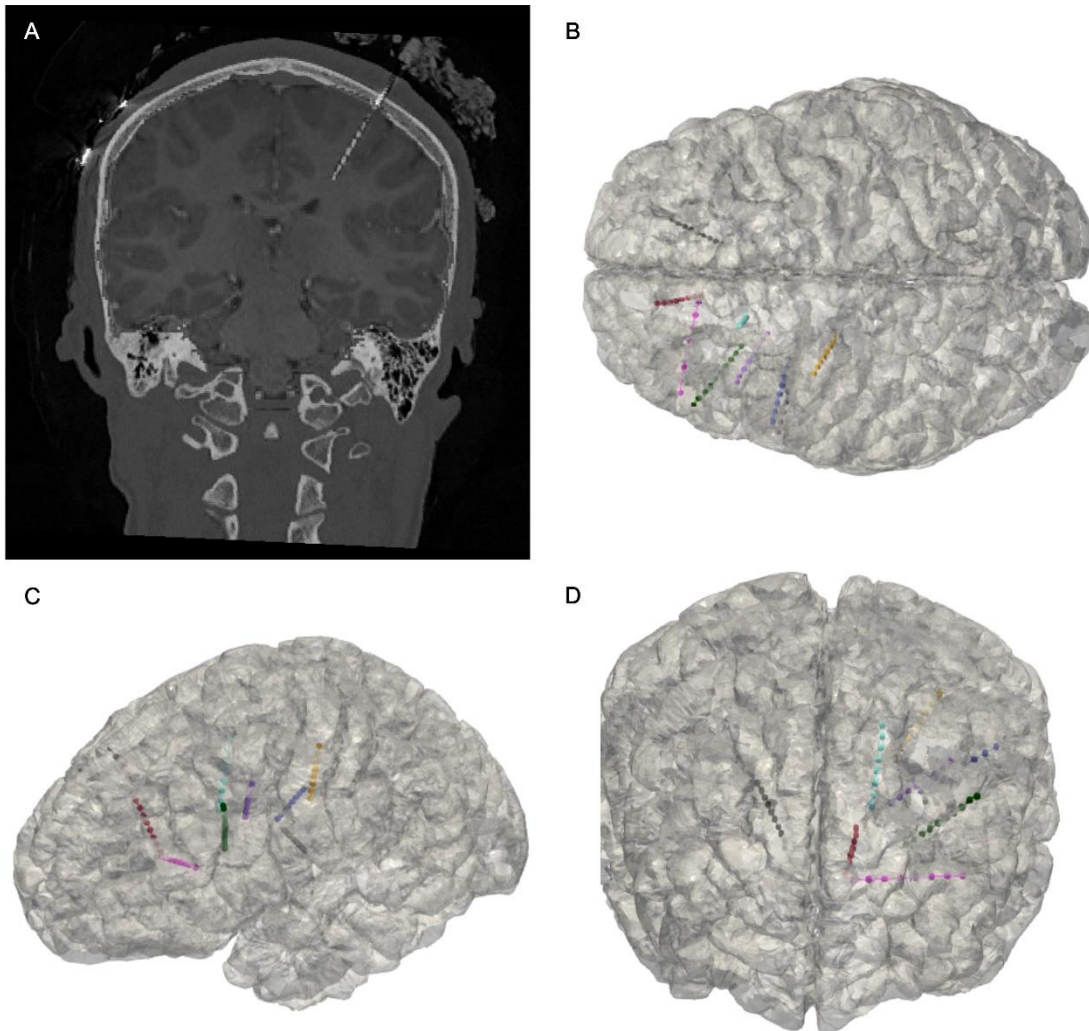
Nanosymposium

260. Vision in Action: Sensory Motor Interactions in Gaze Control

Location: SDCC 7

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 260.03

Topic: E.01. Eye Movements

Title: Eye movement and basal ganglia disruption in a mouse model of progressive supranuclear palsy

Authors: *R. B. CREED[1], A. B. NELSON[2];
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Abstract: Progressive Supranuclear Palsy (PSP) is a neurodegenerative disease that affects movement, behavior, and cognition. Due to an overlap in symptoms with Parkinson's Disease, PSP is considered an atypical parkinsonian disorder, but PSP patients have distinct clinical and pathological features. Clinically, PSP patients have early gait abnormalities, frequent falls, gaze palsy (slowed saccadic eye movements), and tend not to respond to dopamine replacement therapy. Pathologically, aggregated Tau protein (rather than alpha synuclein) accumulates in the brain of PSP patients. As in other neurodegenerative disorders, a combination of cellular dysfunction and cell loss is believed to drive disease symptoms. However, a lack of animal models for PSP has hindered investigation of the causal links between neuropathology, cellular and circuit dysfunction, and symptoms. Here we have utilized the Thy1-hTau.P301S mouse model of tauopathy to determine whether Tau pathology (1) is sufficient to recapitulate key PSP phenotypes in mice and (2) results in aberrant neural activity in motor control nuclei. We find Tau transgenic mice have impaired motor performance in both the open field and accelerating rotarod test. We also find that Tau transgenic mice have tau pathology in several basal ganglia nuclei. The basal ganglia are a group of subcortical nuclei involved in motor and cognitive control, which were recently identified as an initial site of Tau pathology in PSP patients. Lastly, using head-fixed measurements of eye movements, we find tau transgenic mice have decreased spontaneous and evoked saccade velocity. Overall these findings highlight the utility of tau transgenic mice to modelling PSP and provides a platform to investigate the changes in neural structure and function that drive the movement abnormalities seen in disease.

Disclosures: R.B. Creed: None. **A.B. Nelson:** None.

Nanosymposium

260. Vision in Action: Sensory Motor Interactions in Gaze Control

Location: SDCC 7

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 260.04

Topic: E.01. Eye Movements

Title: Comparison of spatial direction encoding properties of spiking and local field potential signals in the superior colliculus during a saccade task

Authors: *M. R. HEUSSER, C. BOURRELLY, N. J. GANDHI;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The superior colliculus (SC) is an oculomotor structure in the midbrain known to be involved in the integration of sensory information into a goal-directed eye movement. Neurons across its dorsoventral axis exhibit transient bursts of activity following the appearance of a visual target and/or preceding a rapid eye movement to that target, and many exhibit sustained activity during the intermediate delay period. The discriminability of spatial information encoded by neurons contained within a laminar column along this dorsoventral axis is thought to be limited to a narrow range of directions and amplitudes. We sought to challenge this notion by characterizing the time course of target direction information present in the spiking activity of SC neural populations and compared the neurons' encoding properties with a second, often overlooked signal modality, the local field potential (LFP). We recorded activity across many channels in a laminar column of the SC while rhesus monkeys (*Macaca mulatta*) performed a delayed saccade task to one of eight equidistant targets, a task which temporally separates the three main epochs of sensorimotor integration - visual, delay, and motor. To obtain a singular measure of the amount of spatial information encoded across all channels, we employed a separate offline classification algorithm for each sliding time window and signal modality. For both spiking and LFP activity, decoding performance was highest during the visual and motor epochs. During the delay period, spiking activity exhibited spatial discrimination properties akin to those observed in the visual epoch, while target direction was represented more broadly during the motor epoch. For LFP activity, direction encoding properties were similar to spiking activity profiles during the visual and motor epochs. Spatial information in the delay period was largely absent in LFP signals, often confined to the target in the preferred direction. Overall, this study demonstrates that the encoding of direction information across layers of the SC is broad in discriminability and dynamic in nature.

Disclosures: M.R. Heusser: None. **C. Bourrelly:** None. **N.J. Gandhi:** None.

Nanosymposium

260. Vision in Action: Sensory Motor Interactions in Gaze Control

Location: SDCC 7

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 260.05

Topic: E.01. Eye Movements

Support: Grant # MOP-130444

Title: Prefrontal neural activity predicts future gaze errors in a cue-conflict task

Authors: *V. BHARMAURIA[1], A. SCHÜTZ[2], P. A. KHOOZANI[1], X. YAN[1], H. WANG[1], F. BREMMER[2], J. D. CRAWFORD[1];
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Abstract: Prediction in the brain plays a vital role in perception, cognition, decisions, and motor plans. To predict future events, the brain must integrate past and current information for optimal plans. How does the brain plan movements in the presence of unstable cues? To this goal (using eye as a model), we recorded neural activity in the monkey frontal cortex in a quasi-predictable cue-conflict task. Animals were trained to remember visual targets (T) (and saccade head-unrestrained in future) relative to a landmark (L) that was subtly shifted (L') with a fixed amplitude but randomly in a circular fashion around original L. These landmark shifts caused a shift in the final post-saccadic gaze position. In our previous studies (Bharmauria et al. 2020, 2021), we fit spatial models along a continuum from target (T)-to-future gaze (G) coding (in eye coordinates) to frontal (FEF) and supplementary (SEF) eye field activity and found a reactive shift in gaze coding in response to the landmark shift. Here, we analyzed 147/68 spatially tuned FEF/SEF neurons during the period before the landmark shift. At the behavioral level, we found that the final gaze endpoint relative to the target can be described as a linear combination of a target-position specific under/overshoot and a deviation parallel to the landmark shift. Based on these statistics, it should be possible to predict some components of the resulting gaze errors. To test this, we examined early (pre-landmark shift) activity in this task using the same continuum (T-G) approach as above. We found a significant transient predictive shift in frontal cortex activity (especially SEF) toward coding the future shifted gaze position. This suggests that the monkey brain had learned to estimate variable future gaze errors in this task, and that this is manifested as a predictive shift in the SEF neural activity. Such learned error estimators might allow the brain to optimize behavior and mitigate spatial uncertainty.References:Bharmauria, V. et al. Integration of eye-centered and landmark-centered codes in frontal eye field gaze responses. Cerebral Cortex bhaa090, https://doi.org/10.1093/cercor/bhaa090 (2020).Bharmauria, V., Sajad, A., Yan, X., Wang, H. & Crawford, J. D. Spatiotemporal Coding in the Macaque Supplementary Eye Fields: Landmark Influence in the Target-to-Gaze Transformation. eNeuro 8, (2021).

Disclosures: V. Bharmauria: None. **A. Schütz:** None. **P.A. Khoozani:** None. **X. Yan:** None. **H. Wang:** None. **F. Bremmer:** None. **J.D. Crawford:** None.

Nanosymposium

260. Vision in Action: Sensory Motor Interactions in Gaze Control

Location: SDCC 7

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 260.06

Topic: E.01. Eye Movements

Title: Sensory tuning in the neuronal eye-movement commands of the primate superior colliculus

Authors: *M. P. BAUMANN, A. F. DENNINGER, A. R. BOGADHI, Z. M. HAFED;
Univ. of Tübingen, Tuebingen, Germany

Abstract: In primates as in other animals, movement control is critical for successful interaction with the environment. However, movement does not occur in complete isolation of sensation, and this is particularly true in the case of eye movements and vision. Here, the superior colliculus (SC) plays a fundamental role, issuing saccade motor commands in the form of strong peri-movement bursts that are very widely believed to specify both saccade metrics (direction and amplitude) and kinematics (speed). However, a great majority of models of saccade control by the SC rely on observations with small light spots as the saccade targets. Instead, we asked rhesus macaque monkeys to "look" at different images, akin to natural behavior, and we recorded SC saccade-related motor bursts. We tested, as the saccade targets, gratings of different contrasts, spatial frequencies, and orientations; images of animate and inanimate objects; and black versus white stimuli. Despite matched saccade properties across trials within a given image manipulation, the SC motor bursts were strongly different for different images; that is, they were sensory-tuned. Such sensory tuning in the SC movement commands was even sharper than that in passive visual responses: the difference in movement burst strength between the most and least preferred image features (for the same saccade vector) was larger than that in visual bursts at stimulus onset, consistent with known pre-saccadic enhancement of perception. Most intriguingly, even neurons classified as being more motor than visual exhibited strong sensory tuning in their saccade-related bursts, and local field potentials around our recording electrodes reflected the different sensory features present at the time of saccade triggering. Since SC motor bursts are relayed virtually unchanged to the cortex (Sommer & Wurtz, 2004), one implication of our results is that the visual system is primed not only about the sizes and directions of upcoming saccades, as is traditionally believed, but also about the movement targets' visual sensory properties. Consistent with this, we additionally found that saccade-target visual features significantly modulate two highly classic peri-saccadic perceptual phenomena in humans: suppression and mislocalization. Both phenomena are believed to depend, at least in part, on corollary discharge associated with saccade-related neuronal movement commands. Our results provide novel insights about the functional role of SC motor commands, and they motivate extending theoretical accounts of corollary discharge beyond just spatial movement-related reference frames.

Disclosures: M.P. Baumann: None. **A.F. Denninger:** None. **A.R. Bogadhi:** None. **Z.M. Hafed:** None.

Nanosymposium

260. Vision in Action: Sensory Motor Interactions in Gaze Control

Location: SDCC 7

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 260.07

Topic: E.01. Eye Movements

Title: Neural dynamics of eye-movement generation in the primate prefrontal cortex

Authors: *C. LIBEDINSKY, R. HERIKSTAD;
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Abstract: The transition from planning to executing a movement involves the reorganization of cortical activity, such that information encoded in a movement preparation subspace transitions to information encoded in a near-orthogonal motor execution subspace (Kaufman et al. 2014, Elsayed et al. 2016, Economo et al. 2018, Inagaki et al. 2022). The near-orthogonality of these subspaces allows the preparation of movements without their execution. When movements are triggered by a sensory go-cue, a quick non-selective response in motor areas (or condition-invariant signal) mediates this reorganization of cortical activity (Kaufman et al. 2016, Guo et al. 2014, Inagaki et al. 2022). To identify an information subspace it is important to determine the precise time window in which information is encoded within that space. If the time window is too large, there is a risk of conflating different subspaces and different types of information. If the window is too small, there is a risk of missing relevant information. Previous studies have identified the different activity subspaces using relatively large time windows (>200ms), defined a-priori by the researchers conducting the studies. We hypothesized that a data-driven approach to determine window sizes may reveal novel activity subspaces, especially in periods with quickly-evolving neural dynamics, such as those observed prior to movement execution.
We analyzed the activity of neurons in area 8a, which includes the frontal eye field (FEF), a prefrontal region involved in the control of eye movements. Using the data-driven approach to determine window sizes, we found that the "movement execution subspace" was in fact a mixture of multiple different subspaces. These include a "movement-onset-invariant subspace" that allows the precise prediction of eye movement timing, which was followed by a "movement-specific subspace". We show that these identified subspaces are specific to the FEF, since they could not be identified in the more anterior area 9/46. A dynamical system model integrates these results into a coherent framework to understand the mechanisms of eye-movement initiation.

Disclosures: C. Libedinsky: None. **R. Herikstad:** None.

Nanosymposium

260. Vision in Action: Sensory Motor Interactions in Gaze Control

Location: SDCC 7

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 260.08

Topic: E.01. Eye Movements

Title: Cortical correlates of transsaccadic object orientation vs. shape change discrimination: an fMRI study

Authors: *B. R. BALTARETU[1,3], W. D. STEVENS[2], E. FREUD[2], J. D. CRAWFORD[2];
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Abstract: Previously, Dunkley et al. (2016) showed transsaccadic perception effects in parietal cortex (i.e., supramarginal gyrus, SMG) for object orientation, whereas Baltaretu et al. (2021) implicated occipital cortex (cuneus) in transsaccadic spatial frequency perception. Based on this, we hypothesized a fundamental difference in transsaccadic detection of object orientation vs. identity. To test this, we used a double-dissociation fMRI task to examine transsaccadic discrimination of object orientation vs. shape change and examined the functional networks involved. 21 participants fixated a cross ±15.4° of centre, where an object was subsequently presented (rectangle, barrel, or hourglass) oriented at ±45° from vertical. The fixation cross remained in the same position (Fixation condition) or shifted (Saccade condition), followed by the same object re-presented at the orthogonal orientation (Orientation change) or another object at the initial orientation (Shape change). Change in object orientation or shape in each trial was indicated via button press. Given the suspected involvement of SMG and cuneus in processing these feature changes across saccades, we conducted a region-of-interest analysis on these two sites. Consistent with our hypotheses, right SMG showed a significant interaction of eye movement and feature change. Moreover, cuneus showed a main effect of eye movement and a main effect of feature change. These results implicate posterior parietal cortex and medial occipital cortex in the transsaccadic discrimination of multiple object features.

Disclosures: B.R. Baltaretu: None. **W.D. Stevens:** None. **E. Freud:** None. **J.D. Crawford:** None.

Nanosymposium

261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.01

Topic: E.05. Brain-Machine Interface

Title: The role of stimulus periodicity in spinal cord stimulation-induced artificial sensations in rodents

Authors: J. SLACK[1,2,3], S. ZEISER[1,2], A. BRUNTON[1,2], *A. P. YADAV[1,2,3];
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Abstract: Introduction: Artificial sensory feedback is crucial for optimal control of brain-machine interfaces and neuroprosthetic devices. Previously, we demonstrated that epidural stimulation of the dorsal columns of the spinal cord, popularly known as spinal cord stimulation (SCS), can be used to generate artificial sensory perceptions in rodents and non-human primates. We observed that, in animals, sensory perceptual thresholds decreased as the frequency of stimulation increased. Although most traditional pulse trains are periodic in nature, naturalistic pattern of neuronal activity is often aperiodic. To mimic naturalistic sensations, it is crucial that aperiodic patterns of stimulation and their impact on sensory detection and discrimination be investigated. Here, we plan to test whether there is any relation between the level of periodicity of SCS pulse-train, perceptual thresholds, and stimulation frequency.
Methods: Rats were implanted with bipolar stimulation electrodes at the T3 spinal level on the dorsal surface of the spinal cord. They were trained to choose between two reward ports in a two-alternative forced-choice task as part of a SCS-induced sensory detection and discrimination training paradigm. Aperiodic stimulation trains were generated based on a gamma distribution modulated by the coefficient of variation (CV) of the inter-pulse interval. Rats were initially trained to detect sensations induced by a stimulation pulse train of 0.8 CV. Thereafter, the aperiodicity of the pulse train was randomly varied between 0 and 1 within a session to determine perceptual detection thresholds as a function of CV. Experiments were repeated at multiple frequencies (5Hz - 200Hz).
Results: We observed that sensory perception detection thresholds decreased with increasing CV at multiple stimulation frequencies (5Hz - 200Hz). Moreover, detection thresholds decreased with increasing frequency at all tested values of CV (0 to 1). Across multiple rats (n=4) and across all CV values, the variance of average detection thresholds was lower at higher frequencies.
Conclusions: Our results suggest that as the randomness i.e. aperiodicity of the pulse train increases, rats can detect SCS-induced sensations at a lower stimulus amplitude as compared to a perfectly periodic pulse train. In addition to that, the impact of aperiodicity on detection thresholds is greater at lower frequencies than at higher frequencies. Overall, periodicity of the SCS pulse train is an important parameter which can be modulated to generate naturalistic stimulation patterns. Further exploration of this crucial parameter is necessary to provide optimal artificial sensory feedback.

Disclosures: J. Slack: None. **S. Zeiser:** None. **A. Brunton:** None. **A.P. Yadav:** None.

Nanosymposium

261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.02

Topic: E.05. Brain-Machine Interface

Title: Sensory stimulation strategies for BCI-FES neuroprostheses to improve grasping function after paralysis

Authors: *E. L. GRACZYK[1,3], B. C. HUTCHISON[1,3], P. BHAT[1,3], K. E. ALFARO[1,3], W. D. MEMBERG[1,3], R. F. KIRSCH[1,3], J. P. MILLER[2,3,4], B. AJIBOYE[1,3];
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Abstract: Regaining reaching and grasping is the top functional priority for persons with tetraplegia. Our team has developed a neuroprosthetic system called Reconnecting the Hand and Arm to the Brain (ReHAB), which combines brain computer interfaces (BCI) with functional electrical stimulation (FES), to restore movement and sensory function to people with high tetraplegia. Restoring touch to users of rehabilitative systems is critical because touch helps establish emotional connection in social interactions and plays a key role in dexterous object manipulation, which is required for activities of daily living. While prior research has explored how intracortical microstimulation (ICMS) can improve the function of BCI-controlled robotic limbs, it is unclear how to optimally restore sensation for users of BCI-FES systems, in which the participant moves their own paralyzed hand and arm to complete tasks. A participant with C4 sensory-incomplete spinal cord injury (AIS B) enrolled in the ReHAB clinical trial and was implanted with six microelectrode arrays in the sensorimotor cortical network and nine multi-contact nerve cuff electrodes in the contralateral arm. In this study, we characterized and compared several strategies of sensory restoration for BCI-FES users, including ICMS, peripheral nerve stimulation (PNS), and residual natural touch. ICMS delivered to electrodes in primary somatosensory cortex (S1) elicited the sensation of pressure on the tips of the index, middle, and ring fingers. PNS delivered to the median and ulnar nerves evoked sensation on the fingers, hand, and forearm, and the perceived intensity was reliably modulated across a broad dynamic range. Both PNS- and ICMS-evoked sensations remained stable in location for over 18 months. Touch applied to the participant's hand evoked detectable and discriminable percepts, as well as cortical responses in S1 and the inferior frontal gyrus (IFG) that modulated to the magnitude and rate of onset of the stimulus. Cortical responses in S1 also modulated to the pulse width of PNS applied to the nerves. Future work will implement each of these sensory feedback strategies into closed-loop task performance with the BCI-FES system to determine functional impacts. Additional study is needed to improve the resolution and duration of ICMS-evoked percepts and develop approaches to optimally integrate sensory PNS with motor FES. Future work will also examine the benefits of spatiotemporal patterns of ICMS and PNS. By restoring touch sensation to people with high tetraplegia, we will enhance upper extremity function and improve psychosocial experience, leading to higher degrees of independence and quality of life.

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261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.03

Topic: E.05. Brain-Machine Interface

Support: NS107714

Title: Touch sensations evoked by microstimulation of human somatosensory cortex depend on recent stimulation history

Authors: *C. GREENSPON[1], T. CALLIER[1], C. VERBAARSCHOT[3], N. SHELCHKOVA[1], A. R. SOBINOV[1], P. JORDAN[6], E. FITZGERALD[1], N. C. BOLES[1], D. SATZER[1], P. WARNKE[1], M. L. BONINGER[4], J. L. COLLINGER[4], R. A. GAUNT[5], J. E. DOWNEY[2], N. G. HATSOPOULOS[1], S. J. BENSMAIA[2];
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Abstract: Intracortical microstimulation (ICMS) of somatosensory cortex evokes touch sensations described as natural or nearly so. This phenomenon can be exploited to restore the sense of touch to individuals who are living with spinal cord or peripheral nerve injuries resulting in loss of sensation. For this strategy to be viable, however, the sensations must not only be informative, they also need to be stable. To test the stability of ICMS-evoked sensations, we carried out a series of psychophysical experiments with three human participants who have upper limb paralysis and who have arrays of microelectrodes implanted in somatosensory cortex. The participants judged which of two consecutively presented pulse trains evoked the stronger sensation. We then investigated the degree to which the participants judgments differed depending on the order in which the two stimuli in each pair were presented. To the extent that presentation order affected performance, we inferred the evoked sensations might depend on recent stimulation history. Consistent with this, we observed a strong and systematic effect of presentation order over the majority of electrodes tested: On most (but not all) stimulating electrodes, the participants were more likely to judge the second stimulus as more intense. The effect was stronger when the first stimulus was more intense and disappeared when the interstimulus interval was long (5 sec). These effects were not consistent with a simple response bias because (1) they varied across electrodes for each participant and (2) they were observed in all three participants. We then tested the hypothesis that perception was altered by having participants perform a magnitude estimation task in which they judge the perceived magnitude of a test stimulus presented shortly after a conditioning stimulus. We found that perceived

magnitude was modulated by the conditioning stimulus when both conditioning and test stimuli were presented on the same electrode, and not otherwise. The strength and sign of the modulation effect on a given electrode was consistent with the participants' performance on amplitude discrimination task on that electrode - eg. electrodes where subjects reported the second stimulus as being more intense also reported higher magnitudes. We demonstrated that this conditioning effect has behavioral implications by showing that participants judgments of force based on ICMS exhibit strong order effects. Finally, we show that biomimetic sensory feedback reducing this perceptual instability by minimizing the incidence of long, strong ICMS trains, which are necessary to trigger the perceptual instability of ICMS-evoked sensations.

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Nanosymposium

261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.04

Topic: E.05. Brain-Machine Interface

Title: A participant-driven approach to designing artificial tactile sensations using intracortical microstimulation of the human somatosensory cortex

Authors: *C. VERBAARSCHOT[1,4], V. KARAPETYAN[1,2], C. M. GREENSPON[5], M. BONINGER[1,2,3], B. SORGER[4], R. A. GAUNT[1,2,3];
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Abstract: Tactile feedback is a key element of effortless motor control and seems to be a prerequisite for achieving such abilities in people with prosthetic limbs. Intracortical microstimulation (ICMS) of the somatosensory cortex is one approach to provide such feedback, and an attractive option in individuals with spinal cord injury. Despite progress on understanding the relationship between stimulation parameters and perception, the vast number of potential parameters limits systematic searches, leaving the experimenter to determine which parameters are tested and how to encode the mechanical properties of objects in trains of ICMS pulses. In this study, we put the participant in control of their own stimulation parameters and tested (1)

whether ICMS can convey useful object characteristics (e.g. compliance, microstructure), and (2) whether selected ICMS parameters can evoke distinct object-specific sensations.

Three individuals with tetraplegia had microelectrode arrays implanted in their somatosensory cortex, which evoked sensations on their right hand. Participants used their left hand to interact with a tablet interface that presented one of five object images and generated multi-electrode ICMS trains when touched. Participants could control four stimulation parameters in a blinded fashion: amplitude, pulse frequency, stimulus onset/offset transients, and the degree of stimulus overlap between consecutive electrodes. We asked participants to select ICMS parameters that best represented each object over several sessions. To assess what the created sensations felt like and determine how distinguishable they were, ICMS trains were replayed in the absence of visual context, and participants were asked to (1) describe their tactile characteristics using a survey and (2) select the object that best matched this sensation.

All participants described vivid object characteristics of compliance, texture, structure, and temperature when ICMS was paired with vision. Without visual context, similar object characteristics were still reported, but with increased variance within objects. Two participants could assign the correct object to a replayed ICMS train with about 34% accuracy (chance: 20%), and peak performances of 45-65% on individual sessions. Moreover, a linear classifier trained on participant specific ICMS parameters selections, could distinguish objects significantly above chance (31% accuracy), suggesting that there is at least some consistency in parameter selections across sessions. These results suggest that it is possible to convey object characteristics via ICMS, which in combination with visual input, can feel quite natural.

Disclosures: C. Verbaarschot: None. **V. Karapetyan:** None. **C.M. Greenspon:** None. **M. Boninger:** None. **B. Sorger:** None. **R.A. Gaunt:** F. Consulting Fees (e.g., advisory boards); consultant for Blackrock Microsystems, Salt Lake City, on the scientific advisory board of Braingrade Gmbh, on the scientific advisory board of Neurowired LLC.

Nanosymposium

261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.05

Topic: E.05. Brain-Machine Interface

Support: DARPA Award HR001120C0120

Title: Motor and somatosensory cortex evoked responses from intracortical microstimulation of human somatosensory cortex

Authors: *L. OSBORN[1], B. CHRISTIE[2], D. P. MCMULLEN[3], A. S. PAWAR[4], R. W. NICKL[5], B. A. WESTER[7], P. A. CELNIK[6], M. S. FIFER[9], F. TENORE[8];
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Abstract: Evoked neural activity can be used to characterize cortical networks. Prior work showed evoked responses in the motor cortex as a result of ipsilateral intracortical microstimulation (ICMS) to the somatosensory cortex (Osborn et al, 2020). In this study, a human participant with a spinal cord injury (C5/C6 ASIA Impairment Scale Grade B) had microelectrode arrays implanted in the motor and somatosensory cortices of both hemispheres of the brain. We characterized the cortical responses evoked by ICMS delivered to somatosensory cortex. When delivering biphasic ICMS pulses at 100 Hz to an electrode in the left somatosensory cortex, we elicited a tactile sensation in the right ring fingertip. We systematically varied the number of pulses and measured the neural activity. We observed an evoked response with a positive peak amplitude ~20 ms after stimulation onset in two electrodes in a separate array across the central sulcus in the ipsilateral motor cortex, similar to previous observations. The amplitude of the evoked response increased with 4 ICMS pulses compared to 1 pulse. For 8 and 12 pulses only, though the ICMS noise artifact dominated the first 50 ms of the signal, a negative evoked response emerged ~80 ms after stimulation onset. We did not observe evoked neural responses in the contralateral hemisphere. The participant could perceive trials with 8 and 12 pulses, but not trials with 1 and 4 ICMS pulses. We also delivered 12 ICMS pulses to an electrode that elicited a tactile sensation in the palm of the hand and systematically increased stimulation amplitude from 10 µA to 35 µA. We observed an evoked response, measured by a neighboring electrode on the same array in the somatosensory cortex, which increased in amplitude with ICMS amplitude. The evoked response occurred ~80 ms after stimulation onset; however, after 30 µA the peak shifted to ~120 ms. We did not observe evoked activity in the motor cortex or contralateral hemisphere. These results suggest that ICMS of somatosensory cortex can modulate evoked neural activity in the ipsilateral motor or somatosensory cortices, though there is variability that depends on the location, amplitude, and duration of the stimulation. Importantly, these results also offer the potential for better understanding the relationship between sensorimotor neural connections and how they are modulated. This research was developed with funding from the Defense Advanced Research Projects Agency (DARPA). The views, opinions and/or findings expressed are those of the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government.

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Nanosymposium

261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.06

Topic: E.05. Brain-Machine Interface

Title: Sensorimotor interplay revealed via microstimulation of human somatosensory cortex

Authors: *N. SHELCHKOVA[1], J. E. DOWNEY[2], C. M. GREENSPON[4], C. S. VERBAARSCHOT[5], A. R. SOBINOV[1], E. OKOROKOVA[1], Q. HE[2], C. SPONHEIM[3], A. F. TORTOLANI[1], D. D. MOORE[6], M. T. KAUFMAN[3], P. WARNKE[1], D. SATZER[1], M. BONINGER[7], J. L. COLLINGER[7], R. A. GAUNT[8], N. G. HATSOPOULOS[1], S. J. BENSMAIA[2];
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Abstract: Closed-loop control of a prosthetic limb involves decoding motor intent from motor cortex (MC) while conveying somatosensory feedback via ICMS delivered to somatosensory cortex (SC). As new decoders are developed to operate within this closed-loop paradigm, it is unclear to what extent ICMS-evoked activity in SC might also cause activity in MC. In at least one example of a closed-loop brain computer interface, decoders were effective with the assumption that ICMS to SC evokes only negligible activity in MC. However, the documented anatomical and functional connectivity between the two regions suggests that ICMS-evoked activity in SC may be transmitted to MC, and this would, in principle, disrupt decoder performance.
To test this possibility, we delivered ICMS pulse trains to SC while measuring the neuronal activity evoked in MC in three human participants with tetraplegia implanted with electrode arrays in both SC and MC. First, we found that ICMS applied to SC was liable to activate the majority of channels in MC in two participants. Some of this activity was seemingly direct and possibly monosynaptic, as evidenced by reliably pulse-locked responses with latencies ranging from 2 to 6 ms. Second, in two of the participants, the spatial pattern of activation in MC depended on the location of the stimulating electrode in SC in a systematic way. Specifically, MC channels that preferably encoded specific digits tended to be activated by SC channels with projected fields on the same digits. In other words, the body maps in MC and SC are somatotopically linked. Third, we showed that the ICMS-evoked activity was task dependent: The same ICMS trains had different impacts on MC activity depending on the behavioral state (rest, reach, object grasp, object transport). Finally, we demonstrate that ICMS-evoked MC activity can disrupt decoding of motor intent in a way that cannot be easily corrected for given the dependence of the effect on behavioral state. This work establishes that MC and SC are interconnected in a manner relevant to brain-machine interfaces.

Nanosymposium

261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.07

Topic: E.05. Brain-Machine Interface

Support: DARPA HR001120C0120

Title: Interaction between intracortical microstimulation of human somatosensory cortex and residual somatosensation in an individual affected by incomplete tetraplegia

Authors: *M. S. FIFER[1], L. E. OSBORN[1], B. CHRISTIE[1], D. P. MCMULLEN[2], A. S. PAWAR[3], R. W. NICKL[3], J. M. YAU[4], B. A. WESTER[1], S. J. BENSMAIA[5], P. A. CELNIK[3], F. V. TENORE[1];
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Abstract: Providing artificial touch sensation to individuals with somatosensory deficits due to injury or neurological disease is increasingly possible using intracortical microstimulation (ICMS). Incomplete tetraplegia is more prevalent than complete tetraplegia, and can lead to a mix of lost and spared sensation in the upper limb. Partially retained sensation poses unique challenges for placing and utilizing stimulating arrays in cortex, and raises unique questions about the interaction between ICMS and native cutaneous sensation.
We had the opportunity to work with an individual affected by incomplete tetraplegia with microelectrode arrays implanted bilaterally in motor and somatosensory cortices. Stimulation through the somatosensory arrays yielded percepts localized to the fingertips, more proximal segments of the fingers, and the palms (Fifer et al., 2022). Our team has characterized the perceptual interactions between simultaneous ICMS and vibration, where vibration was applied in the projected field of the ICMS. These overlapping central and peripheral stimuli were able to be identified above chance levels in a modality discrimination tasks, and were judged as nearly simultaneous in a temporal order judgment task (Christie et al., 2022).
In several exploratory sessions, we provided ICMS associated with 2D objects on an interactive tablet—as the user swiped his finger across the screen, entering or exiting one of several circles on the screen would trigger a stimulation pulse. The cutaneous sensation generated in his thumb fingertip by swiping mixed with the thumb fingertip percept triggered by ICMS, creating a somatosensory illusion that the participant felt as a "divot" in the screen. Additionally, the subject reported that increasing pulse duration (i.e., 200ms, 350ms, 500ms) increased the

perceived diameter of the swiped objects while increasing the pulse amplitude (i.e., 35μA, 58μA, 80μA) increased their perceived depth. Once he identified the effect, he was able to identify the longer and more intensely stimulating objects despite their being visually indistinguishable. This study highlights that there are nontrivial interactions between residual cutaneous sensation and touch evoked by ICMS. Additional work is needed to determine when and to what extent ICMS percepts mask or enhance natural cutaneous sensations.

Disclosures: **M.S. Fifer:** None. **L.E. Osborn:** None. **B. Christie:** None. **D.P. McMullen:** None. **A.S. Pawar:** None. **R.W. Nickl:** None. **J.M. Yau:** None. **B.A. Wester:** None. **S.J. Bensmaia:** None. **P.A. Celnik:** None. **F.V. Tenore:** None.

Nanosymposium

261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.08

Topic: E.05. Brain-Machine Interface

Support: NIH U01 NS108922

Title: Spatial and perceptual determinants of electrode discriminability in a human somatosensory brain-computer interface

Authors: *V. KARAPETYAN[1,2], T. HOBBS[1,2], C. M. GREENSPON[4], T. CALLIER[4], M. L. BONINGER[1,3,2], J. L. COLLINGER[1,3,2], S. J. BENSMAIA[4], R. A. GAUNT[1,3,2];
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Abstract: Intracortical microstimulation (ICMS) in the somatosensory cortex can evoke vivid tactile sensations and be used to provide sensory feedback to individuals living with spinal cord injury. Work in both monkeys and humans has focused on characterizing how varying stimulation parameters affects perception. For example, changing the stimulus electrode changes the location on the body where the sensation is experienced (the projected field) as well as the quality of the percept itself. Varying stimulation amplitude and frequency also affects other factors including intensity and percept quality. Despite these advances in our understanding of ICMS-evoked sensations, it remains unclear how salient any of these perceptual differences actually are.
To address this question, we performed psychophysical experiments with human participants that had two 32-channel microelectrode arrays implanted in area 1 of the somatosensory cortex. Participants judged whether sensations evoked by two consecutively presented ICMS pulse trains - matched for perceived magnitude - were the same or different. Half of the time, the two stimuli were presented through the same electrode, while other times stimuli were delivered though two different electrodes. We reasoned that some electrode pairs would be reliably

distinguished while others would not, depending on both the location and quality of the percepts evoked by each electrode.

In one participant, we selected 58 pairs of electrodes that spanned the two arrays and recorded the perceptual quality and projected field for each electrode during a pre-test survey. Quality was described using a set of verbal descriptors from a validated questionnaire. We used a multivariate model to test the extent to which differences in spatial and perceptual factors contributed to electrode discriminability. The distance between electrodes on the intracortical arrays was a major determinant of discriminability (logistic regression, $R^2 = 0.37$, $p < 0.001$). When the electrodes were more than 3 mm apart, the participant could tell whether stimuli were coming from the same electrode or different electrodes above chance. Percept quality contributed to discrimination only for closely-spaced electrodes and only when the qualities themselves differed by at least two descriptors (binomial test, $z = 2.15$, $p = 0.03$). Understanding the factors contributing to salient perceptual differences will allow us to design stimulation paradigms that improve perceptual and functional performance of bidirectional brain-computer interfaces and may also inform future electrode designs.

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261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.09

Topic: E.05. Brain-Machine Interface

Title: Effects of multi-channel intra-cortical micro-stimulation in human primary sensory cortex: faster reaction times, natural somatosensations and greater percept stability

Authors: *D. A. BJANES[1,2,3], L. BASHFORD[1,2,3], K. PEJSA[1], B. LEE[5,6], C. LIU[6,5,3,4], R. A. ANDERSEN[1,2];
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Rehabil. Ctr., Downey, CA; [5]Dept. of Neurolog. Surgery, [6]USC Neurorestoration Ctr., Keck Sch. of Med. of USC, Los Angeles, CA

Abstract: Somatosensory brain-machine interfaces (BMIs) can create naturalistic sensations by modulating activity of neural populations in the brain. By utilizing different spatial or temporal patterns of intra-cortical micro-stimulation (ICMS) in primary sensory cortex (S1), human patients suffering somatosensory loss can experience both cutaneous and proprioceptive sensory feedback. As evidenced by motor deficits in deafferented patients, rapid somatosensory feedback is critical for dexterous motor ability, in part because visual feedback is much slower than natural occurring somatosensory input. However, somatosensory BMI studies typically report significantly longer cognitive processing latencies for sensations evoked via cortical electrical stimuli than naturally occurring somatosensory or visual percepts. In this study, a human tetraplegic participant was implanted with two 48-channel Utah arrays in primary sensory cortex and data was collected 4 years post-implant. Single- and multi-channel electrical stimulation patterns were used to elicit naturalistic somatosensory percepts in the arm contralateral to the array implants. Reaction times (RTs) to sensations evoked via ICMS were quantitatively compared to RTs from naturally occurring visual and tactile stimuli. Single- and multi-channel ICMS patterns were chosen to produce a stable, reproducible cutaneous somatosensory percepts. A vibrotactor was used to generate naturally occurring tactile sensations in sensate locations near locations of evoked ICMS percepts. We found sensations evoked via multi-channel ICMS were cognitively processed at comparable latencies to naturally occurring tactile stimuli and significantly faster than visual stimuli, as measured via the reaction time task. Further investigation of multi-channel sensations across electrodes on both implanted arrays, showed several improvements over comparable single-channel stimulation patterns. Novel reported somatotopic locations could be evoked via multi-channel stimulation as compared to single-channel ICMS patterns. "Natural" descriptors (tap, pinch, squeeze, grab, etc) were more frequently used than "non-natural" (shock, etc) descriptors from multi-channel ICMS. And improvements in the stability and reliably of evoked somatosensations were observed with multi-channel stimulation. These findings are significant advances toward development of state-of-the-art sensory BMIs and may improve control accuracy and increase embodiment for human users of motor BMI devices.

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Nanosymposium

261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.10

Topic: E.05. Brain-Machine Interface

Title: Development of closed-loop stimulation approaches in cortical visual prostheses

Authors: *F. GRANI, M. VAL CALVO, C. SOTO SANCHEZ, L. SOO, D. WACLAWCZYK, E. FERNANDEZ JOVER;
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Abstract: Cortical visual prostheses based on intracortical electrical stimulation are a potential method to restore vision in blind subjects. An effective prosthesis may include thousands of electrodes implanted in the visual cortex, each of which creates a different phosphene. Besides providing electrical stimulation, each electrode can be used to record the brain signals from the cortex region under the electrode which contains visual processing and brain state information. This information can be used to adjust and improve the electrical stimulation (closed-loop stimulation approach), increasing the safety and usability of the implant. Here we have investigated how features extracted from the local field potential (LFP) collected by intracortical electrodes can be used to build a closed-loop stimulation approach in cortical visual prostheses. We implanted an intracortical microelectrode array (consisting of 96 electrodes) in the visual cortex of a blind participant for 6 months. The subject was able to clearly report visual perception after electrical stimulation. We studied resting state data through the 6 months to assess the stability of the recorded signals. The results show that synchronization measures between electrodes significantly decrease (Wald Test, $p<0.01$), while variance and power spectral density do not show a significant trend. Using the signals acquired during visual perception induced by the electrical stimulation, we found that the LFP phase in the instant before stimulation indicates a brain state in which it is easier to induce visual perception. Indeed, the distribution of LFP phase before stimulation significantly differs in case of perception (most responsive LFP phase) compared to no perception (Mann-Whitney test, $p<0.01$). However, the most responsive LFP phase was different for each stimulation electrode, suggesting a dependence on the neural population under the electrode. Furthermore, using the signals recorded post stimulation we could extract the current threshold to induce perception using the power spectral density in the 4-28 Hz bandwidth. The thresholds obtained with this method correlate well with those obtained with the subject's answers ($r=0.64$). Our results lay the foundation for improving cortical visual prostheses through closed-loop stimulation approaches: stimulating at the right LFP phase could increase the perception probability without increasing the current intensity, and the brain signals' response to stimulation can be used to adjust the perception thresholds. Moreover, the signals acquired by the electrodes provide the long-term stability needed for a chronic implant.

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Nanosymposium

261. Advances in Neural Microstimulation for Sensory Restoration and Feedback in Neural Prosthetics

Location: SDCC 5

Time: Monday, November 14, 2022, 8:00 AM - 10:45 AM

Presentation Number: 261.11

Topic: E.05. Brain-Machine Interface

Title: Chronic stability of 1024-channel Utah-array-based neuroprosthesis in monkey visual cortex

Authors: *X. CHEN[1,2], F. WANG[3], R. N. KOOIJMANS[3], P. C. KLINK[1,4,5], P. R. ROELFSEMA[3,6,7];
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Abstract: Electrical stimulation of visual cortex via a neuroprosthesis induces the perception of dots of light ('phosphenes'), potentially allowing recognition of simple shapes even after decades of blindness. However, restoration of functional vision requires large numbers of electrodes, and chronic, clinical implantation of intracortical electrodes in the visual cortex has only been achieved using 96-channel devices. We evaluated the efficacy and stability of a 1024-channel neuroprosthesis system in non-human primates (NHPs) over >3 years to assess its suitability for long-term vision restoration. We implanted 16 microelectrode arrays consisting of 8x8 electrodes with iridium oxide tips, in primary visual cortex (V1) and area V4 of two sighted macaques. We monitored the animals' health and measured electrode impedances and neuronal signal quality by calculating signal-to-noise ratios (SNR), peak-to-peak signal voltages, and the number of high-amplitude channels obtained during implantation. We delivered cortical microstimulation and performed current thresholding, monitoring the number of channels that successfully yielded phosphenes. The monkeys performed a visual stimulus detection task after 2-3 years of implantation, allowing assessments of fine visual acuity, and histological analysis was carried out at 3-3.5 years post-implantation. The monkeys remained healthy throughout the implantation period and the device retained its mechanical integrity and electrical conductivity (assessed in one animal). However, we observed decreasing signal quality with time, declining numbers of phosphene-evoking electrodes, decreases in impedance on low-impedance channels, and impaired visual acuity at visual field locations corresponding to implanted cortical regions. Tissue dissections revealed encapsulation of arrays and cortical degeneration. Long-term implantation of a high-channel-count device in NHP visual cortex was accompanied by deformation of cortical tissue and decreased stimulation efficacy and signal quality over time.

We conclude that improvements in device biocompatibility and/or refinement of implantation techniques are needed before long-term future clinical use is feasible.

Disclosures: X. Chen: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Phosphoenix BV. **F. Wang:** None. **R.N. Kooijmans:** None. **P.C. Klink:** None. **P.R. Roelfsema:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Phosphoenix BV.

Nanosymposium

262. Addiction: Interaction with Orexin and Oxytocin Systems

Location: SDCC 23

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 262.01

Topic: G.09. Drugs of Abuse and Addiction

Support: AA017072

Title: Differential importance of nucleus accumbens Ox1Rs andAMPARs for female and male mouse binge alcohol drinking

Authors: C. KWOK[1], **K. LEI**[1], **V. PEDROZO**[1], **L. ANDERSON**[1], **S. GHOTRA**[1], **M. WALSH**[1], **L. LI**[1], **J. YU**[1], ***F. HOPF**[2];
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Abstract: Alcohol use disorder extracts substantial social and economic costs, with recent dramatic increases in female problem drinking. Thus, it is critically important to understand signaling differences underlying alcohol consumption across the sexes. Orexin-1 receptors (Ox1Rs) can strongly promote motivated behavior, and we previously identified Ox1Rs within nucleus accumbens shell (shell) as crucial for driving binge intake in higher-drinking male mice. Here, shell Ox1R inhibition did not alter female mouse alcohol drinking, unlike in males. Also, lower dose systemic Ox1R inhibition did reduce compulsion-like alcohol intake in both sexes, indicating that female Ox1Rs can drive some aspects of pathological consumption, and higher doses of systemic Ox1R inhibition (which might have more off-target effects) reduced binge drinking in both sexes. These findings concur with previous studies where males and females exhibit relatively similar reinstatement for cocaine and sucrose, but the role of Ox1Rs is quite different, where systemic Ox1R inhibitors reduce cue- and stress-related reinstatement in males while affecting only stress reinstatement in females. Together, one possibility is that female Ox1Rs are especially important and recruited during more stress-related behaviors, including stress-induced reinstatement and compulsion-like intake, with female Ox1Rs having less role in more basic-level responding (binging and cued reinstatement, or at least where stress is less likely involved). In contrast, male Ox1Rs would contribute to a broader range of motivated behaviors. Finally, in contrast to shell Ox1Rs, inhibiting shell calcium-permeable AMPA receptors (CP-AMPARs) strongly reduced alcohol drinking in both sexes, which was specific to

alcohol since this did not reduce saccharin intake in either sex. Our results together suggest that the shell critically regulates binge drinking in both sexes, with shell CP-AMPARs supporting intake in both sexes, while shell Ox1Rs drove drinking only in males. Our findings provide important new information about sex-specific and -general mechanisms that promote binge alcohol intake and possible targeted therapeutic interventions.

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Nanosymposium

262. Addiction: Interaction with Orexin and Oxytocin Systems

Location: SDCC 23

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 262.02

Topic: G.09. Drugs of Abuse and Addiction

Title: Targeting the oxytocin system to treat stimulant use disorders

Authors: *N. A. EVERETT[1], T. J. DOOLAN[1], S. J. BARACZ[2], M. H. JAMES[3], M. T. BOWEN[1], J. L. CORNISH[2];
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Abstract: The burden of disease associated with methamphetamine addiction is rising globally, and existing psychosocial interventions while effective for some, are ineffective for many. Unfortunately, there are no approved or effective pharmacotherapies for methamphetamine addiction in any jurisdiction. Over a decade of preclinical research has highlighted the potential of the neuropeptide oxytocin as a promising pharmacotherapy for methamphetamine addiction. This has motivated the current clinical investigations, however there been disappointing outcomes from many clinical trials with oxytocin for treating other psychiatric disorders, suggesting a breakdown in translation of oxytocin from rodent models to the clinic. Therefore, preclinical research is ongoing to understand the translatable therapeutic indications, neural mechanisms, and crucially, the pharmacological targeting strategies which will unlock the therapeutic potential of targeting the oxytocin system for addictions. Here, we will present our most recent preclinical experiments describing key efficacy data for oxytocin in methamphetamine self-administration models in rats, as well as the identified peripheral and central mechanisms of action for oxytocin, and hypothesised mechanisms which are under investigation. We will also address the hurdles for translating the oxytocin peptide to the clinic, and will present our solution to this translational problem: the development of novel oxytocin stimulating molecules which have superior physiochemical properties to the oxytocin peptide. We will describe recent progress with these oxytocin-like molecules in methamphetamine models which incorporate rodent behaviour, in vivo neural recordings, and biomarker discovery.

Nanosymposium

262. Addiction: Interaction with Orexin and Oxytocin Systems

Location: SDCC 23

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 262.03

Topic: G.09. Drugs of Abuse and Addiction

Support: DA042110

Title: Correction of sleep disturbances during abstinence following hypocretin-receptor antagonism in fentanyl-dependent rats.

Authors: *M. R. JONES, B. SAWYER, B. E. SCHMEICHEL;
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Abstract: Fentanyl is a potent synthetic opioid that has been shown to produce sleep disturbances, and the deterioration of sleep quality is associated with drug abuse and relapse in humans. The hypocretin/orexin neuropeptide system is a plausible pharmacological target, and dual-hypocretin antagonists such as lemborexant may mitigate sleep disturbances associated with fentanyl dependence. The current study characterizes sleep macroarchitecture (time spent asleep or awake) and microarchitecture (the number of bouts, and NREM sleep spindle characterization) prior to fentanyl vapor exposure (baseline), following one week of drug abstinence, and four weeks of drug abstinence in female and male rats. Females and males showed a reduction in the amount of time spent in rapid eye movement (REM) sleep following one week of abstinence. The pre-treatment of lemborexant the following day increased the amount of time spent in REM, compared to vehicle at both one and four weeks of abstinence. While there was no effect of fentanyl abstinence on the amount of time spent in non-rapid eye movement (NREM) sleep and wakefulness, lemborexant increased the amount time spent in NREM and decreased the amount of time spent awake. Examination of microarchitecture demonstrated a decrease in the number of NREM bouts at one week of abstinence, which lemborexant subsequently brought back to baseline levels at weeks one and four. Abstinence from fentanyl did not impact the number of NREM sleep spindles, but indicated a trend showing a decrease in intra-spindle frequency at one week of abstinence. Lemborexant, however, increased the number of spindles at weeks one and four of abstinence. Presently, findings indicate that fentanyl abstinence produces changes in sleep macroarchitecture, particularly REM sleep disruptions, which may be alleviated by lemborexant. This highlights the need for further examination of the relationship between sleep disturbances and drug abstinence, and the use of dual-hypocretin antagonists as therapeutic intervention.

Nanosymposium

262. Addiction: Interaction with Orexin and Oxytocin Systems

Location: SDCC 23

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 262.04

Topic: G.09. Drugs of Abuse and Addiction

Title: Preclinical evidence supporting the use of suvorexant for the management of cocaine use disorder

Authors: M. S. PALADINO[1], S. L. O'CONNOR[1], N. KRISHNAKUMAR[1], J. H. SUCHOJAD[1], M. M. BILOTTI[1], J. WISKERKE[2], *M. H. JAMES[1];
[1]Rutgers Univ., Piscataway, NJ; [2]Dept. of Biomed. & Clin. Sciences/ Ctr. for Soc & Affect Neurosci. (CSAN), Linköping Univ., Linkoping, Sweden

Abstract: Introduction: There is significant interest in the potential utility of orexin-based compounds for the clinical management of substance use disorders (SUDs). Several dual orexin receptor antagonists (DORAs) are currently approved for the treatment of insomnia, which has raised the possibility of repurposing these drugs for SUD indications. However, very few preclinical studies have examined whether acute administration of DORAs can reduce drug behaviors at doses that are not sleep-promoting. **Methods:** Male and female Long Evans rats (n=24) were tested for cocaine economic demand following both continuous and intermittent cocaine self-administration schedules (both 1h/day total cocaine access). Rats were then trained on the rodent psychomotor vigilance task (rPVT), which tested their ability to maintain sustained attention over 30mins to earn sucrose rewards. All rats were administered suvorexant (0, 3, 10, 30mg/kg; p.o.) 30mins prior to testing on demand and rPVT tests (within-subjects design). **Results:** Demand for cocaine was higher following the intermittent access schedule of cocaine self-administration (paired t-test; p=0.0188). Prior to undergoing intermittent access, a high dose of suvorexant (30mg/kg) was required to reduce cocaine demand; a similar outcome was achieved with a lower dose (10mg) following intermittent access (main effect of treatment, p=0.0277). At low doses (10mg/kg), suvorexant had limited effects on attention and locomotor outcomes. At higher doses (30mg/kg), suvorexant tended to impair attention and general activity. **Conclusions:** Suvorexant is effective at reducing several cocaine-related outcomes at doses that do not have off-target effects, but only in individuals with a strong addiction-like phenotype. These exploratory data are informative when weighing the potential advantages of repurposing already approved DORAs vs. the development of novel SORAs, which may have fewer off-target effects.

Disclosures: M.S. Paladino: None. **S.L. O'Connor:** None. **N. Krishnakumar:** None. **J.H. Suchojad:** None. **M.M. Bilotti:** None. **J. Wiskerke:** None. **M.H. James:** None.

Nanosymposium

262. Addiction: Interaction with Orexin and Oxytocin Systems

Location: SDCC 23

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 262.05

Topic: G.09. Drugs of Abuse and Addiction

Title: Oxytocin and orexin systems bidirectionally regulate the ability of opioid cues to bias choice during relapse

Authors: G. GIANNOTTI[1], F. MOTTARLINI[2], J. A. HEINSBROEK[1], M. R. MANDEL[1], M. H. JAMES[3], ***J. PETERS**[1];
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Abstract: As opioid-related fatalities continue to rise, the need for novel opioid use disorder (OUD) treatments could not be more urgent. Two separate hypothalamic neuropeptide systems have shown promise in preclinical OUD models. The oxytocin system, originating in the paraventricular nucleus (PVN), may protect against OUD severity. By contrast, the orexin system, originating in the lateral hypothalamus (LH), may exacerbate OUD severity. Thus, activating the oxytocin system or inhibiting the orexin system are potential therapeutic strategies. The role of these systems with regard to specific OUD outcomes, however, is not fully understood. Here, we probed the therapeutic efficacy of pharmacological interventions targeting the orexin or oxytocin system on two distinct metrics of OUD severity in rats - heroin choice (versus choice for natural reward, i.e., food) and relapse. Using a preclinical model that generates approximately equal choice between heroin and food reward, we examined the impact of exogenously administered oxytocin, an oxytocin receptor antagonist (L-368,899), and a dual orexin receptor antagonist (DORA-12) on opioid choice. Whereas these agents did not alter heroin choice when rewards (heroin and food) were available, both agents significantly reduced heroin relapse in the presence of both types of reward cues when no rewards were available. In addition, we observed that the number of LH orexin neurons and PVN oxytocin neurons correlated with specific behavioral economic variables indicative of heroin versus food motivation. These data identify a novel bidirectional role of the oxytocin and orexin systems in the ability of opioid-related cues to bias choice during relapse.

Nanosymposium

262. Addiction: Interaction with Orexin and Oxytocin Systems

Location: SDCC 23

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 262.06

Topic: G.09. Drugs of Abuse and Addiction

Title: Disrupted encoding of rewarding cues in the medial prefrontal cortex after intermittent social stress: Relevance for substance use vulnerability

Authors: H. HARRIS[1], ***A. J. DEL ARCO**[2];
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Abstract: Studies that use Intermittent Social Defeat (ISD) in rats demonstrate that social stress increases cocaine-self administration and suggest that a history of social stress makes individuals more vulnerable to substance use. However, we still do not understand the neurofunctional adaptations that predispose individuals to seek out drugs. The medial prefrontal cortex (mPFC) plays a key role encoding rewarding cues and regulating reward-seeking behavior. Here we investigate whether ISD disrupts the neuronal encoding of rewarding cues in the mPFC during a reward-seeking task. We also determine whether ISD enhances the behavioral effects of psychostimulants (i.e., cocaine). Male Long Evans rats (3-4 months old) were trained to discriminate between a rewarded (continuous tone) and a non-rewarded (intermittent tone) stimulus that was paired to the extension of a lever (right or left, counterbalanced). Pressing the rewarded lever delivered a sugar pellet. After stable performance, rats were implanted with electrode arrays in the mPFC (prelimbic area) and divided in two groups (Control, n= 7; Stress, n= 8). Then, they were exposed to ISD (or handling, Control group) once every three days for ten days (four stress episodes in total). mPFC activity was recorded during the task in both stressed and control animals two weeks after the last stress episode. Motor activity and conditioned place preference were assessed four weeks after the last stress episode to test the behavioral effects of cocaine injections. The results show that both stressed and control animals made more responses (lever presses) to the rewarded cue compared to the non-rewarded cue across sessions. There were not group differences in the number of lever presses in response to both cues. Furthermore, both groups decreased equally the number of lever presses during the extinction of the task, when responding to both cues produced the same effect (i.e., no pellet delivery). mPFC neurons increased/decreased their activity in response to both rewarded and non-rewarded cues. However, the number of activated neurons in response to rewarded cues was significantly lower in stressed animals compared to controls ($X^2(2)= 10.64$, $p< 0.01$). Moreover, and in contrast to controls, stressed animals showed an inhibition of the average population activity in response to

rewarded cues. In addition, stressed animals showed an enhanced motor response to cocaine compared to controls. These results suggest that ISD disrupts the encoding of rewarding cues in the mPFC and that this reward deficit may be associated with a higher vulnerability to substance use.

Disclosures: H. Harris: None. **A.J. Del arco:** None.

Nanosymposium

262. Addiction: Interaction with Orexin and Oxytocin Systems

Location: SDCC 23

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 262.07

Topic: G.09. Drugs of Abuse and Addiction

Title: The Serotonin 2A Receptor Regulates Synaptic Plasticity of Claustrum Cortical Projection Neurons: Implications for Cocaine-Induced Cognitive Deficits

Authors: *T. ANDERSON[1], P. I. ORTINSKI[2];
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Abstract: Many drugs of abuse, including cocaine, cause deficits in cognitive flexibility, rendering users more likely to relapse and less likely to abstain from their drug use. Recently, psychedelic hallucinogens, which are agonists of the serotonin 2A receptor (5HT2AR), have attracted attention for their promising therapeutic potential of increasing cognitive flexibility in humans to treat psychiatric diseases such as substance use disorders. The claustrum (CLA), a subcortical nucleus, has the highest density of the serotonin 2A receptor (5HT2AR) in the brain with extensive connections to other brain areas, most prominently the anterior cingulate cortex (ACC) that is known to modulate both cognitive flexibility and drug-seeking behavior. We propose that the CLA-ACC circuit plays a critical role in regulation of cocaine-induced cognitive flexibility deficits via interactions between 5HT2ARs and glutamatergic synapses in the CLA that impact long-term plasticity and the likelihood of relapse to cocaine use. We first that extended access to self-administered cocaine and microinjections of the hallucinogenic 5HT2AR agonist, DOI, into the CLA impaired set-shift performance on a set-shifting task, a measure of cognitive flexibility. Next, we used whole cell recordings to characterize effects of 5HT2AR signaling on CLA neurons that project to the anterior cingulate cortex (ACC). CLA-ACC neurons were recorded in the presence of 5HT and the 5HT2AR antagonist, ketanserin. 5HT caused a drastic inhibitory response. Significant decreases in sEPSC frequency and amplitude were observed in CLA-ACC neurons after application of 5HT. Blockade of the 5HT2AR with ketanserin eliminated synaptic effects of 5HT, indicating a regulatory role of the 5HT2AR in claustrocortical signaling. Next, we observed spike-timing dependent plasticity (STDP) in CLA-ACC neurons, revealing anti-hebbian long-term depression. DOI, a psychedelic 5HT2AR agonist, reversed this LTD into a robust long-term potentiation. These findings provide the first evidence that the large population of CLA-ACC neurons are under inhibitory control from 5HT

and the 5HT2ARs and suggest that serotonin regulation of long-term synaptic plasticity in CLA may contribute to cognitive flexibility deficits following cocaine exposure.

Disclosures: T. Anderson: None. **P.I. Ortinski:** None.

Nanosymposium

263. Social Cognition and Memory: Circuits and Mechanisms

Location: SDCC 24

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 263.01

Topic: H.06. Social Cognition

Support: 1U01NS108680
                1F31MH125451-01A1

Title: Neural correlates of cooperation in freely moving rhesus macaques

Authors: *M. FRANCH[1], S. YELLAPANTULA[2], A. WRIGHT[1], V. DRAGOI[1];
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Abstract: Social interactions are essential for the well-being and survival of humans, non-human primates, and other animals. Specifically, cooperation is an extremely complex social behavior, involving the perception and monitoring of self and others' actions to determine productive responses towards achieving a common goal. Despite such behavioral intricacy, most of our knowledge about the role of social attention in cognition originates from studies performed in restrained animals viewing synthetic stimuli on a monitor. Consequently, our understanding of how the brain processes naturalistic, visual-social cues to facilitate volitional social decision-making remains limited. Here, we examined the neural correlates of cooperation by wirelessly recording eye-tracking and neural activity from a visual-social cortical network - visual area V4 and dorsolateral prefrontal cortex, dlPFC - while dyads of freely moving and behaving rhesus macaques cooperated for a food reward. In this task, the same perceivable but remote reward was revealed to each animal, and animals could obtain their reward by, at any time, simultaneously pushing and holding buttons which would move trays that delivered reward. Critically, the timing of animals' push responses with respect to each other reveals their actions are coordinated, not random, and monkeys across dyads exhibited a high conditional probability to cooperate (P(self | partner) > 0.5). Additionally, animals are more likely to fixate on social cues, such as the reward or partner monkey, before cooperation than during non-socially relevant periods. Importantly, neuronal ensembles in V4 and dlPFC encode these social cues, while population encoding of self and partner decision to cooperate was greatest in dlPFC. Finally, significantly correlated V4-dlPFC cells contribute more to encoding of social variables within each area. Together, these findings reveal a distributed cortical network representation of visually driven cooperation in the primate brain.

Disclosures: M. Franch: None. **S. Yellapantula:** None. **A. Wright:** None. **V. Dragoi:** None.

Nanosymposium

263. Social Cognition and Memory: Circuits and Mechanisms

Location: SDCC 24

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

**Presentation Number:** 263.02

Topic: H.08. Learning and Memory

Title: Macrostructural properties of parahippocampal cingulum are associated with hippocampal subfield volumes and memory performance in healthy cognitive aging

Authors: *N. MALYKHIN[1], A. AGHAMOHAMMADI-SERESHKI[3], Y. HUANG[2], W. PIETRASIK[2];
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[3]Radiology, Univ. of Calgary, Calgary, AB, Canada

Abstract: Background: The parahippocampal cingulum (PHC) carries afferent and efferent projections from structurally and functionally distinct medial temporal lobe structures and provides the majority of inputs and outputs to/from the hippocampus. It is unknown if age-related reductions in hippocampal subfields contribute to structural alterations in PHC, especially to its macrostructural properties. The aim of this study was to investigate if age-related decline in fibre composition of PHC is related to volume reductions in specific hippocampal subfields and how it contributes to episodic memory function. **Methods:** A total of 129 healthy volunteers (18-85 years old) were recruited for this study. Images were acquired using a T2-weighted 2D FSE and a diffusion tensor imaging (DTI) sequences. The hippocampal subfields (dentate gyrus (DG), cornu ammonis (CA1-3), and subiculum (Sub)) were manually traced using reliable volumetric protocol. We used DTI-tractography to delineate PHC and to examine its microstructural (fractional anisotropy (FA), axial and radial diffusivities) and macrostructural measures (tract volume, fiber counts, fiber length, number of fibers per voxel). Participants were administered the Wechsler Memory Scale, 4th edition to assess performance in the visuospatial, verbal, and logical memory. **Results:** We found that total hippocampal volume was positively correlated with PHC tract volume ($r=0.459$, $p<0.001$) and fiber counts ($r=0.227$, $p=0.01$). PHC tract volume was also associated with hippocampal subfield volumes: CA1-3 ($r=0.459$, $p<0.001$), DG ($r=0.274$, $p=0.002$), and Sub ($r=0.459$, $p<0.001$). In addition, total CA1-3 volume correlated with the number of fibres ($r=0.315$, $p<0.001$), their length ($r=0.181$, $p=0.04$), and number of fibers per voxel ($r=0.178$, $p=0.044$). There were no significant associations between hippocampal subfield volumes and microstructural properties of the PHC (all ps>0.05). Fiber count predicted performance on verbal ($r=0.254$, $p=0.003$), logical ($r=0.221$, $p<0.011$), and visuospatial memory ($r=0.447$, $p<0.001$), while number of fibres per voxel predicted performance on verbal ($r=0.182$, $p=0.037$) and visuospatial memory ($r=0.321$, $p<0.001$). Finally, axial diffusivity ($r=0.293$, $p<0.001$) and fiber length ($r=0.217$, $p=0.012$) also predicted performance on visuospatial memory. **Conclusions:** Our results provide initial in-vivo evidence for association between

hippocampal subfields structure and microstructural integrity of PHC across adult lifespan. Our findings suggest that fiber composition of PHC may not only be more specific to hippocampal structure, but also more sensitive than FA to predict memory function.

Disclosures: N. Malykhin: None. **A. Aghamohammadi-Sereshki:** None. **Y. Huang:** None. **W. Pietrasik:** None.

Nanosymposium

263. Social Cognition and Memory: Circuits and Mechanisms

Location: SDCC 24

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 263.03

Topic: H.08. Learning and Memory

Support: 1R01MH123713-01A1

Title: The geometry of cognitive maps under dynamic cognitive control

Authors: *S. A. PARK, M. ZOLFAGHAR, J. RUSSIN, D. MILLER, R. O'REILLY, E. D. BOORMAN;
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Abstract: Recent work has shown that abstract, non-spatial relationships between task-relevant states or entities are organized into map-like neural representations. Here, we investigate how these cognitive maps interact with changing task goals in the context of cognitive control, where the features most relevant to the current goal benefit from top-down biasing. Classic computational neuroscience studies of cognitive control have focused on explicitly presented categorical features rather than map-like representations retrieved from memory, and have typically found facilitation of task-relevant features and suppression/compression of task-irrelevant features. Here, we explore the relationship between cognitive control and the geometry of map-like representations by combining neural network models and fMRI of the same task. Previously, we found that although only one of two task attributes was behaviorally relevant for current decisions, hippocampus (HC), entorhinal cortex (EC), and orbitofrontal cortex (OFC) spontaneously organized pairwise relationships into 2D map-like representations. Consistent with the predictions of the neural-network models, new analyses of the fMRI data show that task-irrelevant dimensions were compressed relative to task-relevant dimensions dynamically as a function of which dimension is currently relevant, in dorsomedial and dorsolateral frontal (DLFC and DMFC) and posterior and medial parietal cortex (PMC). Furthermore, the model's underlying 2D representations were also affected by task demands in a different way: representations were warped along the 2D axis that remains unchanged across conditions requiring focus on each dimension separately. This finding was confirmed by fMRI analyses showing that this same warping phenomenon occurs in the HC, and that the degree of warping was correlated with individual differences in cognitive control. Further simulations showed that

this warped geometry reflects the natural tendency of neural networks to learn context-invariant maps, consistent with behavioral and fMRI results.
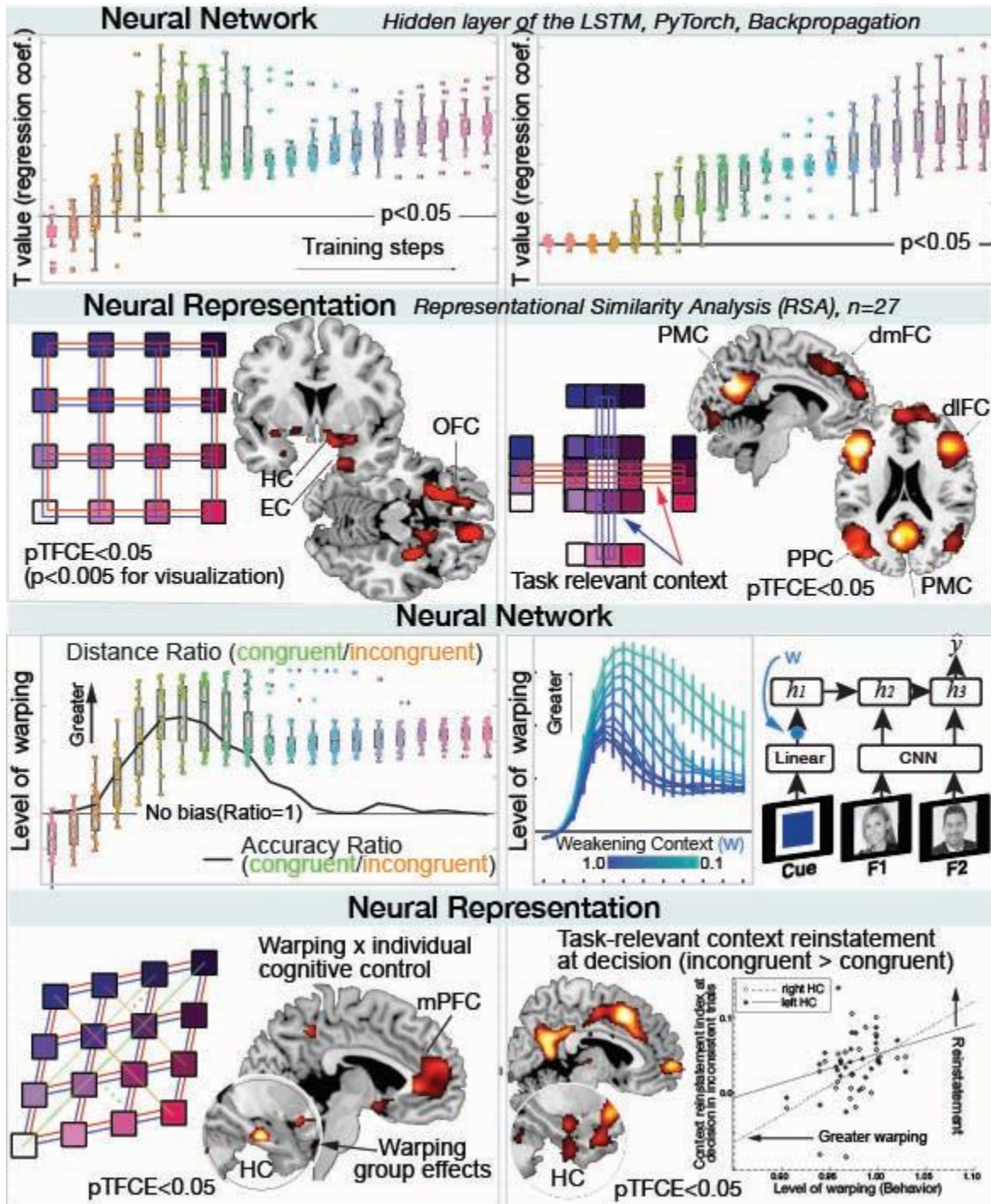
Disclosures: S.A. Park: None. **M. Zolfaghar:** None. **J. Russin:** None. **D. Miller:** None. **R. O'Reilly:** None. **E.D. Boorman:** None.

Nanosymposium

263. Social Cognition and Memory: Circuits and Mechanisms

Location: SDCC 24

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 263.04

Topic: H.07. Long-Term Memory

Title: Delayed but not acute domain-specific retroactive interference effects on consolidated motor memory

Authors: S. CROMBEZ[1], **F. KIEKENS**[1], **E. PLETTINCKX**[1], **F. SAVER**[1], **V. RAMAKRISHNAN**[2], **C. FINETTO**[3], ***K.-F. HEISE**[3,1];
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Abstract: It has been proposed that memories continue to be susceptible to modification in the moment of reactivation during retrieval even long after consolidation. However, solid behavioral evidence to support this hypothesis in humans is still pending because previous interference paradigms used to destabilize existing memories have investigated memory modification mostly in the early stage when the memory trace is still labile. To investigate the modifiability of well-consolidated procedural memory, we used an established retroactive interference paradigm in combination with a variant of the serial response time task. In this task, two layers of sequential information (spatial and temporal) were provided implicitly through visual cues associated with 1-2 simultaneous keypresses on a standard computer keyboard. Participants (N = 66) practiced the task online on seven consecutive days and were instructed to press the indicated keys precisely for the cued duration. Before the start of the study, they were randomly assigned to one of three interference conditions (spatial, temporal, and no interference), which were applied in session six and followed up 24 hours later in session seven. Temporal and spatial precision of sequential keypresses improved over the first five days describing an expected asymptotic exponential function (estimated with a non-linear least-squares fit) reaching a plateau within five days of practice indicating an advanced level of consolidation. Investigating the precision change from before to after the interference intervention with a linear mixed-effects model revealed that none of the interference conditions induced an acute change in precision in either of the sequences immediately after the interference intervention (Type II Wald test SEQUENCE CONDITION x INTERFERENCE CONDITION $X^2(6) = 5.6330$, p>.4). At 24 hours follow-up, we found a marked decrease in precision, which was specific to the interference intervention targeting the respective sequential information. While the spatial interference condition led to isolated precision declines in the spatial sequence (ß = -2.74, 95% CI [-5.12, -0.36], p < .05), temporal interference affected the precision of the temporal sequence (ß = -4.97, 95% CI [-7.33, -2.62], p < .001) after 24 hours. Notably, the effect of interference was evident after repeated practice, i.e., across 20 repetitions of each of the sequences. Based on our results, we suggest that 1) a simple behavioral interference can destabilize motor memory that has previously been well

consolidated, and 2) the interference effect itself potentially requires sleep-based consolidation mechanisms to unfold.

Nanosymposium

263. Social Cognition and Memory: Circuits and Mechanisms

Location: SDCC 24

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 263.05

Topic: H.06. Social Cognition

Title: Social grooming is controlled by a thalamo-preoptic neuronal pathway

Authors: *D. KELLER[1,2], T. LÁNG[1], M. CSERVENÁK[2], G. PUSKA[2,3], J. BARNA[1], V. CSILLAG[4,5], I. FARKAS[6], D. ZELENA[6,7], F. DÓRA[1,2,8], S. KÜPPERS[9], L. BARTECZKO[9], T. USDIN[10], M. PALKOVITS[8], M. HASAN[11], V. GRINEVICH[9], Á. DOBOLYI[2];
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Abstract: Social touch is an essential component of communication. Little is known about the underlying pathways and mechanisms. The hypothalamus is a major regulatory center of rodent social behavior. It is also likely to be involved in the control of instinctive behaviors in humans.

It is conceivable that ascending sensory pathways carrying information on social touch might project directly to the hypothalamus. Here, we discovered a novel neuronal pathway from the posterior intralaminar thalamic nucleus (PIL) to the medial preoptic area (MPOA) is involved in control of social grooming. First, we determined the effect of chemogenetic stimulation of PIL neurons on social interactions between familiar adult female rats. Activity-dependent tagging of PIL neurons was performed in rats experiencing physical social contacts. The selective chemogenetic stimulation of the preoptic area-projecting PIL neurons was performed using double viral injections and also by CNO administration directly into the preoptic area. We found that neurons in the PIL and MPOA were naturally activated by physical contact between female rats and also by chemogenetic stimulation of PIL neurons. Chemogenetic activation of these neurons increased social grooming between familiar rats as did selective activation of the PIL-MPOA pathway. Neurons projecting from the PIL to the MPOA express the neuropeptide parathyroid hormone 2 (PTH2) and central infusion of its receptor antagonist diminished social grooming. We showed its increased expression in the PIL in response to social interaction. Finally, we showed similarity in the anatomical organization of the PIL-MPOA circuit in the rat and human brain. We propose that the discovered PIL-MPOA neuronal pathway facilitates physical contacts in both rodents and human. Therefore, the pathway as well as the PTH2 neuropeptide and its receptor should be investigated in the future in disorders where deficits in direct social interactions are found, such as autism spectrum disorder.

Nanosymposium

263. Social Cognition and Memory: Circuits and Mechanisms

Location: SDCC 24

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 263.06

Topic: H.06. Social Cognition

Title: The involvement of the hippocampal CA2 region in social fear memory

Authors: *P. KASSRAIAN, S. K. BIGLER, S. A. SIEGELBAUM;
Dept. of Neurosci., Columbia Univ., New York, NY

Abstract: Animals need to generalize defensive behaviors to newly encountered stimuli that are likely to threaten their safety on the basis of previously learned fear responses, while at the same time distinguishing between threatening and non-threatening stimuli. While adaptive responses to fearful social stimuli are necessary for survival, the maladaptive processing of fearful social

stimuli, such as an overgeneralization of fear responses to safe social stimuli, is a hallmark of a wide range of neuropsychiatric disorders. Although the hippocampus has been hypothesized to play a role in fear specificity, the exact mechanisms and regions underlying fear specificity, and in particular social fear specificity, remain unknown. Here we show that the hippocampal CA2 region, which has previously been found to be essential for social novelty discrimination of familiar from non-familiar conspecifics, serves as a critical locus for social fear specificity - the ability to distinguish between safe and threatening conspecifics. To this end we employed a social fear conditioning paradigm where a subject mouse was simultaneously introduced to two conspecifics but only received footshocks when it interacted with one (CS+) and not the other (CS-). Twenty-four hours after the paradigm, the conditioned mouse displayed aversive behavior to the CS+ but not CS- mouse. We examined the role of CA2 in this fear specificity by chemogenetic or optogenetic silencing of this region during social fear conditioning. CA2 silencing by either method led to a marked decrease of social fear specificity but not social fear itself as the conditioned animal avoided equally the CS+ and CS- animals. This suggests a broader role of CA2 for the regulation of social behaviors beyond social novelty detection, including the recognition of a potentially threatening conspecific for the initiation of defensive behaviors. Such findings are consistent with results implicating oxytocin receptors, which are highly enriched in CA2, in social fear acquisition and extinction. Finally, our findings indicate that CA2 is important for social memory of distinct individuals with identical degrees of social novelty, based on the recollection of detailed episodic memories of prior encounters.

Disclosures: P. Kassraian: None. **S.K. Bigler:** None. **S.A. Siegelbaum:** None.

Nanosymposium

263. Social Cognition and Memory: Circuits and Mechanisms

Location: SDCC 24

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 263.07

Topic: H.06. Social Cognition

Title: Social-like behavior is still inducible in the evolutionarily asocial Mexican cave tetra by the ketogenic diet intervention.

Authors: *M. YOSHIZAWA[1], A. TRAN[2], J. CASHON[2], R. BALMILERO-UNCIANO[2], M. WONG[3,4], R. LEE[4,5], M. IWASHITA[2];
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Abstract: In animals, ranging from arthropods to mammals, social affinity is recurrently lost in adapting to ecological demands. Recent comparative genomic studies involving honeybees and the Mexican cavefish revealed that the dysregulated genes in these socially deprived populations are significantly enriched in homologs of autism risk-associated genes. Do these genetically solitary populations still socialize or had lost such ability? Recently, the ketosis-inducing ketogenic diet treatment improved the sociality score of Autism Diagnostic Observation Schedule-2 (ADOS-2) in patients with autism. The ketogenic diet induces ketosis instead of glycolysis in the whole body, and also makes the liver release ketone bodies including beta-hydroxybutyrate. This relatively simple treatment is suspected to adjust multiple dysregulated autism risk alleles, however, the mechanisms are still largely unknown. We then hypothesized that the ketone body (beta-hydroxybutyrate) induces social behaviors by adjusting many dysregulated autism-risk genes. Here we used the Mexican tetra fish Astyanax mexicanus as an experimental model. A. mexicanus exists in two forms: blind cave-dwelling (cavefish) and sighted riverine (surface fish) forms. Cavefish were derived from the surface fish-type ancestor, adapted to the food-sparse dark environments, displayed asociality, and exhibit 58.5% of the same directional gene expression changes (up- or down-regulations out of ~4,000 dysregulated genes) seen in patients with autism. In contrast, surface fish show typical behaviors and genetic conditions. One month of the ketone body treatment significantly promoted social affinity in cavefish but had no effect on surface fish. These results suggest that the ketone body could be the key molecule that activates the social-regulating neural network(s) including the dopaminergic network. Indeed, a D2 antagonist, aripiprazole, increased social affinity in asocial cavefish. We then share updates on the detailed ketone body influence on brain gene expressions and neural activities.

Disclosures: M. Yoshizawa: None. **A. Tran:** None. **J. Cashon:** None. **R. Balmilero-Unciano:** None. **M. Wong:** None. **R. Lee:** None. **M. Iwashita:** None.

Nanosymposium

263. Social Cognition and Memory: Circuits and Mechanisms

Location: SDCC 24

Time: Monday, November 14, 2022, 8:00 AM - 10:00 AM

Presentation Number: 263.08

Topic: H.06. Social Cognition

Title: Antagonistic regulation between social affinity and repetitive behavior underpinned by non-visual sensors in asocial Mexican cavefish

Authors: *M. IWASHITA[1], M. YOSHIZAWA[2];
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Abstract: In many animal species, ranging from crustaceans to mammals, vision induced social affinity are well investigated but not so much in non-visual based ones. Visual processing could be a confounding factor to mechanistic understanding for the neural networks of social affinity. We therefore developed an assay system that quantify non-visual based collective behavior in the sighted riverine (surface fish) and non-sighted cave-dwelling populations (cavefish) of the Mexican tetra, Astyanax mexicanus. A. mexicanus surface fish show strong affinity each other in the lighted condition (schooling and shoaling) but reported as not in the non-visual dark. Cavefish were reported as asocial. These reports used the conventional detection methods, inter individual distance (IID) and nearest neighbor distance (NND), which could fail to detect many detail of nearby interactions. To characterize the detail of the collective behavior without visual stimulus, idTracker software-based method was developed. Through tracing each fish's X-Y coordinates within four fish group, we found the social surface fish showed strong affinity to each other without visual stimulus. Cavefish also showed much lower level but the significant social affinity which were enhanced in a familiar environment, which was the first example that evolutionarily asocial population showed plasticity in their social level. We also developed a method to quantify repetitive circling which were seen in cavefish but not in surface fish. Among cavefish, repetitive circling was in antagonistic relationship with social affinity, which is similar to what are seen in mammals. These results will open the path to understand how non-visual based social affinity is regulated, and how evolutionarily asocial population reduced social affinity. Such knowledge will provide further understanding in the social regulating neural network by avoiding vision-based confounding factors.

Disclosures: M. Iwashita: None. **M. Yoshizawa:** None.

Nanosymposium

264. Non-Invasive Neuromodulation Using Ultrasound, TMS, and Other Approaches

Location: SDCC 25

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 264.01

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH grant R01NS110554

Title: Mechanisms underlying the control of neuronal excitability with magnetogenetics

Authors: *M. HERNANDEZ MORALES[1,2], V. HAN[3], J. CHEN[3], S. HAN[3], K. MORALES-WEIL[3,2], C. LIU[3,2];
[1]Univ. of California Berkeley, [2]Helen Wills Neurosci. Inst., [3]Electrical Engin. and Computer Sci., Univ. of California Berkeley, Berkeley, CA

Abstract: The magnetogenetic technique FeRIC (<u>Fe</u>rritin iron <u>R</u>edistribution to <u>I</u>on <u>C</u>hannels) uses radiofrequency (RF) magnetic fields to activate ion channels coupled with ferritin, named FeRIC channels. Specifically, the non-selective cation channel TRPV4 and the chloride channel TMEM16A were coupled with endogenous ferritins by fusing their intracellular domain with the

ferritin binding motif of the kininogen. Using experimental tools such as genetic engineering, colorimetric assays, and Ca^{2+} imaging, we discovered that the mechanism responsible for the magnetic activation of FeRIC channels is a biochemical pathway. In cells expressing FeRIC channels, RF increases the levels of cytosolic iron triggering the production of reactive oxygen species and oxidized lipids that ultimately activate the FeRIC channels. We also found that several experimental factors, such as the ferritin iron load or the period of FeRIC channels expression, influence the efficacy of RF in activating the FeRIC channels. Remarkably, using voltage imaging, we found that FeRIC allows the control of neuronal spike firing. In neurons expressing TRPV4[FeRIC], RF depolarizes the membrane potential and increases the spike firing rate. Conversely, in neurons expressing TMEM16A[FeRIC], RF decreases the spike firing rate. In conclusion, the discovery of the mechanism responsible for the magnetic activation of FeRIC channels may guide future magnetogenetic designs to improve the magnetic control of neuronal excitability.

Disclosures: **M. Hernandez morales:** None. **V. Han:** None. **J. Chen:** None. **S. Han:** None. **K. Morales-Weil:** None. **C. Liu:** None.

Nanosymposium

264. Non-Invasive Neuromodulation Using Ultrasound, TMS, and Other Approaches

Location: SDCC 25

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 264.02

Topic: I.08. Methods to Modulate Neural Activity

Title: Enhancing the effects of transcranial magnetic stimulation (TMS) using a high-density theta burst paradigm

Authors: *H. LU[1], Q. MENG[1], H. NGUYEN[1], A. VRANA[1], S. BALDWIN[1], C. Q. LI[1], A. GILES[1], J. WANG[2], Y. YANG[1];
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Abstract: Theta burst stimulation (TBS) has been adopted as an efficient TMS paradigm in major depression treatment (Huang et al., 2005). However, its therapeutic efficacy remains similarly modest as conventional 10 Hz repetitive TMS (Blumberger et al., 2018). Inspired by classical theta burst paradigm in slice electrophysiological studies, where each burst consists of multiple (typically ≥ 4) electrical pulses, here we propose a new TMS paradigm called "high-density TBS (or hdTBS)." In this paradigm, each burst consists of up to 6 pulses as opposed to only 3 in conventional TBS. This paradigm thus effectively increased the "dosages" of TMS stimulation while maintaining identical time efficiency as in conventional intermittent TBS (200 s per session). We have developed a prototype stimulator in-house, which was able to deliver hdTBS. We tested this paradigm in the motor cortex of awake rats. A rodent-specific TMS coil with a focality of about 2 mm was used in this study (Meng et al. 2018), as well. Motor-evoked potential (MEP) signal was longitudinally measured with EMG electrodes implanted in the biceps femoris and gastrocnemius muscles of the hindlimb. Peak-to-peak MEP signal was used

as the metric to compare the acute aftereffects of hdTBS across different TMS conditions (3, 4, 5 or 6 pulses/burst). Results demonstrated that, in comparison to 3-pulse TBS, hdTBS with 5 and 6 pulses/burst significantly augmented the acute aftereffects. Importantly, we observed no signs of adverse effects in all rats (N=15) that had received multiple sessions of hdTBS, suggesting translational potentials of this paradigm. Acknowledgement: This work was supported in part by NIDA IRP, NIH.

Disclosures: H. Lu: None. **Q. Meng:** None. **H. Nguyen:** None. **A. Vrana:** None. **S. Baldwin:** None. **C.Q. Li:** None. **A. Giles:** None. **J. Wang:** None. **Y. Yang:** None.

Nanosymposium

264. Non-Invasive Neuromodulation Using Ultrasound, TMS, and Other Approaches

Location: SDCC 25

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 264.03

Topic: I.08. Methods to Modulate Neural Activity

Title: Using TMS-EEG to probe fronto-parietal connectivity of individual subjects

Authors: *J. GOGULSKI[1,2], C. C. CLINE[1], J. M. ROSS[3,1], S. PARMIGIANI[1], J. T. TRUONG[1], M. SARKAR[1], F. DONATI[4], D. VIJAYA[1], C. KELLER[1,3];
[1]Stanford Sch. of Med., Stanford, CA; [2]Dept. of Clin. Neurophysiol., Helsinki Univ. Hosp. and Univ. of Helsinki, Helsinki, Finland; [3]Veterans Affairs Palo Alto Healthcare System, and the Sierra Pacific Mental Illness, Research, Education, and Clin. Ctr. (MIRECC), Palo Alto, CA; [4]Dept. of Hlth. Sci., Univ. of Milan, Milan, Italy

Abstract: The dorsolateral prefrontal cortex (dlPFC) is the primary non-invasive brain stimulation target for the treatment of psychiatric conditions. dlPFC is functionally and anatomically connected to the parietal cortex and changes in these connections might underlie the clinical effects of transcranial magnetic stimulation (TMS) treatment. Thus, a clinic-ready readout of fronto-parietal network (FPN) connectivity with high signal to noise is needed. TMS paired with electroencephalography (TMS-EEG) provides a noninvasive, causal readout of neural activity with millisecond resolution, sufficient to capture effective brain connectivity. However, it is unknown if the FPN can be probed with TMS-EEG, and if so, what the optimal stimulation parameters are. Thus, optimizing the stimulation parameters (location and coil angle) within the dlPFC to effectively activate the FPN, while minimizing non-brain artifacts, would be of high value.

In this study, we systematically examined the effect of modifying the stimulation target within the left dlPFC on target engagement of fronto-parietal network (FPN), as measured by amplitude of early parietal TMS-evoked potentials (TEPs). Due to inter-individual differences in anatomy, we hypothesized that for each individual there would be an optimal angle/location combination in the dlPFC that maximally engaged the parietal cortex, but that the optimal angle/location would vary across subjects. For each subject, TMS was applied to six different locations within the dlPFC with two different coil angles (45 and 90 degrees to mid-sagittal plane). Each condition (150 trials) was repeated within session to assess test-retest reliability. Preliminary results (three subjects, two TMS-EEG sessions) demonstrated that the peak-to-peak magnitude of early (20 to 50 ms) parietal TEPs was affected by the location and angle of the stimulation target within the dlPFC. We observed within-subject specificity (i.e. there was an optimal angle/location for each subject), but this angle/location and its amplitude varied across subjects (range: 0.91 µV to 10.0 µV). These observations were consistent across two sessions within each subject. We also showed that stimulation target affected the magnitude of muscle-related artifacts, with larger amplitude artifacts with more anterior dlPFC targets. Preliminary data suggests that: 1. Parietally measured early TEPs may provide a reliable readout of FPN connectivity; 2. Maximizing the early parietal TEP and minimizing artifacts needs angle/location personalization. This work will help to develop a clinic-ready brain biomarker of FPN connectivity for TMS treatment protocols.

Disclosures: J. Gogulski: None. **C.C. Cline:** None. **J.M. Ross:** None. **S. Parmigiani:** None. **J.T. Truong:** None. **M. Sarkar:** None. **F. Donati:** None. **D. Vijaya:** None. **C. Keller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity in Alto Neuroscience, Inc.

Nanosymposium

264. Non-Invasive Neuromodulation Using Ultrasound, TMS, and Other Approaches

Location: SDCC 25

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 264.04

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R00NS100986

Title: Controlled, effective ultrasonic neuromodulation through the skull

Authors: T. RIIS[1], ***J. KUBANEK**[2];
[1]Biomed. Engin., Univ. of Utah, Salt Lake City, UT; [2]Biomed. Engin., Univ. of Utah, 36 S Wasatch Dr, UT

Abstract: Transcranial focused ultrasound provides noninvasive, reversible approaches for precise and personalized manipulations of brain circuits, with the potential to transform our understanding of brain function and treatments of brain dysfunction. However, the effectiveness and safety of these approaches have been limited by the human skull, which attenuates the

ultrasound strongly and unpredictably. There is currently no approach that could account for the ultrasound attenuation by the skull.To address this lingering barrier, we have developed an approach that directly measures and compensates for the attenuation of the skull of a given subject. We have implemented the approach in hardware and demonstrated that it accurately restores the operator's intended intensities beneath the skull. Moreover, we show that the approach is crucial for effective ultrasonic neuromodulation. When applied, there is reliable, dose-dependent, and safe stimulation of neural structures inside the skull. This method and hardware unlock the potential of ultrasound-based approaches to provide effective, safe, and reproducible precision therapies of the brain.

Disclosures: T. Riis: None. **J. Kubanek:** None.

Nanosymposium

264. Non-Invasive Neuromodulation Using Ultrasound, TMS, and Other Approaches

Location: SDCC 25

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 264.05

Topic: I.08. Methods to Modulate Neural Activity

Support: R01MH116981

Title: Sonogenetic fPET: Combining TRPV1-mediated sonogenetics with functional PET for noninvasive functional neural circuit mapping

Authors: *Y. YANG[1], C. WEIXEL[1], H. CHEN[1,2];
[1]Washington Univ. in st louis, st louis, MO; [2]Radiation Oncology, Washington Univ. Sch. of Med., St louis, MO

Abstract: Our ability to map the functional connectivity of neural circuits is largely hindered by the lack of a neuromodulation technique that can noninvasively control specific neural circuits. Recently, a promising technique—sonogenetics—has emerged that uses focused ultrasound (FUS) to remotely activate selected types of neurons virally transduced to express ultrasound-sensitive ion channel TRPV1. This technique has demonstrated combined advantages of noninvasiveness, high spatiotemporal precision, cell-type selectivity, and accessibility to the whole brain region. This study aimed to develop sonogenetics fPET, which integrates sonogenetics with functional positron emission tomography (fPET) to probe the functional connectivity of neural circuits in the whole brain. As a proof of concept, sonogenetics was used to control the basal ganglia circuit. Neurons in the striatum were modified to express ultrasound-sensitive ion channel, TRPV1, with an intracranially unilaterally injected viral vector (AAV5-CaMKII-TRPV1 or AAV5-CaMKII-mCherry). At 4-6 weeks after the virus injection, FUS was applied to the striatum to activate the TRPV1^{+} neurons, and 18F-fluorodeoxyglucose (FDG) was intravenously administered at the start of the FUS sonication. Simultaneously, fPET imaging was performed for the TRPV1^{+} mice and TRPV1^{-} mice. CT and fPET images were co-registered with a 3D mouse brain atlas to evaluate the global functional connectivity. fPET images found

significantly higher FDG uptake in the sonogenetics-targeted left striatum than in the contralateral non-targeted right striatum in TRPV1$^+$ mice. While in the TRPV1$^-$ mice, ultrasound alone did not induce any detectable changes (Fig. 1A). Brain functional connectivity maps revealed that sonogenetics also changed the activity of the global network (Fig. 1B). This study developed sonogenetics fPET by integrating TRPV1-mediated sonogenetics and fPET to map the mouse brain functional connectivity. Sonogeentic fPET provides a next-generation brain mapping tool with the potential to be scaled up to large animals and humans.
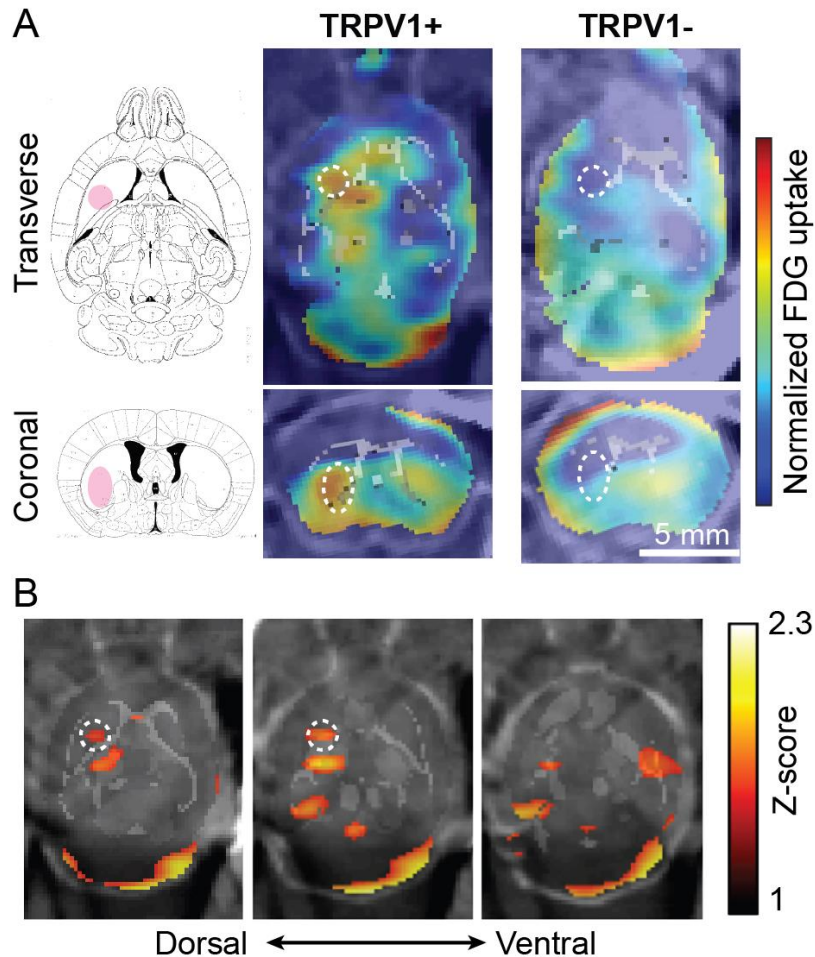
Figure 1. Sonogenetic fPET for mouse brain functional connectivity mapping. **(A)** Sonogenetics fPET shows global neural activity (indicated by the FDG uptake) followed by sonogenetic activation of the left striatum. Left: The location of the sonogenetic-targeted brain site in the striatum is indicated as a pink dots in the brain atlas. Right: transverse (top) and coronal (bottom) normalized FDG uptake images captured during FUS sonication in TRPV1+ mouse and TRPV1- mouse. Enhanced FDG uptake was observed in sonogenetic targeted region, indicating neural activation by sonogenetics. **(B)** Functional connectivity maps display the global network of activity changes induced by sonogenetic activation of the left striatum. The sonogenetic targeted brain site in the striatum is highlighted by dotted circles. N = 6.

Disclosures: Y. Yang: None. **C. Weixel:** None. **H. Chen:** None.

Nanosymposium

264. Non-Invasive Neuromodulation Using Ultrasound, TMS, and Other Approaches

Location: SDCC 25

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 264.06

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH R01 MH112142

Title: Alternating functional connectivity with focused ultrasound exposure in dorsal striatum of nonhuman primates

Authors: *D. LIU[1], **F. A. MUNOZ SILVA**[1], **A. POULIOPOULOS**[2], **E. KONOFAGOU**[2], **V. P. FERRERA**[1];
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Abstract: Focused ultrasound has emerged as a non-invasive technique to reversibly disrupt the blood-brain barrier, or as a means of neuromodulation. This study aims to investigate the effect of focused ultrasound on the pattern of functional connectivity in NHP brains using resting state functional MRI. Two conditions of FUS exposures were applied to the caudate nucleus of the anesthetized NHPs: 1) FUS exposures only (FUS-neuromodulation: 500kHz, 800kPa peak pressure, 2 Hz pulse repletion, 10 ms pulse length, 2% duty cycle, 2 min total duration, 2 NHPs); 2) Same FUS exposure but combined with microbubbles (4-5μm diameter, 2.5×10^8 microbubbles/kg, 2 NHPs). A continuous series of 4 runs (60 min, 15 min/run) resting state fMRI for each NHP were performed starting 45 min after ultrasound exposures. As control, baseline fMRI scans were performed on all 6 NHPs for a total of 16 runs (2-4 runs for each NHP) without FUS exposures. The pre-processing and analysis of fMRI data were performed using FSL 6.0.3 and Matlab 2020a. The seed-based correlations among the FUS target and the other regions were calculated. The resulting correlation coefficients were transformed using Fisher's Z transform and fed into a 5000-resample non-parametric permutation test ($p<0.05$ represents a significance difference) to compare the BOLD activity among baseline, FUS neuromodulation and FUS-BBB opening. The average seed-based correlation maps and the difference map among these three conditions were also determined. When FUS exposures without microbubbles were applied to caudate, an increased mean correlation between FUS target and dorsomedial prefrontal (9m and 10mr, $p<0.05$), superior temporal (RT, RTM, and RTp, $p<0.001$) and insular cortex (Ial and Id, $p<0.001$) were found, slightly decreased correlation were found in some regions in dorsolateral prefrontal cortex (8Bd, 8Bs and 8Ad, no statistical significance, $p>0.05$) and premotor cortex. In contrast, the same FUS exposure combined with microbubbles resulted in a significant increased mean correlation between FUS target and most regions in dorsomedial and dorsolateral prefrontal cortex (8Bm, 9m, 8Bd, 8Bs, 8Ad and 9d, $p<0.001$), anterior cingulate cortex (24c and 24c', $p<0.001$) and ventrolateral prefrontal cortex. In conclusion, the same FUS exposure with and without microbubbles showed different patterns of functional connectivity, which may indicate different modulating effects on neural activity with FUS neuromodulation only and FUS mediated BBB opening. Further studies will be focusing on the role of microbubble doses on dramatic changes of functional connectivity.

Disclosures: D. Liu: None. **F.A. Munoz Silva:** None. **A. Pouliopoulos:** None. **E. Konofagou:** None. **V.P. Ferrera:** None.

Nanosymposium

264. Non-Invasive Neuromodulation Using Ultrasound, TMS, and Other Approaches

Location: SDCC 25

Time: Monday, November 14, 2022, 8:00 AM - 9:45 AM

Presentation Number: 264.07

Topic: I.08. Methods to Modulate Neural Activity

Title: Sonogenetics for locomotor behavior modulation in freely moving mice

Authors: *K. XU, Y. YANG, Z. HU, Y. YUE, H. CHEN;
Washington Univ. in St. Louis, St. Louis, MO

Abstract: Neuromodulation tools are critical for establishing the causal link between neural circuits and behavior. We developed sonogenetics by using focused ultrasound (FUS) to noninvasively activate genetically defined neuron populations overexpressed with thermosensitive TRPV1. While we previously demonstrated successful mouse locomotor behavior modulation in a deep brain region (striatum), the application to target superficial brain regions was not demonstrated. Targeting superficial brain regions using FUS-induced heating requires careful FUS parameter selection to avoid confounding effects associated with skull heating and tissue damage. Therefore, we evaluated the feasibility and safety of TRPV1-mediated sonogenetics to modulate the locomotor behavior of freely moving mice by targeting the motor cortex. C57/BL6 mice (female, 8-10 weeks old) were used. AAV was injected to the left motor cortex to express TRPV1 (TRPV1+, n=7) in excitatory neurons. After a month, a wearable FUS device targeting the motor cortex was attached to the mouse head to control neuronal activity by activating TRPV1 through FUS sonication at different acoustic pressures for causal control of rotational behavior. Control mice were injected with AAV without TRPV1 (TRPV1-, n=7). After FUS sonication, the mouse brains were harvested for immunohistochemical staining and safety analyses. Upon FUS stimulation at 0.7 MPa, TRPV1+ mice displayed rotational behavior in the direction contralateral to the stimulation site, while TRPV1- mice did not display rotational preference in either direction (Figure 1). However, increasing the acoustic pressure to 1.1 MPa did not achieve successful neuromodulation in TRPV1+ mice, which is potentially due to the inhibitory effect related to FUS heating seen in TRPV1- mice. Immunohistological analysis of the mouse brain after FUS stimulation at 0.7 MPa did not find signs of inflammation or apoptosis (GFAP, Iba1, and Caspase-3). These findings demonstrate that TRPV1-mediated sonogenetics can achieve safe and effective neuromodulation in superficial brain regions.
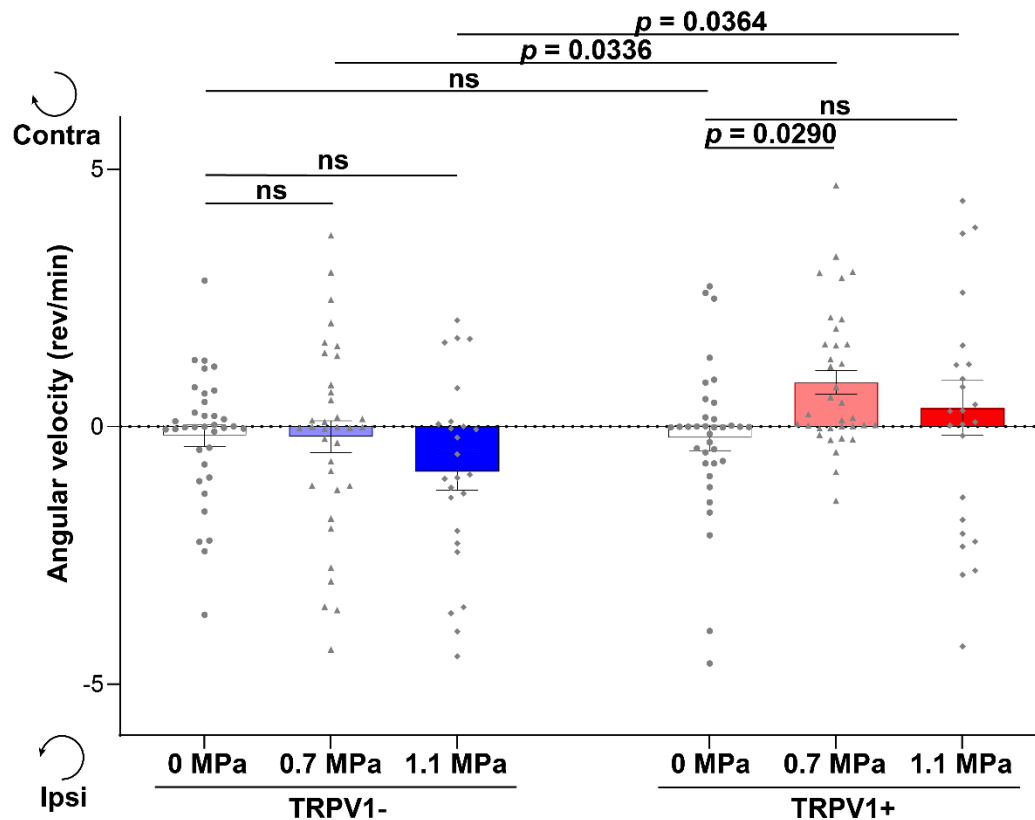
Figure 1. Ultrasound neuromodulation at different acoustic pressures in mice without and with overexpression of TRPV1. Angular velocities of TRPV1- and TRPV1+ mice revealed successful TRPV1-mediated sonogenetic neuromodulation at 0.7 MPa, but not 1.1 MPa. Statistics: Mean ± SEM, two-way ANOVA with Bonferroni post-hoc comparison.

Disclosures: K. Xu: None. **Y. Yang:** None. **Z. Hu:** None. **Y. Yue:** None. **H. Chen:** None.

Nanosymposium

343. Axon Growth, Dynamics, and Transport

Location: SDCC 7

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 343.01

Topic: A.05. Axon and Dendrite Development

Title: Ctip2/bcl11b orchestrates subcerebral projection neuron axon development via cell-autonomous and non-cell-autonomous functions

Authors: *Y. ITOH, M. B. WOODWORTH, L. C. GREIG, A. ENGMANN, J. J. HATCH, K. X. LIU, J. D. MACKLIS;
Harvard Univ., Cambridge, MA

Abstract: Axonal projection is regulated by both cell-intrinsic competency and extracellular cues/environment, although how these elements are coordinated remains elusive. Subcerebral projection neurons (SCPN) reside in deep layer V of neocortex, and extend their primary axons through the striatum ("internal capsule") to targets caudal to the cerebrum–the brainstem and spinal cord. Investigating conditional *Ctip2*/*Bcl11b* mutant mice with gene deletion in cortex (*Emx1-Cre*) and/or striatum (*Gsx2-Cre*), we identify that Ctip2 functions in multiple independent neuron populations to control SCPN axon development. *Ctip2* expressed by SCPN is required cell-autonomously for axonal outgrowth, pathfinding, and connectivity; *Ctip2* expressed by striatal ("medium spiny") projection neurons non-cell-autonomously regulates SCPN axon fasciculation within the internal capsule and cerebral peduncle; double conditional *Ctip2* mutant mice exhibit synergistic and more pronounced SCPN axon projection defects, largely recapitulating those observed in *Ctip2*-null mice. These results indicate that *Ctip2* orchestrates SCPN axon growth and guidance with distinct functions in two distinct but interacting neuron populations, via both cell-autonomous and non-cell-autonomous mechanisms. To directly investigate subcellular molecular mechanisms by which *Ctip2* cell-intrinsically controls SCPN axon projection targeting, we purified SCPN axonal growth cones and somata *in vivo* during development, and investigated their local subcellular transcriptomes. This "subcellular RNA mapping" identifies distinct, subcellularly-specific transcriptomic changes in the growth cone or/and soma compartments of *Ctip2*-mutant SCPN. Together, these results demonstrate that *Ctip2* controls multiple aspects of precise SCPN axon development by coordinated intrinsic and extrinsic mechanisms.

Disclosures: Y. Itoh: None. **M.B. Woodworth:** None. **L.C. Greig:** None. **A. Engmann:** None. **J.J. Hatch:** None. **K.X. Liu:** None. **J.D. Macklis:** None.

Nanosymposium

343. Axon Growth, Dynamics, and Transport

Location: SDCC 7

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 343.02

Topic: A.05. Axon and Dendrite Development

Title: Selective axonal transport through branch junctions is directed by growth cone signaling and mediated by KIF1/kinesin-3 motors

Authors: *S. TYMANSKYJ, B. CURRAN, L. MA;
Thomas Jefferson Univ., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Development of a functioning nervous system requires the establishment of individual neurons communicating with multiple targets, achieved by a single axon generating multiple branches. These branches differ in lengths and targets, and as such have different metabolic and protein needs. Delivery of proteins from the cell body to the correct targets rely on the orchestration of microtubule-based transport by several kinesin families of motor proteins, but if and how protein or membrane cargos are regulated along the axon, especially at the branch junctions, is largely unknown. Here, using cultured primary rodent sensory neurons, we demonstrate that cargo delivery is in fact regulated through branch junctions where they can target specific branches. We show that anterograde transport of LAMP-1 and synaptic vesicles through axonal branch junctions is highly selective, influenced by branch length and more strongly by growth cone motility. We further show that not all cargos are responsive to the same cues with secretory vesicles such as BDNF being largely unresponsive. To further demonstrate this regulation, we employed an optogenetic tool based on the receptor activity for positive (TrkA) or negative (PlexinA4) growth signaling to focally activate one specific growth cone. Using this method, we found that signaling from the growth cone can rapidly direct transport through branch junctions located a distance away from the site of activation. Using shRNA knockdown, we further demonstrate that such transport selectivity of LAMP1 vesicles is differentially regulated and mediated by the KIF1/kinesin-3 family motors. We propose that this transport regulation through branch junctions could broadly impact neuronal development, function, and regeneration.

Disclosures: S. Tymanskyj: None. **B. Curran:** None. **L. Ma:** None.

Nanosymposium

343. Axon Growth, Dynamics, and Transport

Location: SDCC 7

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 343.04

Topic: A.05. Axon and Dendrite Development

Title: Biogenesis and trafficking of Clathrin and related endocytic proteins in Axons and Synapses

Authors: *R. SHARMA[1], A. GANGULY[3], N. BOYER[1], F. WERNERT[4], S. PHAN[2], D. BOASSA[2], L. PARRA[2], U. DAS[2], G. CAILLO[4], X. HAN[5], J. YATES[5], C. LETERRIER[4], S. ROY[1];
[1]Pathology, [2]Univ. of California San Diego, LA JOLLA, CA; [3]Univ. of Rochester, New York, NY; [4]Aix Marseille Univ., Marseille, France; [5]The Scripps Res. Inst., LA JOLLA, CA

Abstract: Clathrin has established role in non-neuronal cells, with clathrin cages enclosing membrane infoldings, followed by rapid disassembly and reuse of monomers. However, in neurons, clathrin is conveyed in slow axonal transport over days to weeks, and the underlying transport/targeting mechanisms, mobile cargo structures, and even its precise presynaptic localization and physiologic role are unclear. We found that unlike in dendrites, where clathrin

cages rapidly assemble and disassemble; in axons, clathrin and related proteins organize into stable "transport packets" that are unrelated to endocytosis and move intermittently on microtubules, generating an overall slow anterograde flow. At synapses, multiple clathrin packets abut - but are not within - synaptic vesicle (SV) clusters, and clathrin packets also exchange between synaptic boutons in a microtubule-dependent "superpool." While clathrin packets are stable during axonal transport, surprisingly, those within synaptic boundaries are dynamic - continuously exchanging between local clathrin assemblies - and depletion of synaptic packets impairs SV recycling. Despite the dogma that slow transport cargoes are not synthesized in the ER-->Golgi, our experiments suggest that the axonal clathrin packets are generated by the biosynthetic pathway. Collectively, the data provide a conceptual framework for understanding clathrin trafficking and presynaptic targeting that has functional implications.

Disclosures: R. Sharma: None. **A. Ganguly:** None. **N. Boyer:** None. **F. Wernert:** None. **S. Phan:** None. **D. Boassa:** None. **L. Parra:** None. **U. Das:** None. **G. Caillo:** None. **X. Han:** None. **J. Yates:** None. **C. Leterrier:** None. **S. Roy:** None.

Nanosymposium

343. Axon Growth, Dynamics, and Transport

Location: SDCC 7

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 343.05

Topic: A.05. Axon and Dendrite Development

Title: Redox environment and Semaphorin 3a effects on rat hippocampal neurites assessed by microfluidic chemical isolation with SLIM imaging

Authors: *M. NORSWORTHY[1], **M. U. GILLETTE**[2], **G. POPESCU**[3], **M. SAKAKURA**[4];
[1]Univ. of Illinois Champaign Urbana, Champaign, IL; [2]Dept. of Cell & Developmental Biol., Univ. of Illinois, Urbana-Champaign Neurosci. Program, Urbana, IL; [3]Univ. of Illinois At Urbana-Champaign, Urbana, IL; [4]Univ. of Illinois Urbana Champaign, Urbana, IL

Abstract: Redox environment and Semaphorin 3a effects on rat hippocampal neuritesassessed by microfluidic chemical isolation with SLIM imaging Norsworthy, Miles[1], Masa Sakakura[2,4], Gabriel Popescu[2,4], and Martha U. Gillette[1,3,4]Dept. of Cell & Developmental Biology[1], Electrical& Computer Engineering[2],Neuroscience Program[3], and Beckman Institute forAdvancedScience &Technology[4],University of Illinois at Urbana-Champaign, Urbana, IL 61801Brain function emerges when $>80 \times 10^9$neurons formappropriate connections.Amongthe myriad factors that influence the ability of neurons to properly wire connections areredox states (Torres & Forman, 2003) & secreted signals, such as semaphorins(Kolodkin et al, 1992). However, interactions between these signals at neuronal &sub-neuronal levels are unknown. We

investigated sub-neuronal differences inglutathione (GSH) redox state & the interaction of redox states with semaphorin 3a(Sema3a) signaling within rat hippocampal neurons. We showthat hippocampalneurons do not maintain a reduced or oxidized GSH state in axons or dendrites at 2, 4,& 7 days*in vitro*. We also demonstrate that bath applicationof oxidizing reagents orSema3a alone diminishes hippocampal axon length, but has no effect on axon lengthwhen applied together. Furthermore, we investigate the long-term (days) & short-term(seconds to minutes) effects of redox reagents & Sema3a on the dendrites & axons ofhippocampal neurons.This is accomplished with microfluidic devices that permitdirectional flows of reagents as well as chemical isolation of different portions of theneurons to better simulate the distinct microenvironments that neuronal processesexperience within the developing brain. Long-term effects are measured by theorientation of axons & dendrites of cultured neurons in relation to the flow of reagents &short-term effects are measured via neurite isolation & Spatial Light InterferenceMicroscopy (SLIM) within microfluidic devices. SLIM is used to measure changes in drymass with accuracy comparable to atomic force microscopy, but at speeds orders ofmagnitude greater (Wang et al., 2011). Combining chemical isolation of axons &dendrites cultured in microfluidic devices with SLIM allows for accurate, label-free,real-time measuring of dry-mass changes in response to a localized signal of redoxreagents &/or Sema3a.This work elucidates the effects of redox conditions on thesignaling of Sema3a in ways not possible with more conventional techniques & furtherexpands understanding of brain function at neuronal & sub-neuronal levels.Support:NSF DGE 17-35252Beckman Institute Vision & SpiritAward NIH R21 MH117377NIH RO1GM129709

Disclosures: M. Norsworthy: None. **M.U. Gillette:** None. **G. Popescu:** None. **M. Sakakura:** None.

Nanosymposium

343. Axon Growth, Dynamics, and Transport

Location: SDCC 7

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 343.06

Topic: A.05. Axon and Dendrite Development

Title: Gap-43 phosphorylation sites dependent upon JNK are closely related to axon growth and regeneration

Authors: *M. IGARASHI[1], M. OKADA[2];
[1]Niigata Univ, Grad Sch. Med. Dent. Sci., Niigata Univ, Grad Sch. Med. Dent. Sci., Niigata, Japan; [2]Dept Neurosurg, Niigata Univ, Brain Rs Inst., Niigata, Japan

Abstract: The growth cone is an important structure that is involved in both processes, and GAP-43 (growth associated protein-43 kDa) is believed to be the classical molecular marker.

Previously, we used growth cone phosphoproteomics to demonstrate that S96 GAP-43 in rodents is a highly phosphorylated site that is phosphorylated by c-jun N-terminal protein kinase (JNK) [1]. We also revealed that phosphorylated pS96 antibody recognize growing axons in the developing brain and regenerating axons in adult peripheral nerves. In rodents, T172 and S142 are the additional putative JNK-dependent phosphorylation site that is modified at a lower frequency than S96. Here, we characterized this site using pT172- and pS142-specific antibodies. We confirmed that these two sites were detected by co-expressing mouse GAP-43 and JNK1. These antibodies labeled growth cones and growing axons in developing mouse neurons. Comparison of amino acid sequences indicated that rodent S142 and T172 correspond to human S151 and T181, we confirmed that these antibodies recognized human phospho-GAP-43 using activated JNK1, and also that its immunostaining patterns in neurons differentiated from human induced pluripotent cells (hiPSCs) were similar to those observed in mice. These results indicate that S142 and T172 residue is phosphorylated by JNK1 and that pS142 and pT172 antibodies are new potential molecular markers for axonal growth in both rodents and human [2, 3].Ref.: 1) iScience 4: 190 ['18]; 2) Mol Brain 14: 66 ['21]; 3) Neurochem Res (in press)

Disclosures: M. Igarashi: None. **M. Okada:** None.

Nanosymposium

343. Axon Growth, Dynamics, and Transport

Location: SDCC 7

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 343.07

Topic: A.05. Axon and Dendrite Development

Support: NSERC
        FRQS

Title: Regulation of the axonal translatome in dopaminergic circuits during development.

Authors: *C. GORA[1], S. HUSSEIN[2], E. METZAKOPIAN[3], M. LÉVESQUE[1];
[1]CERVO brain research center, Québec, QC, Canada; [2]Mol. biologym medical biochemistry and pathology, Laval Univ., Quebec, QC, Canada; [3]Natl. Inst. For Med. Res., London, United Kingdom

Abstract: Midbrain dopaminergic (mDA) neurons play important roles in controlling a variety of brain functions. An abnormal development of the dopaminergic circuits can lead to different brain disorders. In addition, degeneration of mDA neurons is the leading cause of Parkinson's disease. Dopamine neurons in the midbrain form a heterogeneous set of neurons that innervate different regions of the brain. However, the developmental mechanisms regulating the precise organization of these neuronal circuits remain poorly understood. Recent discoveries have revealed that a proportion of the proteins used for the navigation of developing axons are produced locally in axons. This project aims to reveal the cellular and molecular mechanisms regulating the precise development of dopaminergic sub-circuits. To identify mRNAs locally

translated in mDA axons, we used transgenic mice in which ribosomes in mDA neurons are specifically tagged (RiboTag mice). Using these mice, we isolated mRNAs associated with the ribosomes in mDA axons innervating different brain regions and at different developmental time points. Analysis of axonal mRNA content was performed by RNA-sequencing. Our results indicate that specific set of mRNAs are present in axons innervating the striatum, the nucleus accumbens or the prefrontal cortical areas. We are now validating and study the role of axon guidance molecules present in mDA axons. In addition to uncover the axonal translatome of the dopaminergic system, this project help to better understand the development of the dopaminergic circuits.

Disclosures: C. Gora: None. **S. Hussein:** None. **E. Metzakopian:** None. **M. Lévesque:** None.

Nanosymposium

343. Axon Growth, Dynamics, and Transport

Location: SDCC 7

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 343.08

Topic: A.05. Axon and Dendrite Development

Title: APP and GABA$_B$ receptor functionally interact to regulate neurite growth

Authors: *D. BARBER[1], S. BHANDARI[2], C. LACY[2], K. SHUKLA[2], S. HOUMAM[2], S. SIMONS[2], H. RICE[2];
[1]Oklahoma Ctr. for Geroscience and Healthy Brain Aging, Neurosci. Program, [2]Oklahoma Ctr. for Geroscience & Healthy Brain Aging, Dept. of Biochem. & Mol. Biol., Univ. of Oklahoma Hlth. and Sci. Ctr., Oklahoma City, OK

Abstract: Neurodevelopment is the first stage of life in which the brain undergoes vast anatomical and physiological changes often impacting life in its final stages. The amyloid precursor protein (APP), a protein central to the pathogenesis of Alzheimer's disease, also contributes to many crucial processes in the brain throughout neurodevelopment, including neurogenesis, neuronal migration and neurite growth. Recently, we discovered that sAPPα functions as a GABA$_B$R1a-isoform specific ligand to modulate synaptic transmission in neurons. The GABA$_B$ receptor has been implicated in a number of parallel neurodevelopmental processes including neurite outgrowth. Both APP and GABA$_B$ have yet to be investigated as part of the same developmental pathway and their novel interaction provides the impetus for such an inquiry. Utilizing a combination of immunohistochemistry (IHC), primary neuronal cultures of C57/Bl6 mice, and confocal microscopy we investigated the influence soluble APP protein, sAPPα, had on the longest neurite of E18 primary neurons at 72 hours post treatment with

500nM sAPPα. Neurites were identified by immunostaining with Beta III Tubulin a neurite marker, MAP2 a dendritic marker, and SMI-312 an axon marker. We found that treatment with either: Baclofen a GABA$_B$ agonist, sAPPα, or a 17 amino acid peptide corresponding to the specific binding region of sAPPα to the sushi domain of the GABA$_B$ receptor significantly reduced the outgrowth of the longest neurite of primary neurons (N (3 Trials) = 196-214,226-262,195-365 neurons/trial | Median = 81.00, 65.65, 84.04, IQR = 47.61-118.7, 43.90-101.1, 53.05-132.3) compared to untreated controls (N (3 Trials) = 67-178 neurons/trial | Median = 106.8, IQR 69.55-146.1) (Kruskal Wallis Test (One-Way ANOVA) P= <0.0001). In addition to this, we found that sAPPα required the specific binding region to elicit this impact on neurite outgrowth by treating with a form of sAPPα lacking the crucial extension domain responsible for the interaction. These results reveal a potential mechanism by which sAPPα and the GABA$_B$ receptor influence neurodevelopment. In addition, GABA$_B$ and APP function are known to be disturbed in a number of neurodevelopmental disorders including Autism Spectrum Disorder, Fragile X Syndrome, and Angelman Syndrome. We are concurrently investigating this mechanism as a novel inroad into therapeutic targets regarding Fragile X Syndrome and its known disturbances to neurodevelopment namely involving both the GABA$_B$ receptor and APP.

Disclosures: D. Barber: None. **S. Bhandari:** None. **C. Lacy:** None. **K. Shukla:** None. **S. Houmam:** None. **S. Simons:** None. **H. Rice:** None.

Nanosymposium

344. Ionotropic Receptors: Trafficking, Modulation, and Regulation

Location: SDCC 1

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 344.01

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

Support: Agencia Nacional de Promoción Científica y Tecnológica

Title: Role of the TM2-TM3 loop in the potentiation mechanism of the a9a10 cholinergic nicotinic receptor by extracellular calcium.

Authors: *S. L. GALLINO[1], P. CRAIG[2], J. C. BOFFI[3], P. PLAZAS[4], B. ELGOYHEN[1,5]; [1]INGEBI-CONICET, Ciudad de Buenos Aires, Argentina; [2]Inst. de Química Biológica, Facultad de Ciencias Exactas y Naturales (IQUIBICEN, UBA/CONICET), Ciudad de Buenos Aires, Argentina; [3]Inst. For Anat. and Cell Biology/Heidelberg, Inst. For Anat. and Cell Biology/Heidelberg, Heidelberg, Germany; [4]Inst. de Farmacología, Facultad de Medicina, Ciudad de Buenos Aires, Argentina; [5]Facultad de Medicina, Univ. de Buenos Aires, Inst. de Farmacología, Ciudad de Buenos Aires, Argentina

Abstract: The α9α10 nicotinic acetylcholine receptor (nAChR) is expressed in cochlear hair cells. This nAChR mediates the inhibitory synapse between efferent fibers and outer hair cells. The inhibition results from calcium entry through the nAChR, in the presence of acetylcholine (ACh), followed by the activation of a Ca^{2+} dependent potassium current. The α9α10 nAChR

plays a key role in auditory neural circuits at the post-hearing onset. Hence, a deep understanding of the modulatory effects of this cholinergic receptor in auditory circuits would be extremely useful for the development strategies towards hearing loss. The α9α10 nAChR is a pentameric cation-permeable ion channel that is composed of α9 and α10 subunits. Each nAChR subunit comprises a large extracellular amino-terminal domain, four transmembrane domains (TM1-TM4), a long cytoplasmic loop between TM3 and TM4 and a C-terminal domain. Expression of rat α9 and α10 nAChR subunits in *Xenopus laevis* oocytes yields functional α9 and α9α10 receptors, but not α10 homomeric nAChRs. One of the functional differences between α9 and α9α10 nAChRs is the modulation of their ACh-evoked responses by extracellular Ca^{2+}. While α9 nAChRs responses are blocked by Ca^{2+}, ACh-evoked currents through α9α10 nAChRs are potentiated by Ca^{2+} in the micromolar range and blocked at millimolar concentrations. In order to identify the structural determinants responsible for Ca^{2+} potentiation, we generated several chimeric and mutant α10 subunits, expressed them in *Xenopus* oocytes and performed electrophysiological recordings under two electrode voltage clamp. Our results suggest that the TM2-TM3 loop of the α10 subunit contains structural determinants responsible for the potentiation of the α9α10 nAChR by Ca^{2+}. Furthermore, we identified α10 E71 and E 202 as possible key residues of two potential Ca^{2+} binding sites involved in this potentiation. Moreover, to elucidate the mechanism of this potentiation by extracellular Ca^{2+} we performed molecular dynamics simulations of the interaction of Ca^{2+} with different nAChRs models (α9 and α10 homomeric receptors, and α9α10 heteromeric receptor in both grouped and alternated configurations). The result of this study shows that both heteromeric α9α10 and homomeric α9 nAChRs exhibit similar calcium binding in the environment of their TM2-TM3 loops. Therefore, our hypothesis is that the TM2-TM3 loop of the α10 subunit contributes with structural determinants that are key for the gating of the α9α10 nAChR, once ACh binding has occurred in the presence of extracellular Ca^{2+}.

Disclosures: S.L. Gallino: None. **P. Craig:** None. **J.C. Boffi:** None. **P. Plazas:** None. **B. Elgoyhen:** None.

Nanosymposium

344. Ionotropic Receptors: Trafficking, Modulation, and Regulation

Location: SDCC 1

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 344.02

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH NINDS F31NS120586
         NSF IOS#1941073

Title: An inter-tissue feedback signal that couples muscle activity to glutamate receptor trafficking in distal upstream interneurons

Authors: *B. J. RENNICH[1], M. HODUL[1], P. JUO[2];
[1]Grad. Program in Neurosci., Tufts Grad. Sch. of Biomed. Sci., Boston, MA; [2]Tufts Univ. Sch. of Med., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Regulation of the number of AMPA-type glutamate receptors (GluRs) in the postsynaptic membrane controls synaptic strength and is a major mechanism underlying synaptic plasticity. It is becoming increasingly apparent that extracellular signals can modulate synaptic plasticity by controlling GluR surface levels. However, we are only beginning to understand the mechanisms by which secreted factors influence glutamatergic synapses and circuit function, especially those mediated by signals that act at a distance between different tissues or cell-types. In *C. elegans*, there are many extrasynaptic secreted signaling molecules, including neuromodulators and over 200 neuropeptides (many with unknown function), that could potentially regulate synaptic and circuit function. Here, we identify an inter-tissue signal in *C. elegans* that couples changes in muscle activity with GLR-1/GluR surface abundance in distal upstream interneurons that control locomotion. Mutants lacking the neuromuscular junction (NMJ) acetylcholine receptor (AChR) subunits *unc-29* or *unc-38*, exhibit a compensatory increase in surface levels of GLR-1 in the backward locomotion command interneuron AVA. This increase in surface GLR-1 could be rescued by expressing wild type cDNAs of *unc-29* or *unc-38* specifically in the body wall muscle of the respective mutant, revealing a feedback pathway that appears to couple NMJ signaling with GLR-1 trafficking in AVA. Chronic loss of muscle contraction in *unc-54*/muscle myosin mutants also results in increased surface GLR-1 levels in AVA, suggesting that lack of muscle contraction is sufficient to trigger the feedback pathway. Acute loss of muscle activity induced with temperature-sensitive alleles of *unc-54*/myosin or *twk-18*/potassium channels, was sufficient to trigger the feedback pathway in larval L4 animals, suggesting that the change in surface GLR-1 levels can be engaged on a relatively short timescale and cannot be attributed to a developmental defect. Finally, loss of function mutations in *unc-31*/CAPS, which mediates the release of neuropeptide-containing dense-core vesicles, blocks the feedback pathway triggered by either *unc-29*/AChR or *unc-54*/myosin mutants. Together, our results identify a novel inter-tissue signal dependent on *unc-31*/CAPS that couples muscle activity with surface levels of GluRs in distal upstream interneurons. We propose that this compensatory feedback signal adjusts the strength of motor circuit excitability in response to changes in muscle contraction and may be engaged under conditions of declining muscle function.

Disclosures: B.J. Rennich: None. **M. Hodul:** None. **P. Juo:** None.

Nanosymposium

344. Ionotropic Receptors: Trafficking, Modulation, and Regulation

Location: SDCC 1

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 344.03

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

Title: Discrepancy between NMDA receptor effects at synapse and dendrite in patient derived GRIN1 mutant mouse leads to unexpected treatment opportunity

Authors: *S. VENKATESAN[1], A. J. RAMSEY[2], E. K. LAMBE[3];
[2]Pharmacol. and Toxicology, [3]Dept Physiol., [1]Univ. of Toronto, Toronto, ON, Canada

Abstract: GRIN1 neurodevelopmental disorder is a rare disease caused by mutations in the obligate GluN1 subunit of the NMDA receptor (NMDAR). Y647S+/- mutation in the transmembrane region of GluN1 causes intellectual disability and seizures in a patient, with unknown effects on NMDAR function and synaptic integration. To determine appropriate treatment strategies, we sought to identify the nature of NMDAR deficits using transgenic mice of both sexes heterozygous for the Y647S mutation compared to littermate controls. Patch-clamp electrophysiology in prefrontal layer 5 pyramidal neurons revealed seemingly paradoxical results. Some aspects of NMDAR signaling are diminished, but others are amplified/prolonged. Electrically evoked synaptic NMDAR EPSCs are significantly smaller in Y647S mice, yet whole-cell currents evoked by bath-applied NMDA are significantly larger. This contradictory pattern is also observed on examining dendritic plateau potentials that require NMDARs for synaptic integration. The amplitude of plateau potentials is smaller in Y647S mice, but their duration is significantly and unexpectedly prolonged. We hypothesize that this pattern in Y647S mice arises from a combination of deficient synaptic NMDARs along with an impairment in typical NMDAR recruitment of calcium-activated potassium channels to act as brakes on postsynaptic activity. Consistent with this hypothesis, a drug potentiating calcium-activated potassium channels (NS309) is successful in reducing whole-cell currents evoked pharmacologically with NMDA. NS309 also restores appropriate timing to dendritic plateau potentials in Y647S mice. These findings give insight into dynamic interactions between NMDARs and proximal ion channels and identifies a new research direction for GRIN disorder treatment.

Disclosures: S. Venkatesan: None. **A.J. Ramsey:** None. **E.K. Lambe:** None.

Nanosymposium

344. Ionotropic Receptors: Trafficking, Modulation, and Regulation

Location: SDCC 1

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 344.04

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

Title: Optimization of photoswtichable NMDA receptor positive allosteric modulators

Authors: *Y. GE[1], Z. ZHANG[2,3], G. LE[2], P. AXERIO-CILIES[1], M. DONG[2,4], A. WOOLLEY[2], Y. WANG[1];

[1]Univ. of British Columbia, Univ. of British Columbia, Vancouver, BC, Canada; [2]Univ. of Toronto, Toronto, ON, Canada; [3]East China Univ. of Sci. and Technol., Shanghai, China; [4]Qingdao Univ., Qingdao, China

Abstract: Light control of glutamate receptor ion channel activities, including using small molecule photoswitch for manipulating N-methyl-d-aspartate glutamate receptor (NMDAR) functions, is an important method for studying brain function. Our previous virtual drug screening has identified several NMDAR positive allosteric modulators (PAMs), some of which contain an arylhydrazone backbone that can undergo E/Z isomerization under appropriate conditions. In this study, combining analytical chemistry approaches and electrophysiology recordings of the HEK293 cells transiently expressing rat NMDARs containing GluN1/GluN2A or GluN1/GluN2B subunits, we optimized the photochemical and pharmacological properties of these NMDAR PAMs. We found that one arylhydrazone compound named 217 readily underwent *E*-to-*Z* isomerization under blue light, resulting in over 100-fold less potency in modulating the currents gated through two prominent NMDAR subtypes; moreover, another compound named HILKOD underwent reversible *E*-to-*Z* and *Z*-to-*E* isomerization using blue light (450nm) and UV light (310nm), respectively. The two photoisomers also had substantially different bioactivities on NMDARs. To demonstrate the usefulness of these novel photoswitches, we showed that optical control of NMDAR activities during electrophysiology experiments was possible with compound HILKOD. We concluded that arylhydrazone compounds could be useful photoswitches for NMDARs, and further optimizing these compounds might result in highly effective research tools for precise manipulation of NMDAR activities.

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Nanosymposium

344. Ionotropic Receptors: Trafficking, Modulation, and Regulation

Location: SDCC 1

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 344.05

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

Title: A Dual Role for LAR-RPTP in Regulating Long-distance Transport and Synaptic Retention of AMPARs

Authors: D. PIERCE[1], Z. LENNINGER[1], R. L. DOSER[1], K. KNIGHT[1], A. STETAK[2], *F. J. HOERNDLI[1];

[1]Biomed. Sci., Colorado State Univ., Fort Collins, CO; [2]Biozentrum, UniBasel, Basel, Switzerland

Abstract: The AMPA subtype of ionotropic glutamate receptors (AMPARs) plays an essential role in excitatory synaptic transmission, learning, and memory. The majority of AMPA receptors are made in the cell body and are transported by molecular motors to synapses. Maintaining a proper number of synaptic receptors requires a coordinated regulation of production of receptors, export from the soma and delivery at synapses.This is a major logistical process for neurons and essential for circuit function and behavior. Although recent studies have shown that long-distance synaptic transport is regulated by neuronal activity, little is known about mechanisms that would coordinate somatic export and synaptic delivery and removal. Here we show that loss of the PTP-3A isoform of the receptor tyrosine phosphatase PTP-3 (the C. elegans homologue of vertebrate LAR-RPTP) leads to a ~60% decrease in AMPAR transport; this affects synaptic delivery of AMPARs and synaptic functions necessary for long-term associative olfactory memory in *C. elegans*. We reveal that the PTP-3A isoform is necessary postsynaptically in adult neurons for the regulation of AMPAR transport, delivery, and removal. Interestingly, while complete loss of PTP-3A leads to defects in transport and local synaptic trafficking of AMPARs, loss of PTP-3 phosphatase function only affects local synaptic cycling and retention. Finally, we show that the N- terminal of PTP-3A regulates transport, whereas the C-terminal regulates synaptic retention of AMPARs. Altogether, our results suggest a model in which the two domains of PTP-3/LAR RPTPs have specific complementary roles in coordinating somatic export and local retention of AMPARs essential for long-term associative memory.

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Nanosymposium

344. Ionotropic Receptors: Trafficking, Modulation, and Regulation

Location: SDCC 1

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 344.06

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

Title: Neuronal alternative splicing of gephyrin C4 cassettes modulates condensate formation in vitro and synaptic scaffolding in cultured neurons

Authors: *F. LIEBSCH, A. BODENHAUSEN, A. F. LÜTZ, G. SCHWARZ;
Univ. of Cologne, Koeln, Germany

Abstract: Gephyrin is the key postsynaptic scaffolding protein at glycinergic and a subset of gamma aminobutyric acid (GABA)-ergic inhibitory synapses. Neurons express different gephyrin splice variants; however, it is unknown whether all isoform act to the same extent as synaptic organizers. To characterize the scaffolding properties of neuronal gephyrin splice variants, specifically those differing in the incorporation of C4 cassettes, we studied the

biophysical properties of recombinantly produced isoforms and assessed synaptic scaffolding of fluorophore-tagged variants in dissociated neurons. Recombinantly produced isoforms, namely gephyrin P1 (without additional splice cassette), - C4a, - C4c, or - C4d, displayed similar binding affinities with a soluble pentameric model of the glycine receptor intracellular loops, as measured by isothermal titration calorimetry. Biomolecular condensate formation is a process that has been implicated in inhibitory postsynaptic density sheet formation. Interestingly, purified isoforms containing either C4a or C4d cassettes displayed more rapid phase separation with the model interaction partner than the other variants, as determined by time-resolved microscopy and turbidity kinetic assays. For the synaptic scaffolding analysis, the formation of cytosolic aggregates that are commonly observed upon exogenous gephyrin expression in neuronal and non-neuronal cells was circumvented by using adeno-associated virus-mediated expression of C4 isoforms in individual dissociated hippocampal CamKII-expressing neurons. An automated and quantitative analysis revealed that while P1 did not show any localization preference, C4a was enriched in the neuron's soma and distal localizations, while C4c and C4d localized mainly to distal inhibitory synapses. Our results suggest that inhibitory synapse heterogeneity may be influenced - at least in part - by mechanisms relating to C4 cassette splicing. Taken together, the alternative splicing of gephyrin could be one of the factors influencing the organization of molecules at diverse synapse populations.

Disclosures: F. Liebsch: None. A. Bodenhausen: None. A.F. Lütz: None. G. Schwarz: None.

Nanosymposium

344. Ionotropic Receptors: Trafficking, Modulation, and Regulation

Location: SDCC 1

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 344.07

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

Title: Gaba$_a$ receptor subunits in human oligodendrocyte progenitor cells

Authors: *B. A. GUTIERREZ GREBENKOVA[1], R. O. ARELLANO[2], A. LIMON[1];
[1]UTMB, Houston, TX; [2]UNAM, Queretaro, Mexico

Abstract: GABA$_A$ receptors (GABA$_A$Rs) in oligodendrocyte progenitor cells (OPCs) are thought to be an important target for the treatment of demyelinating diseases. In general, GABA$_A$Rs are pentameric complexes built from 3 different subunits encoded from a pool of 19 genes (α1-6, β1-3, γ1-3, δ, ε, π, θ, ρ1-3), still, the subunit combination/s of the receptors in human OPCs remains unknown, delaying their study as potential therapeutic targets. Our aims in the present study were to identify the subunits expressed in GABA$_A$Rs in human OPCs and to predict their stoichiometry. For the expression analysis, we used 8 available transcriptomic

datasets from human cerebral cortex of adults without known neurological disorders. We identified OPCs in the sequencing data by selecting cells positive to PDGFRα, an OPC cell surface marker. We normalized the expression units (UMIs) of each dataset by obtaining the fractional contribution (FC) of subunits to the total pool of mRNA available, where FC is the percentage of the sum of the expression levels of each subunit gene in each cell over the sum of all 19 gene subunits. We then evaluated the most likely stoichiometry of the GABA$_A$R in human OPCs by performing a multiple correlation analysis of the FC data using JMP software. We found mRNA expression of most GABA$_A$R subunit genes among all analyzed OPCs. The most abundant subunits were α1-3, β1-3 and γ1-3, while all others were barely detected. In our multivariate analysis, knowing that most GABA$_A$Rs in other cells are heteropentameric complexes formed by 2αs, 2βs, and 1γ subunit, we looked at the correlations formed by those subunits, finding α1β2γ2, α2β1/2γ2/3, and α3β3γ1 as the best combinations in the indicated order. To the best of our knowledge, this is the first report to identify GABA$_A$R subunits in human OPCs, as well as their likely stoichiometry. Our next goal is to characterize functionally and pharmacologically these receptor subunit combinations in native GABA$_A$Rs isolated from human OPCs.

Disclosures: B.A. Gutierrez Grebenkova: None. **R.O. Arellano:** None. **A. Limon:** None.

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

Location: SDCC 33

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 345.01

Topic: D.01. Somatosensation

Support: University Startup Fund

Title: High-precision automatic quantification of mice scratching behavior using deep learning

Authors: *H. YU[1], J. XIONG[2], J. ARSUAGA[2], W. LUO[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of California Davis, Davis, CA

Abstract: With the rapid development of artificial intelligence recent years, deep learning neural networks have started to be used in behavior analysis of model animals in scientific research. Many proof of principle studies show that deep learning neural networks have the ability to recognize and quantify the complex behaviors. In itch field, scratching behavior is quantified to indicate the itch intensity, as itch is defined as an unpleasant sensation that provokes the desire to scratch. Mice is the most widely used model animals for studying the mechanisms of itch in the physiological and pathological conditions. However, till now, this quantification process is mainly conducted by watching videos and manually counting mouse scratching bouts or time, which is tedious, time consuming, and limits the large scale genetic or drug screenings. In this study, we developed a method and achieved a high-precision automatic quantification of mice scratching behavior using deep learning. First, we designed a video recording box to achieve

high-quality recording of freely-moving mice in a stable environment. Then we intradermally injected chloroquine, an itch-inducing compound, into the nape of 10 mice and recorded 40 videos containing scratching behavior. We manually annotated the scratching behavior in these videos, and trained the deep learning neural networks with 32 videos. The best model is evaluated on the 8 testing videos. The recall and precision are also above 95%. We also tested the generalization of the prediction model on other itch models with recall and precision above 85%. The In summary, we developed a ready-to-use system for high-precision automatic quantification of mice scratching behavior. This strategy can also be extended for automatic analysis and quantification of other behaviors in model animals.

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

Location: SDCC 33

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 345.02

Topic: D.01. Somatosensation

Title: Piezo1 transduces mechanical itch in mice

Authors: *R. Z. HILL[1], M. LOUD[1], A. E. DUBIN[2], B. PEET[1], A. PATAPOUTIAN[1];
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Abstract: Itch triggers scratching, a behavioral defense mechanism that aids in the removal of harmful irritants and parasites. Chemical itch is triggered by many endogenous and exogenous cues, such as pro-inflammatory histamine that is released during an allergic reaction. Mechanical itch can be triggered by light sensations such as wool fibers or a crawling insect. In contrast to extensively studied chemical itch pathways, the mechanisms underlying mechanical itch transduction are largely unknown. Here we show that the mechanically activated ion channel PIEZO1, which was not previously implicated in somatosensation, is selectively expressed by itch-specific sensory neurons co-expressing somatostatin and natriuretic polypeptide precursor b

and is required for their mechanically activated currents. Loss of PIEZO1 function in peripheral neurons and in somatostatin-expressing neurons greatly reduces mechanically evoked scratching behaviors in mice. We also show that neuronal PIEZO1 is essential for both acute and chronic itch-evoked sensitization. Finally, mice carrying a gain-of-function *Piezo1* allele display enhanced mechanical itch behaviors. Our studies unveil the polymodal nature of itch sensory neurons and highlight a novel and unexpected role for PIEZO1 in the sensation of itch.

Disclosures: R.Z. Hill: None. **M. Loud:** None. **A.E. Dubin:** None. **B. Peet:** None. **A. Patapoutian:** None.

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

Location: SDCC 33

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 345.03

Topic: D.02. Somatosensation – Pain

Title: AIBP regulates TLR4-expressing lipid rafts in dorsal root ganglion neurons, reducing TRPV1 functionality and reversing polyneuropathic pain

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Abstract: Previous studies have shown that ligand- and voltage-gated receptors localize to cholesterol-enriched membrane lipid rafts. In several cell types, including nociceptive dorsal root ganglion (DRG) neurons, these lipid rafts express toll-like receptor 4 (TLR4) and the capsaicin receptor TRPV1. Activation of TLR4, as with lipopolysaccharide, leads to lipid raft clustering and enlargement, providing optimal conditions for TLR4 homo- and hetero- dimerization. ApoA-I binding protein (AIBP), a protein regulating membrane cholesterol, binds to activated TLR4, resulting in cholesterol depletion from TLR4-rafts, disrupting lipid raft function, blocking TLR4 dimerization and subsequent signaling. Prior work suggests that blocking TLR4 diminishes pain associated with chemotherapy-induced peripheral neuropathy (CIPN), as produced by cisplatin or paclitaxel. In murine paclitaxel-induced neuropathy, we examined TLR4 expression and dimerization, lipid raft content, and TRPV1 proximity to lipid rafts in DRG neurons by flow cytometry. We further assessed the association of TPRV1 to TLR4 by proximity ligation assays in whole-mount DRGs. We evaluated the functional implications of these interactions by the *in vivo* response to capsaicin in the hind paw and evaluation *ex vivo* of TRPV1 activation by capsaicin in isolated DRG neurons. We found that paclitaxel: i) induced persistent mechanical allodynia, ii) increased the size of neuronal lipid rafts, iii) increased TLR4expression, iv) increased phosphorylation of TRPV1, and v) increased proximity of

TRPV1 to TLR4 in the lipid rafts. These events were uniformly reversed by intrathecal AIBP. Furthermore, the nociceptive response to capsaicin in the paclitaxel mouse is increased, and intrathecal AIBP restores it to normal (naïve) levels. This AIBP effect is accompanied by reduced phosphorylation of DRG TRPV1 and reduced capsaicin-evoked calcium influx. Together, these results suggest that nociceptive signaling is regulated by the assembly of nociceptive receptor and ion channel complexes with TLR4 in the environment of lipid rafts in DRG neurons. These findings support our hypothesis that persistent pain phenotypes mediated by TLR4 signaling may initiate a coordinated activation of a neuraxial signaling complex organized by the TLR4-bearing membrane lipid rafts on DRG neurons.

Disclosures: J. Navia Pelaez: None. **L. Gonzalez:** None. **J. Borges Paes Lemes:** None. **G. Goncalves Dos Santos:** None. **J. Lu:** None. **T.L. Yaksh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor listed in patent applications related to the topic of this paper and scientific co-founder of Raft Pharmaceuticals LLC. **Y.I. Miller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor listed in patent applications related to the topic of this paper and scientific co-founder of Raft Pharmaceuticals LLC.

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

Location: SDCC 33

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 345.04

Topic: D.01. Somatosensation

Title: IL-4 and IL-13 directly promote neurite outgrowth and branching in murine sensory neurons

Authors: *M. K. JHA, Y. HARA, A. HICKS;
Immunol. & Inflammation, Sanofi, Cambridge, MA

Abstract: Type 2 inflammation is a key driver of the pathophysiology of multiple atopic or allergic diseases characterized by neuronal hyperinnervation and direct apposition of nerve terminals with immune cells. Although the role of neuroinflammatory axis in type 2 inflammation is ill-explored, recent studies suggest a potential role of type 2 cytokines interleukin (IL)-4, IL-13, and IL-31 in multiple type 2 inflammatory disease sensory symptoms, including itch in atopic dermatitis, pain and dysphagia in eosinophilic esophagitis, and loss of smell and taste in chronic rhinosinusitis with nasal polyposis. Targeting to these type 2 cytokines and the neuroimmune axis with biologics can be a promising strategy to attenuate these sensory symptoms of type 2 inflammatory diseases. A recent study demonstrates a significant role of IL-31 in sensory nerve elongation and branching. However, the impact of IL-4 and IL-13 on sensory neuron architecture during type 2 inflammatory responses is completely unknown. To investigate whether IL-4 and IL-13 modulate neurite outgrowth and branching in sensory neurons, we

performed immunocytochemical staining to visualize neurons using antibody against PGP9.5 and/or colocalize with the antibody against IL-4 receptor alpha (IL-4 Rα), a common receptor for both IL-4 and IL-13 cytokines. We found that IL-4 Rα is widely expressed in mouse dorsal root ganglia (DRG) neurons, which relay sensory neural messages from the periphery to the central nervous system. Interestingly, DRG neurons exposed to IL-4 and/or IL-13 cytokines had significantly increased neurite outgrowth and branching. Excitingly, the impact of IL-4 and/or IL-13 on neurite outgrowth and branching of the sensory neurons was fully comparable with that of IL-31, suggesting that IL-4, IL-13, and IL-31 are equally competent in modulating sensory axon architecture. Taken together, this study suggests that IL-4 and IL-13 signaling may play a key role in neuronal hyperinnervation in type 2 inflammatory diseases and the therapeutic benefit of targeting these cytokines in the management of sensory symptoms of these diseases may be a result of restoration of sensory neuron architecture.

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

Location: SDCC 33

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 345.05

Topic: D.02. Somatosensation – Pain

Title: Subtype specificity of collateral sprouting mediated skin reinnervation after peripheral nerve injury

Authors: *S. JEON[1], A. PRADEEP[1], L. MCDONOUGH[1], A. LATREMOLIERE[1], L. K. CRAWFORD[2], M. J. CATERINA[1];
[1]The Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]2University of Wisconsin - Madison Sch. of Vet. Med., Madison, WI

Abstract: Partial denervation, as a result of partial sciatic nerve transection, in skin results in collateral sprouting of spared sensory nerves into neighboring denervated territory. Collateral sprouting provides a potential means of restoring sensory function without surgery, but it occurs concomitantly with unwanted events such as neuropathic pain. A better understanding of

collateral sprouting mechanisms and consequences is therefore needed. In this study, we performed whole-mount immunofluorescence staining of hind paw skin in the mouse spared nerve injury (SNI) model, in which the tibial and peroneal branches of the sciatic nerves are ligated and transected, leaving the sural nerve intact. Seven days after SNI, we observed a large reduction in immunoreactivity (IR) for peptidergic (CGRP) and myelinated (NF-H) nerve fibers in the middle of the ipsilateral hind paw skin formerly supplied by the tibial nerve. However, at 28 and 56 days after injury, progressive reinnervation by CGRP$^+$ and NF-H$^+$ nerve fibers into denervated, apparently from saphenous and sural territories, was observed. We next asked whether low-threshold mechanoreceptors (LTMRs) participate in collateral sprouting after nerve injury, by performing SNI surgery on labeled LTMR mouse lines. Seven days after injury, we observed an obvious reduction in nerve terminal structures for Aβ RA-LTMRs, Aδ RA-LTMRs, Aβ SAI-LTMRs, Aβ Field-LTMRs, and C-LTMRs in the ipsilateral hind paw. Even 56 days after SNI, no collateral sprouting of any LTMR populations into the denervated region was evident. However, sympathetic nerve fibers had begun to sprout into the denervated skin by day 28 and increased until day 56, and these sympathetic neurons formed LTMR-like nerve circumferential endings on hair follicles. To define the functional recovery of collateral sprouts in denervated skin after nerve injury, we performed optogenetic experiments in Pirt^{Cre};Rosa26$^{LSL-ChR2-EYFP}$ and Calca^{Cre};Rosa26$^{LSL-ChR2-EYFP}$ mice, which express the light-gated ion channel channelrhodopsin-2 in all sensory neurons and in peptidergic nociceptors, respectively. Light-induced paw withdrawal frequency on the ipsilateral hind paw of both mouse lines dropped dramatically by 3 days after SNI, but returned gradually to near basal level over 28 days. Mechanical sensitivity, assayed utilizing von Frey monofilaments and an Austerlitz pin applied to the midline of the hind paw, also showed a marked decrease 3 days after SNI, but returned gradually to basal level by 28 days, without evidence of hyperalgesic overshoot. These results suggest that collateral sprouting contributes to functional recovery of sensory nerves in denervated skin.

Disclosures: **S. Jeon:** None. **A. Pradeep:** None. **L. McDonough:** None. **A. Latremoliere:** None. **L.K. Crawford:** None. **M.J. Caterina:** None.

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

Location: SDCC 33

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Presentation Number: 345.06

Topic: D.02. Somatosensation – Pain

Support: CIHR FDN-154336
         CIHR Canada Graduate Scholarships Doctoral Award

Title: Beclin 1 regulates inflammatory pain hypersensitivity in a sex-dependent manner

Authors: *T. H. TAM, Y. TU, W. ZHANG, S. FARCAS, M. W. SALTER;
The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Chronic pain affects approximately 1 in 5 individuals, yet safe and effective treatments are lacking, likely due to the diversity of cellular mechanisms underlying pain. Autophagy is a lysosome-mediated degradation pathway and autophagy dysfunction is implicated in many neuropathologies. Yet, whether autophagy regulates pain sensitivity is unclear. Here, we addressed this by targeting a critical protein involved in autophagy initiation, beclin 1 (*Becn1*). As a model of inflammatory pain, complete Freund's adjuvant (CFA) was administered subcutaneously to the hind paw of mice. CFA induces hypersensitivity to mechanical stimuli, as assessed by von Frey assay. Mice with monoallelic deletion of *Becn1* (*Becn1$^{+/-}$*) have greater CFA-induced mechanical hypersensitivity compared to wild type, but only in males (M), not in females (F) (14 day time course area over the curve, *Becn1$^{+/-}$* vs *Becn1$^{+/+}$*; M: $p<0.01$, n=8; F: $p>0.05$, n=8). Conversely, intrathecal administration of a beclin 1-activating peptide reverses CFA-induced mechanical hypersensitivity in wild type male mice, but had a limited effect in females (2h time course area under the curve (AUC), beclin 1-activating peptide vs control scrambled peptide; M: $p<0.05$, n=7-8; F: $p>0.05$, n=7-8). We have previously demonstrated that the male-specific pain pathway is dependent on brain-derived neurotrophic factor (BDNF) upregulation of NMDA receptor activity. Here, we found that intrathecal administration of Y1036, a BDNF inhibitor, reverses CFA-induced mechanical hypersensitivity in wild type males, but not in *Becn1$^{+/-}$* mice (1h time course AUC, *Becn1$^{+/-}$* vs *Becn1$^{+/+}$*; $p<0.05$, n=7-9). Co-administering beclin 1-activating peptide with BDNF prevents BDNF-induced mechanical hypersensitivity (30 min post-injection, BDNF+peptide vs BDNF; $p<0.01$, n=6-7). Furthermore, *Becn1$^{+/-}$* male mice express higher levels of the GluN2B subunit of the NMDA receptor in the spinal dorsal horn compared to wild type ($p<0.05$, n=7-10). Taken together, we conclude that loss of beclin 1 contributes to mechanical hypersensitivity in a male-specific manner, where loss of beclin 1 activity drives the BDNF-NMDA receptor pain pathway.

Disclosures: T.H. Tam: None. **Y. Tu:** None. **W. Zhang:** None. **S. Farcas:** None. **M.W. Salter:** None.

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

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Presentation Number: 345.07

Topic: D.02. Somatosensation – Pain

Support: 1R43NS119087-01A1

Title: Novel drug target: TAK1 regulates TNF-mediated inflammatory and neuropathic pain

Authors: *S. SCARNEO, R. FREEZE, T. HAYSTEAD, P. HUGHES;
EydisBio Inc., Durham, NC

Abstract: One of the main drivers of chronic pain is inflammation following tissue injury or nerve injury caused by increased levels of cytokines such as tumor necrosis factor α (TNF). TNF

has been shown to bind TNFR1 receptor located on the terminals of primary afferent nociceptors to directly increase their activity as well as stimulate immune cell activation leading to pro-inflammatory cytokine production. Our preclinical work has identified TGFβ-activated kinase 1 (TAK1) as a key signaling element in the TNF mediated pro-survival/inflammatory response pathway. TAK1 plays a crucial role in facilitating activation of protein kinase-mediated signaling pathways implicated in the pathogenesis of chronic pain processes, and as a result has emerged as a novel target for regulating inflammatory and neuropathic pain. To evaluate the potential therapeutic role of TAK1, our group recently developed the first orally bioavailable and selective TAK1 inhibitor. EYD-001 is a low nM TAK1 inhibitor, that shows µM serum surveillance up to 8 hours post oral gavage. Based on our pre-clinical work, we hypothesize that TAK1 inhibition with EYD-001 will alleviate inflammatory and neuropathic pain by reducing immune responses and nociceptor activity. To test this hypothesis, separate groups of mice (N=12, 6M+6F/group) received EYD-001 (QD,PO), gabapentin (QD, PO) or vehicle (QD,PO) following MSU intra-articular knee injections. Mechanical allodynia (Von Frey), knee edema (caliper) and cytokines (serum and synovial fluid) were evaluated over the course of the study. Results demonstrate that EYD-001 reduced knee edema, mechanical allodynia, and key pain-relevant inflammatory cytokines. Furthermore, histological analysis of the knee's showed EYD-001 treatment reduced inflammation and cartilage damage in female mice but not male mice. Our findings suggest that TAK1 plays an integral role in inflammatory pain signaling through non-neuronal immune cell mechanisms as well as endogenous neuronal mechanisms.

Disclosures: S. Scarneo: A. Employment/Salary (full or part-time):; EydisBio Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EydisBio Inc. **R. Freeze:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EydisBio Inc. **T. Haystead:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EydisBio Inc. **P. Hughes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EydisBio Inc..

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

Location: SDCC 33

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 345.08

Topic: D.02. Somatosensation – Pain

Support: Neurosurgery Pain Research Institute at Johns Hopkins School of Medicine

Title: A synthetic sense-and-actuate system for rerouting hyperactive signaling pathways implicated in chronic pain

Authors: *S.-J. TSAI[1], J. XU[5], Y. GONG[5], S. J. GOULD[2], M. J. CATERINA[1,2,3,4];
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Med., Baltimore, MD; [5]Biochem. and Mol. Biol., Johns Hopkins Univ. Bloomberg Sch. of Publ. Hlth., Baltimore, MD

Abstract: Development of safe and effective pharmacological interventions to treat chronic pain is an ongoing clinical need. Development of new drugs requires careful consideration of target cell population, fine-tuning of efficacy, and prevention of unwanted signaling. One strategy toward such specificity is to establish cell state-driven delivery of therapeutics at appropriate quantities. Here we report the invention of a novel synthetic sense-and-actuate system that translates chronic pain-implicated signaling into potentially analgesic cellular responses. Specifically, this system utilizes active tropomyosin-related kinase A (TrkA) to couple a signal-activated regulator to a signal-activated effector that downregulates mitogen-activated protein kinase (MAPK) pathway via relocation of a modified c-Raf Ras binding domain (RBD) to subcellular sites enriched with TrkA-activated Ras. We also demonstrated this system to be highly modular by showing its ability to receive triggers other than TrkA and to generate outputs from effectors other than RBD. Our results highlight the potential of this synthetic system for attenuating signaling pathways related to pathological pain, as a platform for development and testing of novel pain therapeutics, and as a new general approach to interrogate receptor tyrosine kinase activity in real-time.

Disclosures: S. Tsai: None. **J. Xu:** None. **Y. Gong:** None. **S.J. Gould:** None. **M.J. Caterina:** None.

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

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Presentation Number: 345.09

Topic: D.02. Somatosensation – Pain

Support: ON Kick-Starter Grant 21-077

Title: Efficacy Study of NGF[R100W] in Treating Peripheral Sensory Neuropathy

Authors: *C. WU[1], K. SUNG[2], S. WOO[2], S. BARBER[2];
[1]Univ. Of California San Diego Neurosciences Grad. Program, La Jolla, CA; [2]Univ. of California San Diego, La Jolla, CA

Abstract: BACKGROUND: Nerve growth factor (NGF) provides robust trophic support to peripheral sensory neurons. As such, NGF has been extensively tested as a therapeutic measure for treating various conditions. However, many past clinical trials failed due to painful side effects such as site injection hyperalgesia or myalgia. Recent studies of a mutant NGF (NGF[R100W]) associated with hereditary sensory autonomic neuropathy V (HSAN V) have demonstrated that NGF[R100W] no longer induced pain while still retaining its trophic support bioactivity. Therefore, NGF[R100W] may serve as an effective therapy for treating small sensory fiber degeneration in peripheral sensory neuropathy. **OBLECTIVE:** To define the preclinical

efficacy and effectiveness of NGFR[100W] in rescuing/preventing small sensory small sensory fiber degeneration in vivo. **METHODS**: NGF and NGF[R100W] was injected into the hindpaw of two mouse models of sensory fiber neuropathy: Charcot Marie Tooth Type 2B mice and cisplatin-induced neuropathy. We measured the pain response in mice treated with NGF or NGF[R100W]. The density of small sensory fibers (intra-epidermal sensory fibers, IENFs) in the hindpaw skin was measured and quantitated. **RESULTS:** When injected into wildtype mice, NGF at 0.5 μg induced strong acute pain response, while mice receiving NGF[R100W] even at 10 μg still did not show apparent hyperalgesia to thermal stimuli. When injected into CMT2B and cisplatin-treated mice, the effect of NGF[R100W] in rescuing pain response and IENF density was similar to that by wild type NGF. Our immunohistochemistry data further indicated NGF[R100W] prompted neuronal recovery comparable to that observed in wild-type NGF. **CONCLUSION**: Our study has demonstrated that NGF[R100W] even at high doses did not induce strong pain causing effect in vivo; More importantly, NGF[R100W] was effective in rescuing degenerative small sensory fibers both structurally and functionally. These results provide strong support for further investigating the clinical applications of NGF[R100W].

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Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

Location: SDCC 33

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 345.10

Topic: D.02. Somatosensation – Pain

Title: Low-intensity 10kHz spinal cord stimulation reduces behavioral and neural hypersensitivity in a rat model of painful diabetic neuropathy

Authors: *D. WANG, K. LEE, Z. KAGAN, D. LEE, K. BRADLEY;
Nevro Corp., Redwood City, CA

Abstract: Painful diabetic neuropathy (PDN) is a serious complication of diabetes and occurs in approximately 20% of patients with diabetes mellitus. In a recent randomized controlled clinical trial, 10kHz spinal cord stimulation (10kHz SCS) was shown to be a promising therapy for PDN, in both pain reduction and possible neurological improvements (Petersen et al 2021). In this study, we aimed to observe the behavioral and neural effects of 10kHz SCS on PDN using streptozotocin (STZ)-induced diabetic rats.
We established four testing groups: naïve controls, STZ controls (STZ injection only), STZ+Sham (STZ injection with implanted-but-unstimulated epidural spinal electrode), and STZ+10kHz SCS (STZ injection with implanted-and-stimulated epidural spinal electrode). A single effective dose (60 mg/kg) of the cytotoxic agent STZ caused the rats to become hyperglycemic (>270 mg/dl) within 72 hours and lasted for several weeks. These STZ-injected rats also showed a significant and continuous reduction in body weight in comparing to naïve control rats. In behavioral assessments, we observed that 7 days of continuous (24h/day) low-

intensity (30% of motor threshold) 10kHz SCS appeared to restore von Frey paw withdrawal thresholds to approximately 96% of naïve controls, showing statistically significant improvement from the Pre-SCS baseline after 7 days of 10kHz SCS, and versus STZ control or STZ+Sham animals.

We also performed electrophysiologic testing just prior to termination in all groups. We found normalization of the receptive field area of wide dynamic range dorsal horn neurons was significantly greater for the STZ+Sham group than the areas of both the STZ+10kHz SCS and Naïve group. In addition, we observed that the firing rates of dorsal horn neurons induced by mechanical stimulation were significantly different between groups. 10kHz SCS demonstrated a significantly reduced firing rate in response to paw brush, pinch, or Von Frey filaments stimulation in comparing to STZ+Sham animals.

Our results demonstrate that low intensity 10 kHz SCS resulted in behavioral and electrophysiological outcomes reflective of pain reduction in a commonly used rodent model of painful diabetic neuropathy. This work underscores the clinical findings of significant and robust pain relief in PDN patients using paresthesia-free 10 kHz SCS, and lays the groundwork for further exploration of newer mechanisms not dependent upon dorsal column activation.

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

Location: SDCC 33

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 345.11

Topic: D.02. Somatosensation – Pain

Title: Mast cell activation of the dura mater mediates alcohol withdrawal-induced headache behavior

Authors: H. SON[1], ***Y. KIM**[2];
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Abstract: Mast cell activation of the dura mater mediates alcohol withdrawal-induced headache behavior
Hyeonwi Son[1], Yan Zhang[1], John Shannonhouse[1], Hirotake Ishida[1], Ruben Gomez[1],and Yu Shin Kim[1,2,*]
[1]Department of Oral & Maxillofacial Surgery, School of Dentistry; [2]Programs inIntegrated

Biomedical Sciences, Translational Sciences, Biomedical Engineering,Radiological Sciences, University of Texas Health Science Center at San Antonio

Headache is a severe alcohol withdrawal symptom affecting daily life. There is an unmet need for appropriate therapeutic options. Here, we show that mast cell (MC) activation mediates development of alcohol withdrawal-induced headache. Withdrawing alcohol from alcohol-acclimated mice induces headache behaviors often observed in humans suffering from headaches, including facial allodynia, facial pain expressions, and reduced walking movement. Observed pain behaviors were abolished in MC-dysfunctional mice. We observed *in vivo* spontaneous activation and hypersensitization of trigeminal ganglia neurons in alcohol withdrawal mice but not in MC-dysfunctional mice. Moreover, MC degranulation was increased in dura mater of alcohol withdrawal mice, which was prevented by MC dysfunction. Injection of MC activator into dura mater resulted in activation of trigeminal ganglia neurons and vasodilation, which was accompanied by headache behavior. These results indicate that alcohol withdrawal causes headache via MC degranulation in dura mater. MCs of dura mater are a potential target for treating alcohol withdrawal-related headache.

Disclosures: H. Son: None. **Y. Kim:** None.

Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

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Topic: D.02. Somatosensation – Pain

Support: Internal Grant Noorda COM

Title: Appearance of reactive hypoglycemia in otherwise healthy patients with treatment resistant migraine

Authors: *K. BILLS, W. MAGOFFIN, C. MCKEE, A. NAIK, M. COOK, M. SETTELMAYER;
Noorda Col. of Osteo. Med., Provo, UT

Abstract: Migraine is the most common neurological disorder in the world. It is a multisystemic, multicausal condition characterized by increased neuronal activity in various brain regions including the hypothalamus and trigeminal nerve complex. Reactive hypoglycemia has not been previously characterized as a diagnostically or therapeutically relevant ancillary to chronified migraine. Previous reports have indicated an association between migraine and reduced insulin sensitivity leading to increased average blood glucose levels. In this study twenty-five patients with previously diagnosed chronic migraine were given three-hour glucose tolerance testing of 100 g glucose load after 12-hours of fasting. The average glucose blood levels were as follows: post fasting baseline (82 mg/dL +/- 12 mg/dL), 1-hour post glucose ingestion (110 mg/dL +/- 14 mg/dL), 2-hour post glucose ingestion (80.25 mg/dL +/- 14 mg/dL), and 3-hour post glucose

ingestion (53 mg/dL +/- 6 mg/dL). The flagged reference glucose range at 3-hours was 65-139 mg/dL. The mechanistic pathophysiological causes of altered glucose regulation in migraine are poorly understood. There could be various aspects of the glucose profile that should be taken into consideration by the clinician until greater understanding of the extent to which this issue contributes to migraine is further elucidated.

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Nanosymposium

345. Itch and Pain Mechanisms in Rodents and Humans

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Presentation Number: 345.13

Topic: D.02. Somatosensation – Pain

Support: NINDS Grant R01NS099245
           NINDS Grant R01NS069568

Title: The role of parabrachial in nociception and pain in awake mice

Authors: *J. SMITH[1], Y. JI[2], R. MASRI[2], A. KELLER[1];
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Abstract: The parabrachial nuclear complex (PB) is a nexus for aversion, and for the nociceptive and affective components of pain perception. We have previously shown that, during chronic pain, PB neurons have increased activity and respond to noxious stimuli with prolonged after-discharges – responses that far outlast the stimulus. This phenomenon—like most of what we know about the electrophysiology of pain—has only been observed in anesthetized animals. Anesthesia profoundly alters neuronal responses to nociception and masks their responses to the affective component of pain. We have developed a method to investigate PB in awake, behaving animals by recording single units *in vivo* from head restrained mice. This offered opportunities to study the time course of changes in PB activity by recording repeatedly from the same animals. It also allows us to correlate PB activity with the animal's behavioral state, by using pupil changes as a proxy for internal states. We report that, in PB neurons from both male and female mice, anesthesia leads to decreased activity, specifically a decrease in spontaneous activity and reduced magnitude of the responses to noxious stimuli. We also demonstrate that, in awake mice, evoked response both before and after chronic pain results in a lasting amplification of PB activity. Finally, we show that changes in PB activity are related to changes in arousal, which was captured by increases in pupil diameter states in response to noxious and aversive stimuli.

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345. Itch and Pain Mechanisms in Rodents and Humans

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Presentation Number: 345.14

Topic: D.02. Somatosensation – Pain

Support: Weizman-Ichilov research grant

Title: Lesions to both somatic and affective pain pathways lead to decreased connectivity in the Salience network

Authors: *I. JALON[1,3], A. BERGER[4,6], B. SHOFTY[4,7], N. GOLDWAY[8], M. ARTZI[2,5], G. GUREVICH[5,2], T. HENDLER[9,5], T. GONEN[5], I. STRAUSS[4];
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Abstract: Human pain is a salient stimulus composed of two main components: a sensory\somatic component, carrying peripheral nociceptive sensation via the spino-thalamic tract and brainstem nuclei to the thalamus and then to sensory cortical regions, and an affective (suffering) component, where information from central thalamic nuclei is carried to the anterior insula, dorsal anterior cingulate cortex and other regions. In the current study, we examined how lesions to nodes of the affective and somatic pain pathways affect resting-state network topology in cancer patients suffering from severe intractable pain. Two procedures have been employed: percutaneous cervical cordotomy (n=20), hypothesized to disrupt the transmission of the sensory component of pain along the spino-thalamic tract, or stereotactic cingulotomy (n=12), which refers to bilateral intra-cranial ablation of an area in the dACC and is known to ameliorate the affective component of pain. Both procedures led to immediate significant alleviation of experienced pain and decreased functional connectivity within the salience network. However, only the somatic procedure (cordotomy) led to decreased connectivity within the sensorimotor network. Thus, our results support the existence of two converging systems relaying experienced pain, showing that pain-related suffering can be either directly influenced by interfering with the affective pathway, or indirectly influenced by interfering with the ascending spino-thalamic tract.

Disclosures: I. Jalon: None. **A. Berger:** None. **B. Shofty:** None. **N. Goldway:** None. **M. Artzi:** None. **G. Gurevich:** None. **T. Hendler:** None. **T. Gonen:** None. **I. Strauss:** None.

Nanosymposium

346. Visual Processing During Behavior

Location: SDCC 5

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 346.01

Topic: D.06. Vision

Title: Extraretinal modulation of perisaccadic population codes in macaque V1

Authors: *S. AKERS-CAMPBELL[1,2], J. E. NIEMEYER[3], M. A. PARADISO[1];
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Abstract: Humans and other primates make 2-4 saccadic eye movements each second to direct the central retina towards objects of interest. We isolate the neural and perceptual effects of saccades in macaques by comparing behavioral trials with saccades and trials that involve a simulated saccade and the same visual stimulation, but no actual eye movement. We previously showed that, compared to simulated saccades, true saccades suppress contrast sensitivity to low spatial frequency stimuli that appear at the start of fixation and enhance sensitivity to higher frequencies. Changes in V1 firing rates correlated with these changes in perception that resulted from extra-retinal input (i.e. corollary discharge). Here, we trained decoders on the latency of the first spike after fixation onset and found that spike latency readily distinguishes trials with saccades from simulated-saccades. Neural decoders were then trained on spike rates and this revealed two distinct temporal components of extraretinal modulation. The immediate effect of a stimulus that appeared at fixation onset was response suppression (compared to the simulated-saccade condition), and this suppression occurred at all spatial frequencies. The timing of suppression could be used to accurately predict the time of fixation onset and thus parse the visual response into epochs coinciding to different fixation periods. The temporal precision of suppression presumably explains why first-spike latency accurately distinguishes saccade and simulated-saccade trials. After about 50 ms, firing rates were modulated in a stimulus-dependent manner – low spatial frequency responses were suppressed and higher frequencies enhanced. In this later time epoch, average pairwise noise correlations were reduced and the principal mode of the noise became orthogonal to the incoming visual signal. Finally, spike count decoding was used to determine the time after fixation onset at which neural activity reached 70% correct distinguishing the presence of a stimulus at one of two locations. Consistent with a coarse-to-fine trend, threshold was reached faster at low spatial frequencies. However, saccades had the effect of significantly reducing the time needed to reach threshold at high spatial frequencies. Taken together, the results show the significant extent to which an extraretinal signal modulates the timing and specificity of the V1 response to visual input. Saccade-based changes in noise correlations and coarse-to-fine processing may optimize V1 activity to speed object recognition.

Disclosures: S. Akers-Campbell: None. **J.E. Niemeyer:** None. **M.A. Paradiso:** None.

Nanosymposium

346. Visual Processing During Behavior

Location: SDCC 5

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 346.02

Topic: D.06. Vision

Title: Preemptive gain control in primary visual cortex.

Authors: *B. KREKELBERG, J. GUEZ;
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Abstract: Neurons' dynamic range is limited compared to the wide range of sensory inputs encountered in everyday life. The visual system solves this problem by dynamically adjusting neural response functions by a computation called gain control. However, this optimization for the current input poses a problem in the context of the repeated saccades that primates make to explore visual scenes. Specifically, optimal gain settings based on pre-saccadic input are unlikely to be optimal for the post-saccadic input. We tested the hypothesis that the visual system deals with this problem by preemptively adjusting gain settings with each saccade. We recorded neural activity in the primary visual cortex (V1) of two male macaques using permanently implanted electrodes (N=96). The animals received liquid reward for making a sequence of back-and-forth saccades interspersed with steady fixation. The contrast of the visual input (sinusoidal gratings) changed pseudo-randomly every 330 ms. We used a general linear model to extract, from the multi-unit activity, the response corresponding to each stimulus, the preceding stimulus (i.e., gain control), and the saccade, all separately for each stimulus contrast. Stimulus responses showed the expected transient/sustained responses that increased monotonically with contrast. Gain control was evident as a suppression (low gain) of the response following high contrast stimuli and an enhancement (high gain) of the response following low contrast stimuli. Saccades reset these gain settings; saccades following a high contrast (low gain) stimulus reset the gain to a high value, while saccades following a low contrast (high gain) stimulus reset the gain to a low value. These findings suggest that V1 neurons optimize gain not only to handle current input, but also to prepare for impending changes in visual input. We coin the term preemptive gain control to describe the latter process. In the past, we and others have interpreted firing rate changes at the time of saccades in terms of saccadic omission - the phenomenon that intra-saccadic retinal input is omitted from awareness. Although these interpretations may not necessarily be mutually exclusive, the novel concept of preemptive gain control links peri-saccadic firing rate changes directly to the optimization of post-saccadic vision. This optimization is important because the goal of each saccade is to inspect a new area of interest with the high-resolution fovea; suboptimal gain settings would be contrary to this core goal of active vision. Given that primates make about three saccades each second, preemptive gain control can contribute substantially to the optimization of visual processing.

Disclosures: B. Krekelberg: None. **J. Guez:** None.

Nanosymposium

346. Visual Processing During Behavior

Location: SDCC 5

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM
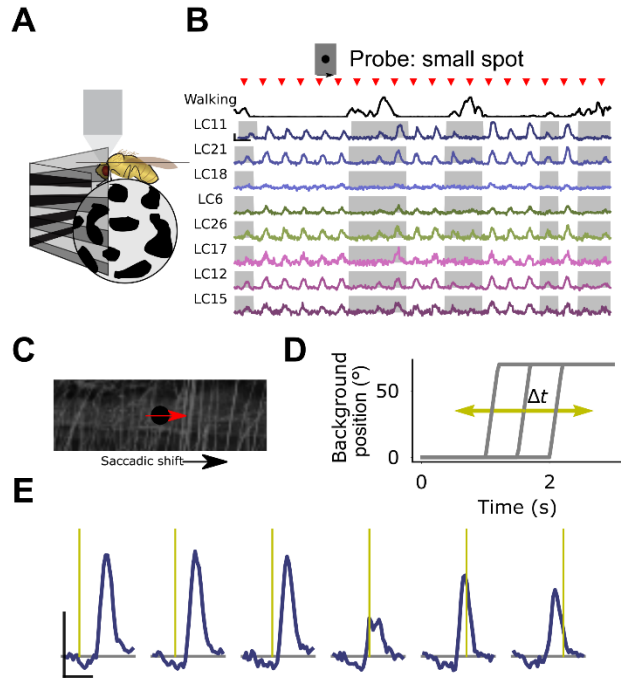
Presentation Number: 346.03

Topic: D.06. Vision

Title: Population representation and gain control in diverse visual projection neurons in the fly

Authors: *M. TURNER, T. CLANDININ;
Stanford Univ., Stanford, CA

Abstract: Animals actively sample their sensory environment. In vision, this often means that movement of the body, head and/or eyes directs gaze to scene locations of interest. As a result, under naturalistic viewing conditions, the image on the retina is subject to continuous self-generated motion. This presents a challenge for the visual system, as visual neurons are tasked with encoding specific features of the external visual world in a rapidly changing context where the dominant sources of visual changes on the retina may be self generated. How do visual neurons reliably encode features of interest under these dynamic conditions? We explore this question using visual projection neurons (VPNs) in Drosophila. VPNs are situated at a critical bottleneck in the fly visual system, carrying highly processed visual information from the optic lobes to the central brain to guide behavior. To explore the relationship between self motion cues and representation of external object motion by populations of VPNs, we developed a new calcium imaging and alignment method to measure responses across many VPN classes simultaneously in a behaving animal. We find that small-object detecting VPNs modulate their gain according to two distinct cues associated with self motion: motor-related cues when the animal walks, and visual scene statistics characteristic of retinal input during self-generated motion. Fig. 1: A. Fly on ball under imaging microscope. B. VPN calcium responses to a repeated small moving spot are measured while fly walking behavior is monitored. Responses are suppressed during walking (gray shaded trials). C. A small spot probes response gain while a widefield image is shifted to mimic Drosophila walking body saccades. D. Timing of saccade is varied. E. Probe responses of a small object detecting glomerulus with different visual saccade timings indicated by the vertical yellow line. Response gain is suppressed when the visual saccade occurs near the probe response onset time.

A

B Probe: small spot

Walking

LC11
LC21
LC18
LC6
LC26
LC17
LC12
LC15

C

Saccadic shift→

D

Background position (°)

50

0

Δt

0   2
Time (s)

E

Nanosymposium

346. Visual Processing During Behavior

Location: SDCC 5

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 346.04

Topic: D.06. Vision

Title: Superior colliculus visual neural sensitivity at the lower limit of natural self-induced image displacements

Authors: *Z. HAFED[1], C.-Y. CHEN[2], F. KHADEMI[3];
[1]Werner Reichardt Ctr. For Integrative Neurosci., Tuebingen, Germany; [2]Dept. of Neurosci., Kyoto Univ., Kyoto-Shi, Japan; [3]Tuebingen Univ., Tuebingen, Germany

Abstract: Image analysis in the visual system is performed by neurons having individual receptive fields (RF's) sampling confined regions of the retinal image. Extrafoveally, and particularly in higher visual areas, RF's can be large; this is also the case in sensory-motor structures like the superior colliculus (SC). Integration of relatively large image regions into individual RF's raises questions about how detailed visual pattern analysis can occur when the local features inside an RF are much smaller than RF size. Among these questions is what nature of visual processing takes place in the SC when compared to other visual areas that are more distant from the motor control apparatuses. For example, does the SC's classically-attributed role in orienting responses mean that SC neurons are incapable of detailed visual pattern analysis? We investigated this question by exploring whether SC neurons are sensitive to the visual pattern consequences of minute image displacements over their RF's. With the head fixed, a lower limit on natural self-induced retinal image motion is that caused by slow ocular position drifts. With stable external stimuli, such drifts introduce image pattern displacements over individual RF's that are much smaller than the RF's themselves. Thus, the local pattern features of the stimuli never really leave the RF's. Yet, theoretical and perceptual works suggest that small displacements associated with ocular position drifts reformat images in meaningful ways for perception. We asked whether SC neurons are sensitive to such reformatting. We employed gaze-contingent retinal image stabilization, combined with gratings of different properties, to identify a direct SC neural correlate of perceptual effects associated with drifts. We presented gratings to SC neuron RF's in two macaque monkeys, either stably on the display (and thus moving on the retina) or using gaze-contingent display updates. Across trials, gaze-contingence was programmed to minimize either the full drift-induced retinal motions of the gratings or the retinal motions' horizontal or vertical components; with horizontal or vertical gratings, the latter meant minimizing retinal luminance variations either orthogonal or parallel to the image patterns. SC neurons robustly reflected the minute spatiotemporal luminance changes caused by ocular position drifts, even extrafoveally with RF's much bigger than the image shifts. These results complement studies in the retina highlighting the impact of visual reformatting by drifts on the retinal output, and they demonstrate that SC neurons can contribute to visual scene analysis with high fidelity despite their relatively large RF's.

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346. Visual Processing During Behavior

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Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 346.05

Topic: D.06. Vision

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                Max Planck Society

Title: Representation of prey-related variables in mouse V1 during prey capture behavior

Authors: *D. GUGGIANA NILO, M. MCCANN, T. BONHOEFFER, M. HÜBENER;
Max Planck Inst. for Biol. Intelligence, Martinsried, Germany

Abstract: Neurons in the mouse primary visual cortex (V1) are traditionally described using classical response properties, such as direction/orientation selectivity. These properties are measured by showing moving gratings to a head-fixed animal while recording the ensuing neural responses. This approach is in stark contrast to what mice see and do in nature, where they experience much more complex visual scenes while engaging in behaviors such as foraging and hunting. It is unclear how V1 cells respond during such behaviors. Here, using hunting as a naturalistic behavior, we show that V1 neurons encode a number of hunting-related variables, such as distance and angle to prey. To acquire such data, we combined video tracking of mice hunting live crickets in an open arena and calcium imaging using miniature microscopes. This allowed us to characterize the tuning of hundreds of V1 cells to several behaviorally-relevant variables. We observed that many neurons showed selective tuning to at least one of such variables. At the population level, however, decoding analyses showed that only a subset of these variables can be decoded. These included previously reported quantities, like mouse speed, but also hunting-relevant variables, such as prey distance. Our results therefore show that, despite the "early" positioning of V1 in the visual pathway, its cells can show complex response patterns to scenes that differ greatly from moving gratings. This highlights the importance of recording neuronal responses in freely-moving animals performing ethologically relevant behaviors, as these responses are a step towards understanding how the brain controls complex behaviors.

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346. Visual Processing During Behavior

Location: SDCC 5

Time: Monday, November 14, 2022, 1:00 PM - 2:45 PM

Presentation Number: 346.06

Topic: D.06. Vision

Support: ONR N00014-21-12324

Title: Laminar organization of spike-field connectivity during behavior

Authors: S. AKELLA[1], A. BASTOS[2], E. K. MILLER[3], ***J. PRINCIPE**[4];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Psychology and Vanderbilt Brain Inst., Vanderbilt Univ., Nashville, TN; [3]MIT, Cambridge, MA; [4]Univ. of Florida, Gainesville, FL

Abstract: Distinctions in neural dynamics of different cortical layers are apparent in both neuronal and local field potential (LFP) patterns. Yet, the associations between spiking activity and LFPs in the context of laminar processing have only been sparingly analyzed. While spike-field connectivity analyses can help decipher the link between the multilevel brain dynamics and behavior, they present a challenging computational problem due to disparities in the signal structure and corresponding signal processing methodologies developed for each modality. Here, we study the laminar organization of spike-field causal flow patterns using a novel multiscale framework that quantifies LFPs as a point process of frequency-specific transient bursts. The framework provides a shared metric space for computations on the signal modalities while limiting time resolution only to the slowest sampled signal. To quantify directional asymmetries between spikes and LFPs in the point process space, we evaluate directed information, an information-theoretic measure of causal influence, using techniques from kernel spike-train representations. In monkeys performing a visual task with modified sample predictability, spike-field causal flow within and across individual columns of visual-area 4 (V4) and prefrontal cortex (PFC) revealed behavior and frequency specificity in their laminar organization. During stimulus processing, gamma bursts (40-80 Hz) in the superficial layers of V4 largely drove intralaminar spiking. These gamma influences also fed forward up the cortical hierarchy, where they modulated laminar spiking in PFC. Connections originating in V4 were generally more pronounced during novel stimulus presentations than during repeated presentations. In the same interval, we observed a reverse phenomenon in the PFC where the direction of intralaminar information flow was from spikes to fields. Here, superficial-layer neurons were the primary drivers of gamma activity, while deep layers neurons modulated beta (8-30 Hz) activity in the column. Moreover, these influences dually controlled top-down and bottom-up processing, with superficial-layer influences being higher to gamma during novel stimuli and deep-layer influences to beta during repeated stimulus presentations. Prestimulus spiking activity in V4 and PFC was primarily driven by deep-layer beta in PFC, where the pathways showed enhanced information transfer during repetitive blocks. While these results corroborate existing theories on the functional roles assumed by individual cortical layers, they further emphasize the complexities of causal interactions between LFPs and spiking activity.

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Nanosymposium

346. Visual Processing During Behavior

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Topic: D.06. Vision

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ICT (2021R1A2C3012159, 2021R1A4A2001803)
KAIST Global Singularity Program for 2020

Title: Visually evoked activity of the SST-expressing interneurons and the SST release in the V1 of mice discriminating visual stimuli in a freely moving state

Authors: *J. YOON, S.-H. LEE;
Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: The activity of interneurons in the visual cortex is critical for visual processing and perceptual behaviors. Among the interneurons, somatostatin-expressing interneurons (SST$^+$ INs) are important for sensory gating via inhibition of the pyramidal neurons or other interneurons. Moreover, a recent study showed that the exogenous application of somatostatin (SST), a neuropeptide potentially released from the SST$^+$ INs, enhanced the visual gain of primary visual cortex (V1) neurons and the visual discriminability of mice. However, it is still unclear when the SST$^+$ INs release the SST peptides and modulate V1 processing in animals performing visual tasks. Here, we present an experimental setup that enables *in vivo* calcium imaging of V1 neurons in mice discriminating visual stimuli in the T-maze. In this setup, mice learn to discriminate the two different static grating orientation stimuli for left or right choices. In the well-trained mice, we measured the activity of SST$^+$ INs in the V1 using a miniaturized 1-photon fluorescence microscope. Interestingly, we found that their activity increased after the stimulus onset but decreased when mice entered the reward zones. This opposite responsiveness was not observed in the excitatory neurons during the task. We next measured the SST release via GRAB$_{SST}$ sensor imaging in the V1 of task-performing mice using fiber photometry. We found that the SST release increased after the stimulus onset, in correlation with the activity increase of SST$^+$ INs during the task. Our data suggest that the activity of SST$^+$ INs increases and the SST is released in the V1 when animals process visual information for their behavioral choices.

Disclosures: J. Yoon: None. **S. Lee:** None.

Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.01

Topic: G.09. Drugs of Abuse and Addiction

Title: Insight into the Transcriptome Driving Maladaptive Drug-Seeking

Authors: *C. LITIF[1], J. GIGLEY[2], N. BLOUIN[2], A.-C. BOBADILLA[3];
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Abstract: Substance use disorder (SUD) is characterized by compulsive and chronic use and seeking of psychoactive drugs. Drug seeking behavior is believed to usurp reward-signaling pathways that otherwise control seeking of non-drug natural rewards such as food acquisition or reproductive efforts. To target maladaptive drug seeking, it is critical to understand the neurobiological components driving drug reward seeking that are exclusive to only drug reward seeking. Neuronal projections between the prefrontal cortex (PFC) and the nucleus accumbens core (NAcore) regions within the brain reward pathway have shown genetic changes related to drug-seeking behavior. Additionally, reward-specific ensembles, i.e., a small population of neurons co-activated during reward seeking, have been identified in the PFC and NAcore. However, the reward signaling transcriptome of the PFC and NAcore regions that solely drive drug seeking have not yet been defined. Thus, our research aims to determine transcription factors related exclusively to drug seeking behavior within the PFC and NAcore. Using targeted recombination of activated populations (TRAP) in FOSCreERT2/+/Ai14 transgenic mice, we conditioned mice using a drug (cocaine) and non-drug (sucrose) dual-reward self-administration model to fluorescently sort (FACS) and characterize (RNAseq) the transcriptomes of reward signaling neurons involved specifically within each reward-seeking ensemble. Identifying drug-specific alterations in gene expression, separate from seeking other non-drug rewards, ultimately advances the understanding and improves intervention of relapse in those struggling with SUD.

Disclosures: C. Litif: None. **J. Gigley:** None. **N. Blouin:** None. **A. Bobadilla:** None.

Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.02

Topic: G.09. Drugs of Abuse and Addiction

Title: Nicotinic Mutant Mice for the Chrna5 Gene as Models of Cloninger Type 1 and 2 Alcohol Use Disorders

Authors: *L. TOCHON[1], N. HENKOUS[1], M. BESSON[2], N. DOMINIQUE[2], U. MASKOS[2], V. DAVID[1];
[1]Univ. Bordeaux, Aquitaine Inst. for Cognitive and Integrative Neurosci. (INCIA), CNRS UMR 5287, Bordeaux, France; [2]Integrative Neurobio. of Cholinergic Systems, Inst. Pasteur, CNRS UMR 3571, Paris, France

Abstract: Human genetic association studies have linked single nucleotide polymorphisms (SNPs) of the CHRNA5 gene, encoding the α5 nicotinic acetylcholine receptor subunit (α5-nAChR), to an increased risk of alcohol use disorders (AUDs).To understand how α5-nAChR subunit mutations may influence alcohol-drinking behavior and preconsummatory traits relevant to AUDs (anxiety, sensation-seeking, impulsivity), we tested male and female transgenic mice expressing either the most common SNP (D398N_α5KI) or a deletion of the CHRNA5 gene (α5KO) in the elevated-plus maze, novelty-place preference and step-down tasks. Their alcohol consumption was then assessed through an intermittent two-bottle choice self-administration protocol. As this is where α5-nAChR is most densely expressed, we investigated the implication of the α5*nAChR-expressing IPN-GABAergic neurons in these changes using neurospecific reexpression of the subunit in α5KOxGAD-Cre mice.α5KI and α5KO mice both showed alcohol over-consumption for highly concentrated solutions but displayed opposite anxiety-related behaviour (hyper vs hypo-anxious) and behavioural control (impulsive-like vs not impulsive, respectively). These opposite phenotypes strongly evoke characteristics of Cloninger's avoidant (Type-I) and sensation-seeking (Type-II) AUDs. Moreover, viral reexpression of α5-nAChR in IPN-GABAergic neurons decreased alcohol consumption and improved the impulsive-like phenotype observed in α5KO. These results support that mutations of the α5-nAChR subunit resulting in loss of α5*nAChRs function shift the alcohol consumption toward high doses, and in contrast, that α5*nAChR-expressing IPN-GABAergic neurons contribute to its control. Furthermore, we hypothesize that α5-nicotinic mutants may provide a preclinical model of Cloninger's AUD subtypes, which could represent a major step toward the development of personalized and more effective therapeutic strategies.

Disclosures: L. Tochon: None. **N. Henkous:** None. **M. Besson:** None. **N. Dominique:** None. **U. Maskos:** None. **V. David:** None.

Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.03

Topic: G.09. Drugs of Abuse and Addiction

Title: Social emotional profiles of two strains of transgenic mice expressing nicotinic receptor mutations involved in alcohol abuse

Authors: *C. PAGEZE, L. TOCHON, J.-L. GUILLOU, N. HENKOUS, R.-M. VOUIMBA, V. DAVID;
Aquitaine Inst. for Cognitive and Integrative Neurosci. - CNRS/University of Bordeaux, Univ. of Bordeaux, Bordeaux, France

Abstract: Human genetic association studies have linked different single nucleotide polymorphisms (SNPs) of the alpha5-subunit of nicotinic acetylcholine receptors to an increased risk of alcohol use disorders (AUDs). Transgenic mice expressing either a common SNP (D398N, α5KI) or a deletion of the CHRNA5 gene altogether (α5KO) are both prone to alcohol over-consumption but display opposite anxiety-related behaviour (hyper vs hypo-anxious) and behavioural control (impulsive-like vs not impulsive, respectively). These opposite phenotypes strongly evoke characteristics of Cloninger's avoidant (Type-I) and sensation-seeking (Type-II) AUDs. To further explore the possibility that α5-nicotinic mutants may provide a preclinical model of Cloninger's AUD subtypes, we investigated their social-emotional profiles as well as the activity of the amygdalo-hippocampal pathway, involved in social-emotional processes. Emotion recognition abilities were assessed using the Affective State Discrimination Task, and rescuing behaviour using the Restrainer Tube Test. Male α5KO mice were impaired in recognition of a stressed affective state and did not display rescuing behaviour towards trapped peers, or were doing so in an inappropriate manner by assaulting them. In contrast, female α5KI mice exhibited normal emotion recognition and improved rescuing behaviour. Moreover, *in vivo* electrophysiological recordings revealed opposite changes in amygdalo-hippocampal activity in relation with these social-emotional profiles, such as a higher potentiation versus a loss of potentiation of the BLA-vCA1 neurotransmission in α5KI and α5KO respectively following high frequency stimulation. These results further support the parallel between social-emotional profiles of AUD type-I and female α5KI mice, and between AUD type-II and male α5KO mice and the implication of the amygdalo-hippocampal pathway in these differences.

Disclosures: C. Pageze: None. **L. Tochon:** None. **J. Guillou:** None. **N. Henkous:** None. **R. Vouimba:** None. **V. David:** None.

Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.04

Topic: G.09. Drugs of Abuse and Addiction

Title: Predator odor (tmt) exposure increases interoceptive sensitivity to alcohol and affects gene expression in the insular cortex

Authors: *R. TYLER[1], **K. VAN VOORHIES**[1], **J. BESHEER**[2];
[1]Univ. of North Carolina Chapel Hill, CHAPEL HILL, NC; [2]Psychiatry; Bowles Ctr. for Alcohol Studies, Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract: Post-traumatic stress disorder (PTSD) increases vulnerability to develop AUD co-morbidity. Alcohol produces interoceptive (subjective) stimulus effects that likely influence drug intake by driving drinking or signaling satiety. Therefore, understanding the role of a stressor on alcohol interoceptive sensitivity is clinically relevant. The present studies used exposure to the predator odor 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) to model a traumatic stress event. Not all individuals that experience trauma develop PTSD, and as such our model captures individual differences in stress reactivity. We use digging and immobility measures during the TMT exposure to create 2 TMT sub-groups (digging/immobility ratio; TMT-1 < 5 and TMT-2 > 5). Prior experiments show TMT-2 rats display increased alcohol self-administration compared to TMT-1 and controls. These experiments follow-up to determine if TMT exposure and these sub-groups affect alcohol interoceptive sensitivity. Male and female, Long-Evans rats were trained to discriminate the interoceptive effects of alcohol (2.0 g/kg, i.g.) from water using a Pavlovian drug discrimination procedure, which serves as an index of interoceptive sensitivity to alcohol. Upon stable discrimination, rats underwent TMT exposure and then remained undisturbed in their home cage for 2 weeks prior to interoceptive sensitivity testing. In males, TMT exposure potentiated the interoceptive effects of 1 g/kg alcohol, and this effect was driven by the TMT-2 sub-group. A pentobarbital substitution test showed potentiated alcohol-like stimulus effects in the TMT-2 group compared to controls, suggesting a GABAergic adaptation. In females, TMT exposure increased the interoceptive effects of 2 g/kg alcohol, and this effect was driven by the TMT-1 sub-group. Next, gene expression analyses were conducted 2 weeks after TMT exposure in male and female rats in the insular cortex (IC), a brain region implicated in alcohol interoceptive sensitivity. Several NMDA receptor transcripts and *GAD1* (inhibitory cell marker) were elevated in the TMT group compared to controls. Parvalbumin gene expression was increased in the TMT-2 group compared to controls, but somatostatin expression was unchanged. Together, these data demonstrate potentiated sensitivity to alcohol in both sexes, and sex differences in the stress-reactive sub-groups driving this effect. Finally, GABAergic, parvalbumin interneurons in the IC may underlie the potentiated sensitivity to alcohol in males.

Disclosures: R. Tyler: None. **K. Van Voorhies:** None. **J. Besheer:** None.

Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.05

Topic: G.09. Drugs of Abuse and Addiction

Title: Molecular characterization of cocaine-relapse ensemble neurons in the prefrontal cortex using single nuclei RNA sequencing in Fos-mRFP transgenic rats

Authors: *K. E. SAVELL[1], R. MADANGOPAL[1], R. PALAGANAS[1], O. R. DRAKE[1], D. Q. PHAM[1], M. B. BRENNER[1], T. L. MARTIN[1], K. D. WOODS[1], M. STEINBERG[2], J. MARTIN[2], C. CHARENDOFF[2], W. AUSTIN[2], J. CHOI[1], M. MERRIMAN[1], S. J. WEBER[1], V. LENNON[1], L. E. KOMER[1], E. VAN LEER[1], B. T. HOPE[1];
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Abstract: Drug-associated memories can drive relapse long after the last instance of drug use. Associative memories, including cue-drug memories, are thought to be encoded within specific patterns of strongly activated neurons (neuronal ensembles) that can be identified using immediate early genes such as Fos. Our lab and others showed that Fos-expressing neuronal ensembles play a causal role in drug-seeking behaviors and identified ensemble-specific transcriptional alterations. However, these studies used pooled samples of Fos-positive and Fos-negative neurons, which contained multiple cell types, and used a targeted analysis of relatively few candidate genes. These results cannot inform which cell types are participating in the drug memory ensemble and cannot distinguish differential transcriptional responses within individual cell types. To address this issue, we combined ensemble-based transgenic tools with recent advances in multiplexed single nuclei RNA sequencing to conduct an unbiased screen of ensemble-specific cell types and transcriptional changes underlying cocaine relapse.
We used male and female Fos-based transgenic rats to label cocaine relapse ensemble neurons in the medial prefrontal cortex (mPFC) following cocaine self-administration training. We trained rats to self-administer cocaine (FR1 reinforcement schedule, 0.75 mg/kg/inf. cocaine paired with a 3.5-s light cue) during twice daily 3 h sessions . Following training and 21 days of abstinence, we tested rats for cocaine seeking (30 min, extinction conditions) and collected brains 3 h after test (peak Fos-driven mRFP expression). We observed reliable cocaine self-administration during training and robust cue-induced cocaine seeking following abstinence. We established a pipeline to isolate nuclei from mPFC following behavioral testing, sort mRFP positive (ensemble) and negative (non-ensemble) neuronal nuclei using fluorescence-activated nuclei sorting, and identify transcriptional signatures using single nuclei RNA sequencing. Our analysis revealed distinct clusters corresponding to known cell types in the mPFC (glutamatergic and gabaergic neurons) that further subcluster into expected layer and interneuron sub-types within mPFC. Using this unbiased approach, ongoing analysis is aimed at characterizing cell-type and ensemble-specific transcriptional signatures that contribute to drug-seeking behaviors. We will employ transcriptional modulators in future experiments to assess causal roles for these cocaine memory-specific genes in relapse.

Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.06

Topic: G.09. Drugs of Abuse and Addiction

Title: Morphological alterations of dendritic spines on Fos-positive ensemble neurons in rat nucleus accumbens following cue-induced cocaine craving

Authors: *D. E. OLIVARES[1], F. J. RUBIO[2], E. M. HILAIRE[2], A. HENRY[2], L. KANE[2], R. MADANGOPAL[2], C. MEJIAS-APONTE[2], B. T. HOPE[2];
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Abstract: Learned associations between discrete cues (or contexts) and drug effects are thought to be encoded by sparsely distributed patterns of neurons called neuronal ensembles and cue-specific synaptic inputs on these ensemble neurons are altered during learning to mediate long-lasting memories. Here we assessed morphological alterations of dendritic spines on medium spiny neurons in nucleus accumbens (NAc) following cue-induced cocaine craving. We trained all rats to lever press for cocaine infusions paired with a 3.5-s light cue. After training, all rats were injected in NAc with a cocktail of AAVs encoding Cre recombinase and Cre-dependent GFP for sparse neuronal labeling. After 3 weeks in their home cages, rats were exposed for 30 min to either the cocaine-paired light cue (relapse test group) or a non-drug-related novel context (ensemble-specific control) or kept in their home cages. Rats were sacrificed 60 min after cue exposure and their brains processed for Fos immunohistochemistry and cleared prior to confocal imaging. Morphological analysis of GFP-labeled spines of Fos-positive and Fos-negative neurons were performed using 3D reconstruction using Neurolucida and CellProfiler.In proximal dendritic segments (<50 mm from soma), Fos-positive neurons had significantly larger spine head diameter than Fos-negative neurons for both relapse and novel context groups. The increases in overall spine head diameters can be explained by an increased proportion of mushroom-like spines with no increase in their spine head diameter. In distal dendritic segments (>70 mm from soma), Fos-positive neurons had larger spine head diameters compared to Fos-negative neurons after novel context exposure. In contrast, after cue-induced cocaine, Fos-negative neurons had larger spine heads relative to Fos-positive neurons. This increase in the relapse group can be explained by larger spine head diameters in mushroom spines in the Fos-negative neurons as well as a change in proportion of mushroom and filopodia/long thin spines. In a previous study, we found ribosomal S6 protein is a more specific marker of cue-activated

synapses. So we are now examining morphological alterations in S6-positive dendritic spines in the above brains to identify synaptic engrams in cue-induced cocaine craving.

Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.07

Topic: G.09. Drugs of Abuse and Addiction

Title: In vivo labeling and molecular characterization of cocaine memory-specific active neurons using the photo-convertible calcium integrator CaMPARI2

Authors: R. MADANGOPAL[1], K. E. SAVELL[1], O. R. DRAKE[1], M. B. BRENNER[1], V. A. LENNON[1], D. Q. PHAM[1], S. J. WEBER[1], L. E. KOMER[1], L. WANG[2], K. SCHAEFER[2], F. J. RUBIO[1], A. LEMIRE[2], V. MENON[3], E. R. SCHREITER[2], *B. T. HOPE[1];
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Abstract: In abstinent drug users, cues previously associated with drug-taking can provoke drug craving and promote relapse long after the last instance of drug use. These maladaptive drug-cue associations are thought to be encoded by sparse patterns of strongly activated neurons (neuronal ensembles) that can be identified by the expression of immediate early genes (IEGs) such as *Fos*. However, IEG-based labels lack the temporal precision needed to label active neurons during short-lasting behavioral events (e.g, lever press or drug infusion) or characterize them immediately after the event when important learning mechanisms are being activated. We employed a photo-convertible calcium integrator, CaMPARI2, to label active neurons in the infralimbic cortex (IL) of rats with sub-second temporal specificity. We applied ultraviolet photoconversion (PC) light during cocaine seeking to rapidly convert CaMPARI2 protein in active neurons from green to red fluorescent state and thus permanently labeled these cocaine-memory specific neurons.
We used male and female Sprague-Dawley rats in all experiments. We delivered AAVs into IL for CaMPARI2 expression, implanted an optical fiber for PC light delivery and inserted a jugular catheter for cocaine self-administration. We trained rats to self-administer cocaine (FR1 reinforcement schedule, 0.75 mg/kg/infusion cocaine paired with a 3.5 s light cue) during twice

daily 3 h sessions before switching to trial-based cocaine self-administration (30 trials/ 3 h session, 1 min lever access/trial). Following training and 21 abstinence days, we tested rats for cocaine-seeking (1 min lever access, extinction conditions) and delivered PC light (1 min, 10 mW, 375 nm) to permanently label cocaine-memory specific active neurons in IL. We observed reliable cocaine self-administration during training and robust cue-induced cocaine seeking following abstinence. We collected brains either immediately after the 1 min test (0-min group) or waited 10 minutes to allow for experience-induced gene expression (10-min group). We used fluorescence activated nuclei sorting to isolate red (active) and green (inactive) CaMPARI2 labeled neuronal nuclei and performed single-nucleus RNA sequencing. We will identify unique molecular alterations (differentially expressed genes, DEGs) induced specifically within cocaine memory ensemble neurons following relapse and investigate whether DEGs are restricted to specific cell type clusters within IL. Understanding the molecular and cell-type basis of drug memories are maintained could help prevent relapse by selectively weakening persistent drug memories, without influencing other memories.

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Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.08

Topic: G.09. Drugs of Abuse and Addiction

Title: Capturing and profiling cocaine-recruited neuronal ensembles in the nucleus accumbens

Authors: *M. SALERY[1], A. GODINO[1], Y. XU[1], J. F. FULLARD[2], P. ROUSSOS[2], E. J. NESTLER[1];
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Abstract: Learned associations between the rewarding effects of drugs and the context in which they are experienced are decisive for precipitated drug-seeking and relapse in abstinent individuals. As learned associative memories have been proposed to be stored in sparse and highly discriminative populations of concomitantly activated neurons, emerging evidence supports the idea that drug-recruited neuronal ensembles could similarly encode addiction-related pathological memories. However, little is known regarding the dynamics and molecular mechanisms of both their recruitment upon initial drug exposure and their later contribution to relapse. In this study we explore the contribution of the subsequent reactivation of an initially-

activated ensemble in supporting the encoding, the strengthening, and ultimately the expression of drug-associated memories. A related goal is to explore the intrinsic or acquired cellular properties that would favor the allocation of specific cells to these functional ensembles and predict their further reactivation. Capitalizing on the activity-dependent labeling of neuronal ensembles in Arc-CreER[T2] mice (Denny et al., 2014), we were able to capture cocaine-activated cells in the nucleus accumbens and permanently tag them with fluorophores or channel-rhodopsin for further characterization, optogenetics, and single-nuclei sorting. We identified a subset of neurons activated at both early and late stages of drug exposure and show that the level of reactivation of the initial ensemble correlates with the amplitude of behavioral sensitization. Similarly, re-exposure to a cocaine-paired context in a conditioned place preference (CPP) paradigm was associated with an increased reactivation of cocaine-recruited ensembles. The behavioral consequences of such reactivation was further assessed using optogenetics-mediated artificial reactivation. We found that the reactivation of ensembles recruited at early- versus late-stages of drug exposure had opposite effects on CPP expression. We then isolated tagged nuclei with FACS and performed single nucleus RNA sequencing to analyze their transcriptional signature. Using activity-dependent transcriptional programs as a marker of recent activation, we successfully isolated a cluster of reactivated cells within the initially activated ensemble. Together, such multiscale and ensemble-specific approaches represent a pivotal step towards a better understanding of the cellular and molecular processes involved in the encoding of pathological memories associated with drug addiction.

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Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.09

Topic: G.09. Drugs of Abuse and Addiction

Title: Serotonin Modulates the Interaction between Prior Social Experience and Alcohol Sensitivity in Crayfish

Authors: *T. HO[1], J. HERBERHOLZ[1,2];
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Abstract: Social isolation causes detrimental effects to human health and has been implicated in alcohol use disorder. Prior studies in humans and other animal species have shown that social isolation correlates with increased alcohol consumption. However, the underlying mechanisms are poorly understood. Our lab has developed the crayfish as a suitable model system to elucidate the neurocellular mechanisms regulating the interplay between social isolation and acute alcohol (EtOH) intoxication. Prior work in our lab has demonstrated that acute EtOH exposure elicits a sequence of discrete and biphasic behavioral modifications in both juvenile and adult crayfish (elevated stance and spontaneous tail-flipping is followed by loss of postural control). Intriguingly, we found that crayfish isolated for 7 days displayed lower behavioral sensitivity to acute EtOH exposure compared to group-housed conspecifics, and this socially-dependent difference was paralleled on the level of single, identified neurons. This includes the lateral giant (LG) interneuron - the command neuron that drives one of the tail-flip escape behaviors in crayfish. Our current work aims to uncover the role of serotonergic modulation in this process. A total of 164 crayfish, *Procambarus clarkii*, of both sexes (80 males and 84 females), all freely behaving, were used in this study. First, we found that chronic depletion of serotonin (5-HT) in the nervous system, through systemic injection of a 5-HT neurotoxin, eliminated the difference in EtOH sensitivity between socially isolated and communally housed crayfish. This suggests the difference is, at least in part, mediated by the serotonergic system. Second, pre-treatment via bath application with fluoxetine, a selective serotonin reuptake inhibitor, facilitated the socially-mediated difference in EtOH sensitivity, further affirming interactions between the 5-HT system, social history, and EtOH sensitivity. Lastly, systemic injections of methiothepin maleate, an antagonist for 5-HT$_{2b}$ receptors in our species, reduced the behavioral sensitivity to EtOH in animals of both social conditions, but the effects were less pronounced in socially isolated crayfish. Taken together, our results suggest that one week of social isolation causes changes in the expression of specific 5-HT receptors and most likely a downregulation of the crayfish 5-HT$_{2b}$ receptor subtype. One promising target for investigation of receptor changes is the LG circuit. Our ongoing work focuses on intracellular electrophysiology and neuropharmacology as well as molecular analyses such as western blotting and single-cell RNAseq of the LG neurons to confirm this hypothesis.

Disclosures: T. Ho: None. **J. Herberholz:** None.

Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.10

Topic: G.09. Drugs of Abuse and Addiction

Title: Investigating a novel therapeutic target for treatment of alcoholism

Authors: *A. GOYAL[1], J. YUEN[2], A. RUSHEEN[1], K. E. BENNET[3], Y. OH[1], K. H. LEE[1], H. SHIN[4];

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Abstract: Alcohol abuse is an extremely prevalent problem. Chronic ethanol (EtOH) intake increases tonic extracellular dopamine (DA) concentrations throughout the limbic system, contributing to addiction. Tracking these tonic DA changes in real-time with high spatiotemporal resolution will enable investigations into the effects of EtOH administration, and the potential of neuromodulation to reverse these tonic DA increases. Our lab has developed the multifunctional apparatus for voltammetry, electrophysiology, and neuromodulation (MAVEN) recording device that measures tonic neurotransmitter concentrations with multiple-cyclic square wave voltammetry (M-CSWV) simultaneously with local field potentials and electrical stimulation across 4 channels. This allows the ability to track tonic DA concentrations changes in several brain regions in response to acute EtOH administration and neuromodulation. The ventral tegmental area (VTA) projects DA to the nucleus accumbens (NAc), contributing to addiction. However, continuous activation of the VTA may activate VTA D2 autoreceptors, thereby inhibiting DA release into the NAc and mitigating the addictive potential of drugs of abuse. To probe this hypothesis *in vivo,* a carbon fiber microelectrode was lowered into the NAc of urethane-anesthetized Sprague-Dawley rats. One hour of baseline electrochemical recording was performed, and then EtOH was administered (2.5 g/kg, i.p.). To capture the entire time course of the EtOH, 3 hours of tonic DA measurements were performed. 30 minutes after EtOH was administered, electrical stimulation (90 Hz, biphasic 200 us pulse width, 0.2 mA) was delivered continuously to the VTA for 30 minutes, and the resulting effects on tonic DA levels were tracked voltammetrically. Tonic extracellular DA concentrations increased in the rat NAc to 200% ± 15% of baseline after acute EtOH administration (n = 5; **Fig 1A**). 90 Hz electrical stimulation of the VTA reversed this increase, such that DA levels during VTA stimulation were not significantly different from pre-EtOH treatment (p > 0.1; n = 4; **Fig 1A-B**). After electrical stimulation was discontinued, the tonic DA levels continued to rise, and returned to 200% ± 12% of baseline (n = 4; **Fig 1A-B**). Overall, electrical stimulation of the VTA reversed the acute DA increase caused by EtOH exposure, suggesting that tonic VTA stimulation may activate D2 autoreceptors and lower DA release to the NAc. These results suggest the exciting possibility that DBS of this area can modulate the addictive potential of drugs of abuse and may perhaps be a treatment for alcoholism. We are now working to assess this possibility in chronic addiction rat models.

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Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.11

Topic: G.09. Drugs of Abuse and Addiction

Title: Intoxicating effects of alcohol depend on acid-sensing ion channels

Authors: G. HARMATA[1], **A. C. CHAN**[3], **M. MERFELD**[3], **R. J. TAUGHER-HEBL**[4], **A. HARIJAN**[5], **J. B. HARDIE**[3], **J. LONG**[3], **G. WANG**[3], **A. KANTI**[5], **N. S. NARAYANAN**[6], **B. J. DLOUHY**[2], ***J. WEMMIE**[3];
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Abstract: Persons at risk for developing alcohol use disorder (AUD) differ in their sensitivity to acute alcohol intoxication. Alcohol effects are complex and thought to depend on multiple mechanisms. Here, we explored whether acid-sensing ion channels (ASICs) might play a role. We tested ASIC function in transfected CHO cells and amygdala principal neurons, and found alcohol potentiated currents mediated by ASIC1A homomeric channels, but not ASIC1A/2A heteromeric channels. Supporting a role for ASIC1A in the intoxicating effects of alcohol *in vivo*, we observed marked alcohol-induced changes on local field potentials in basolateral amygdala, which differed significantly in *Asic1a^{-/-}* mice, particularly in the gamma, delta, and theta frequency ranges. Altered electrophysiological responses to alcohol in mice lacking ASIC1A, were accompanied by changes in multiple behavioral measures. Alcohol administration during amygdala-dependent fear conditioning dramatically diminished context and cue-evoked memory on subsequent days after the alcohol had cleared. There was a significant alcohol by genotype interaction. Context- and cue-evoked memory were notably worse in *Asic1a^{-/-}* mice. We further examined acute stimulating and sedating effects of alcohol on locomotor activity, loss of righting reflex, and in an acute intoxication severity scale. We found loss of ASIC1A increased the stimulating effects of alcohol and reduced the sedating effects compared to wild-type mice, despite similar blood alcohol levels. Together these observations suggest a novel role for ASIC1A in the acute intoxicating effects of alcohol in mice. They further suggest that ASICs might contribute to intoxicating effects of alcohol and AUD in humans.

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Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.12

Topic: G.09. Drugs of Abuse and Addiction

Support: 348NIH6JSC

Title: Identifying sex differences in neuronal activation during aversion-resistant alcohol intake

Authors: *M. E. ARNOLD, A. N. BUTTS, K. N. AMICO, J. R. SCHANK;
Physiol. and Pharmacol., Univ. of Georgia Interdisciplinary Neurosci. Program, Athens, GA

Abstract: Within the last 20 years, there has been an increase in women have been diagnosed with alcohol use disorder (AUD). Females also have been shown to transition from recreational alcohol use to a state of dependence more rapidly than males and begin drinking at a later age compared to men. This suggests a sex difference in alcohol use patterns and highlights a need to examine both sexes in preclinical studies. A major DSM-5 criterion for AUD is compulsive alcohol consumption, which is the continuation of alcohol use despite negative consequences such as an adverse impact on health, loss in workplace productivity, and strained relationships with family and friends. This has been modeled in mice using adulteration of alcohol solution with the bitter tastant quinine. Mice that continue to consume ethanol despite this adulteration are considered to be aversion resistant. Our aim was to characterize aversion-resistant ethanol intake in female mice compared to males during continuous access two-bottle choice and identify potential sex differences in neuronal activation.In our study, male and female C57BL6/J mice voluntarily consumed ethanol for 10 days before adulterating with increasing concentrations of quinine-hydrocholoride. Baseline ethanol consumption was greater in females compared to males, consistent with the findings of many other groups. We found that female mice were more resistant to quinine adulteration than males. Two-way ANOVA analysis revealed a significant effect of quinine concentration ($F_{(2,87)} = 8.233$, p=0.0005) and an effect of sex ($F_{(1,87)} = 10.82$, p=0.0014). We followed this by examining neuronal activation using Fos immunohistochemistry after a quinine-ethanol drinking session, focusing on brain regions with roles in reward, aversion, decision-making, and salience. Three regions exhibited an interaction between sex and quinine adulteration: ventromedial prefrontal cortex (vmPFC), posterior insular cortex (PIC), and ventral tegmental area (VTA). The vmPFC and the PIC displayed the highest level of Fos positive cells in males that consumed quinine-adulterated ethanol, while the VTA exhibited an increase in neuronal activation in females that consumed quinine-adulterated ethanol. Our next step of this study is to identify cellular phenotype of neurons activated during aversion-resistant ethanol intake in male and female mice by using RNAscope procedures in the vmPFC, PIC, and VTA. We believe that this will further dissect the circuitry of aversion-resistant drinking behaviors between males and females.

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Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

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Presentation Number: 347.13

Topic: G.09. Drugs of Abuse and Addiction

Title: Activation of the CRF-CRF1 system is responsible for the decrease in whole-brain modularity during alcohol withdrawal

Authors: *L. L. G. CARRETTE[1], A. SANTOS[1], M. BRENNAN[1], D. OTHMAN[1], A. COLLAZO[2], O. GEORGE[1];
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Abstract: Using single-cell whole brain imaging of immediate early genes, our group recently showed that alcohol-dependent mice in withdrawal exhibit a widespread increase in coordinated brain activity, associated with a decrease in modularity of the whole-brain functional network, compared to naive mice. This decreased modularity and hyperconnectivity has been hypothesized to represent a novel biomarker of alcohol dependence that can be used to evaluate the potential efficacy of novel treatments for alcohol use disorder. However, there is no evidence that current FDA-approved and experimental treatments that reduce alcohol drinking normalize the changes in whole-brain functional connectivity. Here, we examine the effect of an FDA approved treatment for alcohol use disorder, naltrexone (opioid antagonist), and an experimental treatment, R121919 (CRF antagonist), on the functional connectome modularity of naive and alcohol dependent mice in withdrawal. Alcohol dependence was induced in mice by ethanol injections (2 g/kg or saline; i.p., 9 days) with 4-fomepizole (9 mg/kg). On day 10, 24 h in withdrawal, mice were treated with naltrexone (3 mg/kg), R121919 (2 mg/ml) or saline (N = 4F + 4M). Behavior was examined 30 min following treatment by digging and marble burying tests. Then, 90 min later, mice were sacrificed, the brains immunolabeled for FOS, cleared using the iDISCO+ protocol, imaged using light-sheet microscopy, and processed using the ClearMap pipeline to map the functional connectome. Alcohol dependent animals dug significantly more than naive animals, but there was no difference between treatments. The modularity of the alcohol dependent withdrawal network was normalized by R121919 treatment, that mainly acted in the CRFR1 receptors in the cortical plate, causing functional disconnection between the prefrontal cortex and extended amygdala. Naltrexone on the other hand caused a further reduction of the modularity, through broad brain-wide co-repression in reactivity. While naltrexone reduced alcohol intake and withdrawal-induced hyperalgesia in dependent rats in withdrawal, it was also found to cause increased anhedonia-like behaviors, which could be explained by increased reactivity of the lateral habenula and decreased network modularity. These results demonstrate that whole-brain functional connectivity based on immediate early genes can be used to identify the neuronal network mechanisms underlying the behavioral effects of potential medications, identify brainprints of specific compounds, and demonstrate that activation of the CRF-CRF1 system is responsible for the decrease in whole-brain modularity during alcohol withdrawal.

Nanosymposium

347. Cellular, Molecular, and Genetic Mechanisms of Drug and Alcohol Abuse

Location: SDCC 23

Time: Monday, November 14, 2022, 1:00 PM - 4:30 PM

Presentation Number: 347.14

Topic: G.08. Other Psychiatric Disorders

Title: Precision Functional Brain Mapping in Healthy Adults before, during, and after Psilocybin Exposure

Authors: *S. SUBRAMANIAN[1], J. S. SIEGEL[2], D. PERRY[3], R. RENEAU[4], K. FLAVIN[3], J. SCHWEIGER[3], C. HORAN[3], N. METCALF[5], E. J. LENZE[3], N. DOSENBACH[6], A. S. SNYDER[5], G. E. NICOL[3];
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Abstract: Background Psilocybin is a serotonin 2A receptor agonist with rapid-onset antidepressant effects. It produces neurotrophic effects in animal models, but its persisting effects on human brain networks remain unknown. Resting state functional MRI (rsfMRI) is a promising method for visualizing these effects. The precision functional mapping (PFM) strategy increases sensitivity of rsfMRI by controlling for individual variability, and improving data quality; PFM may increase the ability to resolve circuit-level effects of plasticity-inducing drugs. In the present study, we piloted a PFM approach to evaluate acute and persisting effects of psilocybin in healthy adults. **Methods** We conducted a cross-over study to characterize the impact of psilocybin (25mg) versus methylphenidate (active control, 40mg) on the brain using resting state, task, and diffusion MRI. Healthy volunteers ages 18-45 years with previous psychedelic experience were eligible to participate. Subjects underwent 1) baseline imaging, 2) two drug exposure scans, 60-90 minutes after psilocybin or methylphenidate ingestion, and 3) longitudinal imaging for up to two weeks after first drug and second drug exposure. Altogether, up to 40 15 minute rsfMRI scans were obtained. Image quality was determined using head motion (<0.2 mm movement per 15-minute scan). Subjective experience was measured via the Mystical Experiences Questionnaire (MEQ) after each drug exposure session. **Results** Six adults (mean age 32.7 years, SD=9.9; 50%[n=3] female, 83%[n=5] Caucasian) completed the study. We obtained a mean of 34 (SD = 6.7) 15-minute rsfMRI scans per participant. Unique to our dataset, 5 out of 6 individuals had at least one 15-minute high-quality resting state scan on psilocybin with <0.2mm average framewise head motion. All 6 individuals reported significantly higher MEQ scores on psilocybin across all four factors compared to during the control condition

(t-test, p<0.05). Both acute and chronic spatial and organizational variability in brain networks were revealed, as well as network features and topologies that corresponded with structural and task-derived brain features. **Conclusions** This data resource is presented as a resource for neuroscientists, and we propose precision individual connectomics to access the effects of psilocybin on neural networks. This type of data is critical for developing precision dosing and administration protocols for psilocybin and other psychedelic compounds and serves as a model for future mechanistic study of therapeutic effects in the human brain.

Nanosymposium

348. Human LTM: Encoding and Retrieval

Location: SDCC 24

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 348.01

Topic: H.07. Long-Term Memory

Title: Memory's pulse: theta rhythmic sampling underlies episodic memory formation

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Abstract: Episodic memory formation has long been hypothesized to occur at specific phases of hippocampal theta oscillations (3-10 Hz; Hasselmo et al., 2002), but we are only beginning to understand whether humans indeed rhythmically sample experiences into memory (Ter Wal et al., 2021). Here, we apply the behavioral oscillation paradigm to episodic memory for the first time to determine if its formation is rhythmic. This approach leverages the finding that a salient cue resets ongoing neural oscillations, making the timing of oscillation cycles predictable relative to cue onset. Consequently, if memory formation is rhythmic, the successful encoding of the subsequently presented item should exhibit oscillatory fluctuations as a function of the stimulus-onset asynchrony (SOA) between cue and item. Thus, in the current preregistered (https://osf.io/d582f) study, we cued participants (n=120) to perform one of two perceptual judgments (blue flash: indoor/outdoor task; orange flash: size task) on a subsequently presented object. Importantly, the cue-to-object SOAs were varied systematically (200-1100 ms). When subsequent memory for the object-task association was examined as a function of the SOAs, spectral analysis revealed that associative encoding oscillated at the theta frequency, as measured in both accuracy (p<.005) and reaction time (p<.05, uncorrected). Furthermore, the time-frequency analysis showed that theta rhythmic sampling was transient, occurring for 250-350 ms. Remarkably, the phase of theta oscillations in memory formation was synchronized across individuals (p<.001), suggesting common moments optimal for the formation of episodic memories. Furthermore, no behavioral oscillations in classification performance during encoding were observed, supporting the notion that rhythmicity in memory formation is not simply a function of rhythmic attentional sampling. Strikingly, these findings align with the timing of post-stimulus human hippocampal theta oscillations and their relationship to associative memory formation (Herweg et al., 2020). Taken together, the current data provide the first evidence – to our knowledge – that humans do encode episodic memories rhythmically, possibly reflecting fluctuations in neuronal excitability and circuit dynamics in the hippocampus.

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Nanosymposium

348. Human LTM: Encoding and Retrieval

Location: SDCC 24

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 348.02

Topic: H.07. Long-Term Memory

Title: Interleaved learning shapes neural representations in medial prefrontal cortex to enhance categorization of naturalistic stimuli

Authors: S. M. NOH[1], ***N. W. MORTON**[2], A. R. PRESTON[3];
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Abstract: Real-world reasoning often depends on generalizing knowledge from previous exemplars to new stimuli; for example, one may determine the music genre of a new album by comparing it to previously encountered albums. Recent work suggests that learning of real-world stimulus categories may benefit from interleaving exemplars across different categories, compared to blocking presentation of exemplars by category (Kornell & Bjork, 2008, Birnbaum, Kornell, Bjork, & Bjork, 2013). Interleaved learning may emphasize discriminative contrast between different categories, leading to improved generalization (Kang & Pashler, 2012); however, the neural implications of this hypothesis have not yet been tested. Prior work suggests that learning of discriminative contrast may be facilitated by medial prefrontal cortex (mPFC), which is thought to form reduced-dimensionality representations of category-relevant stimulus features (Mack, Preston, & Love, 2020), and hippocampus, which is thought to group exemplars based on their relevant features (Mack, Love, & Preston, 2018). To test these hypotheses, we had participants learn to categorize naturalistic painting stimuli based on the artist of each painting. Consistent with prior work (Kornell & Bjork, 2008), we found that categorization of new exemplars was more accurate for categories learned with an interleaved schedule compared to categories learned with a blocked schedule. To measure learning-related changes in neural representations of individual exemplar paintings, participants were scanned during presentation of each painting before and after learning of the category labels. We found that the similarity of exemplar representations in both hippocampus and mPFC was modulated by learning schedule, with greater separation between categories for paintings learned with the interleaved schedule compared to the blocked schedule. Furthermore, we found that exemplars presented within the same block during interleaved learning formed representations in mPFC that differentiated between same-category and different-category exemplar pairs. Consistent with the discriminative contrast hypothesis, individual differences in the strength of this category differentiation in mPFC correlated with the degree to which interleaved categories were better generalized than blocked categories. Our results suggest that an interleaved schedule focuses attention on category differences, promoting formation of discriminative representations in hippocampus and mPFC that may support successful categorization of new stimuli.

Disclosures: S.M. Noh: None. **N.W. Morton:** None. **A.R. Preston:** None.

Nanosymposium

348. Human LTM: Encoding and Retrieval

Location: SDCC 24

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 348.03

Topic: H.07. Long-Term Memory

Title: Distinct hippocampal contributions to the rapid learning of category exceptions

Authors: *M. GUMUS, M. L. MACK;
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Abstract: Successful memory relies on the ability to record specific details of individual events and generalize acquired information to new experiences. Hippocampus (HPC) is involved in both processes, forming organized concepts from individual episodes to adaptively represent shared and distinct elements. However, the role of HPC in concept formation is largely based on behavioural evidence and neural coding measured at the end of learning after episodic and conceptual memories are formed. Thus, it remains unclear what neural mechanisms are at play early in learning and how initial HPC information processing supports the formation of flexible category knowledge. We combined functional MRI with a computational model of learning, SUSTAIN, to investigate how early learning processes are reflected in neural activity. Participants (N = 37) learned to categorize complex visual objects with multiple features during fMRI scanning. Categories were defined according to a rule-plus-exception structure such that each category included a prototype along with two similar and one exception items that differed from the prototype by one and two features, respectively. Exception items were visually more similar to the prototypes of the opposite category than to their own, thus acting as a behavioural index of flexible learning. Participants were separated into 3 learner groups according to their end categorization accuracy for exceptions: low ($< 50\%$), medium (at 50%), and high ($> 50\%$). Although early learning behaviour was similar across learners, model-based estimates of latent category representations indicated high learners rapidly encoded distinct representations for exceptions. Medium and low learners were characterized by less precise representations that favoured generalization for rule consistent items. Neural signatures of these behavioural differences revealed better exception learning was associated with deactivation of distinct HPC subfields, including dentate gyrus and $CA_{2,3}$, during initial learning. This link between early learning HPC engagement and learning outcome was stronger in anterior portions of HPC. Deactivation within the HPC may indicate the neural underpinning of pattern separation to allow for sparse representations of exceptions. These findings suggest that HPC differentiates initial information in learning to support formation of flexible category knowledge.

Disclosures: M. Gumus: None. **M.L. Mack:** None.

Nanosymposium

348. Human LTM: Encoding and Retrieval

Location: SDCC 24

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 348.04

Topic: H.07. Long-Term Memory

Title: Neural correlates of memory-based decisions in younger and older adults

Authors: *C. M. F. VAN GEEN[1], M. COHEN[1], K. M. LEMPERT[3,1], K. A. MACNEAR[4], F. M. RECKERS[2], L. ZANESKI[2], D. A. WOLK[5], J. W. KABLE[1];
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Abstract: This study aims to uncover the neural mechanisms that support memory-based decision-making in younger vs. older adults, specifically in the social domain. When people decide whether to re-engage with others, various sources of information compete to shape their choice. One source of information people may use is their episodic memory for their previous interactions with someone. Conversely, they may extrapolate across experiences to form semantic expectations about characteristics that are likely to connote positive attributes in a person. For instance, it is known that people infer traits like trustworthiness or generosity from certain facial features (Todorov et al., 2008), even when these are unrelated to actual generosity. Aging has been shown to modulate the weight given to episodic vs. semantic information: older adults exhibit a decline in associative memory, which hinders their ability to make memory-based decisions. However, they demonstrate an increased reliance on semantic features of facial appearance during choice (Lempert et al., 2022). Here, we ask whether age-related differences in neural activity while participants are learning about others' behavior underlie these behavioral effects. We collected fMRI data from 45 younger (ages 21-40) and 39 older (ages 65-85) adults while they performed a memory-based decision-making task. Participants first learned associations between images of faces and reward outcomes. They then decided whether to interact with each of the faces again, or rather with an unknown face chosen at random. Behaviorally, older adults made fewer adaptive decisions than younger adults (p = 0.013), meaning that they were less likely to re-engage with a high-reward face or avoid a low-reward face. During the reward learning phase, we found a parametric effect of the face's perceived generosity in the amygdala and the striatum (whole brain analysis: p < 0.001 voxel threshold with parametric cluster correction to p < 0.05). Furthermore, the magnitude of this effect in the striatum was inversely correlated with the proportion of adaptive choices in the decision task for older but not younger adults (Fisher Z difference in correlation coefficients: p = 0.043). In conclusion, we find that echoing the striatum's role in responding to rewards, both younger and older adults show striatal activation in response to facial features that connote generosity. However, the magnitude of this effect is only negatively correlated with performance in older adults. This suggests that the ability to make decisions based on episodic memory is impaired in older adults, possibly in favor of an increased reliance on over-generalized inferences.

Disclosures: C.M.F. van Geen: None. **M. Cohen:** None. **K.M. Lempert:** None. **K.A. MacNear:** None. **D.A. Wolk:** None. **J.W. Kable:** None.

Nanosymposium

348. Human LTM: Encoding and Retrieval

Location: SDCC 24

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 348.05

Topic: H.07. Long-Term Memory

Title: Age-related differences in frontoparietal function support developmental improvements in memory-based inference

Authors: *C. COUGHLIN[1], M. L. SCHLICHTING[3], N. W. MORTON[1], K. R. SHERRILL[1], M. M. MOREAU[1], A. R. PRESTON[2];
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Abstract: A mature memory system flexibly supports memory for discrete events as well as the ability to infer connections between events, the latter of which allows individuals to extend knowledge beyond direct experience. The extraction of knowledge through memory-based inference improves across childhood; however, little is known about the neurocognitive mechanisms that contribute to this increase. Here, we examine the mechanisms that support successful inference decisions in children and adults. Prior work indicates that adults may infer connections between related events as they are learned, forming integrated representations in memory that support faster and more accurate inference decisions. Frontoparietal representations may allow adults to navigate inferred trajectories through integrated memory representations, speeding inference decisions. Frontoparietal cortex has a prolonged developmental trajectory, raising the possibility that children may perform inference differently from adults. One hypothesis is that children must iteratively retrieve and recombine multiple, individual memories during inference. Such an iterative retrieval strategy would rely on associative retrieval processes supported by the hippocampus, rather than frontoparietal mechanisms that exploit integrated representations formed during learning. To test these ideas, children (7-12 years) and adults (total *N*=74) studied and were tested on direct associations that overlapped due to a shared item (AB, BC pairs). In a surprise inference test, they were then asked to infer an indirect association between elements from overlapping pairs (AC inference). While both memory for direct associations and inference (controlling for memory of component pairs) improved with age, inference improvements were more protracted. Computational modeling of response times further suggested that adults can flexibly perform inference using either integrated representations or an iterative retrieval strategy. We found that all age groups recruited hippocampus more for inference than direct memory, which may reflect the proposed iterative retrieval mechanisms. In contrast, adults uniquely recruited lateral prefrontal cortex (LPFC) and posterior parietal cortex (PPC) during inference decisions, with the degree of engagement predicting faster inference only in adults. Collectively, these results indicate that while children can perform inference successfully, they may not do so as efficiently as adults, who leverage mature frontoparietal function to navigate inferred trajectories through learned memory representations.

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Nanosymposium

348. Human LTM: Encoding and Retrieval

Location: SDCC 24

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 348.06

Topic: H.07. Long-Term Memory

Title: How adolescents and adults use category knowledge to learn and infer value

Authors: *C. INSEL, N. BIDERMAN, Z. SHEHZAD, S. BAMBARDEKAR, L. CONNER, D. SHOHAMY;
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Abstract: Adolescence provides a window of opportunity for learning. Prior research has investigated the mechanisms by which adolescents learn habits from repeated experiences with reward. Yet, a key feature of adolescence is the rapid expansion of knowledge about the world. This growing knowledge provides a scaffold for generalizing any one particular experience to other similar experiences and allows individuals to integrate multiple separate memories to build an internal predictive model. However, while this process of learning and generalization has been extensively studied in adults, it remains unclear how generalization supports value-based inference during adolescence. From childhood to adulthood, connectivity between the striatum and distributed cortical regions strengthens, which may support the developmental emergence of flexible generalization of value. To test this, we designed a reward-based learning task that leveraged object categories as a form of general knowledge. Participants chose between pairs of objects for the chance to receive a monetary reward. Objects were sampled from 33 distinct categories which were, on average, worth different amounts of reward (e.g., balloons = ~80¢, masks = ~20¢), allowing participants to learn the object category value. We tested whether individuals generalized category value to guide decisions when they were presented with novel objects from previously learned categories. To index explicit awareness of the category value structure, participants self-reported category values after learning. We examined age-related differences in 102 participants aged 10 to 25 years-old. Because retrieving and updating category knowledge relies on cortical systems that continue to mature during adolescence, we hypothesized that flexible category generalization would emerge with age. We found that 10-12 year-olds did not use category value to guide decision making. However, generalization increased with age, and older adolescents and adults were more likely to generalize category value. Surprisingly, although younger adolescents did apply category value to guide decision making, they still reported explicit awareness of the category values following the task. This reveals that younger participants learned category value but did not generalize this learning to guide decisions about novel choices. Together, these findings demonstrate that younger adolescents experience a knowledge-behavior gap: they can explicitly express value knowledge but don't apply it to guide value-based decision making. Future work will identify how ongoing brain development supports the emergence of flexible generalization.

Nanosymposium

348. Human LTM: Encoding and Retrieval

Location: SDCC 24

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 348.07

Topic: H.07. Long-Term Memory

Title: Memory distortions reveal different representations for specific and general knowledge

Authors: *M. C. TANDOC[1], C. V. DONG[2], A. C. SCHAPIRO[1];
[1]Univ. of Pennsylvania, [2]Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Information varies in how reliably it appears across experiences. Some information links our experiences together (birds can fly) while other information sets them apart (flamingos have long legs). How do we learn and represent both kinds of information? These forms of knowledge are best supported by opposing forms of representation: Learning shared features benefits from integrating information across instances in support of generalization, whereas learning unique features benefits from keeping instances separate in memory to reduce interference. While a growing body of work suggests that the brain uses both integrated and separated representations to represent whole experiences, items, and events, less is understood about how individual features, which themselves vary in generalizability (most birds can fly but few have long legs), are represented. Across two behavioral experiments, we leverage distortions in color memory (Chanales et al., 2021, *Psychol. Sci*) to evaluate representational shifts in the learning of shared and unique features in a novel semantic domain. Participants learned two categories of novel "satellite" objects: Each satellite had parts shared with members of the same category as well as a part unique to that exemplar. Each part had a color drawn from a 2D color space, with the parts for each category clustered in this space. As participants learned the features, we tested their color memory to track representational change for each feature. Relative to unique features, we found that shared features were misremembered as being more similar to the category's average color. This effect was particularly evident for shared features of novel satellites. This suggests that the representation of shared features in memory may be more integrated with category information relative to unique features. To examine what representations might have given rise to these systematic distortions in feature memory, we trained a neural network model of the hippocampus on these categories. We found that in subregion CA1 of the model, shared features are represented similarly to same-category features, whereas unique features are represented more distinctly. Together, these results demonstrate that features that are more generalizable vs. idiosyncratic are represented in memory according to their different computational needs, with shared features benefitting from integration and unique features benefitting from relative separation. We are currently collecting neuroimaging data that examines how shared and unique features are represented in the brain.

Nanosymposium

348. Human LTM: Encoding and Retrieval

Location: SDCC 24

Time: Monday, November 14, 2022, 1:00 PM - 3:00 PM

Presentation Number: 348.08

Topic: H.07. Long-Term Memory

Title: Higher-contrast images are better remembered during naturalistic encoding

Authors: *S. GILAIE-DOTAN[1,2], **L. BROOK**[1,2], **O. KREICHMAN**[1,2], **S. MASARWA**[1,2];
[1]Sch. of Optometry & Vision Sci., [2]The Gonda Multidisciplinary Brain Res. Ctr., Bar Ilan Univ., Ramat Gan, Israel

Abstract: It is well substantiated that visual memory is affected by semantic and conceptual information. However this is often investigated during task-based instructed encoding and while physical image properties are not parametrically manipulated at critical visibility ranges (as low contrast or small size). We have recently shown that during spontaneous viewing (naturalistic encoding) visual image size significantly influences visual memory and image memorability such that bigger images are better remembered and have higher memorability. Here we reasoned that without task-related modulations, higher contrast images preferentially activate multiple visual stages and would thus be better remembered. Naïve participants (n=39) were asked to freely view presented images (160 images of 7.5 to 60 RMS contrast, memorability scores (LaMem dataset) and mean luminance controlled across contrast levels) without any instructed encoding task or knowledge about the memory aspect of the study. The images in each contrast level were of faces, people, indoor and outdoor scenes. Afterwards, participants were given a surprise recognition test (320 mid-contrast images, 50% already seen). We found that image contrast that critically contributes to image visibility influenced memory and image memorability during naturalistic encoding with higher contrast images remembered significantly better than lower contrast images (∼16% higher accuracy, ∼1.35 times better). As in our previous study we also found here that face images were best remembered and outdoors the least. These results further substantiate the contribution of bottom up physical image dimensions to visual memory during naturalistic non-instructed visual behavior.

Nanosymposium

349. On Neural Connectomics

Location: SDCC 25

Time: Monday, November 14, 2022, 1:00 PM - 3:15 PM

Presentation Number: 349.01

Topic: I.03. Anatomical Methods

Support: RF1MH117820-01A1
         UG3MH126864-01

Title: A pipeline for high resolution axonal connectomics in nonhuman primates and humans

Authors: *P. BALARAM, K. TAKASAKI, R. TORRES, O. GLIKO, S. BHASKARAN, S. SESHAMANI, B. MACLENNAN, E. E. TURSCHAK, S. KINN, A. HELLEVIK, D. VUMBACO, R. REID;
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Abstract: Many neurons send axonal projections across great distances in mammalian brains, to connect and transmit information between brain areas that aren't located in proximity. When these long-range projection axons leave one brain area and travel to the next, they cable together and form dense white matter tracts such as the corpus callosum, which connect multiple areas across whole brains. The organization and structural complexity of individual axons within white matter tracts is well documented in rodents (eg. Peng et al, 2021), but largely unexplored in the bigger brains of nonhuman primates and humans. Most reports of long-range neuronal connectivity in these species are limited to high resolution descriptions of a few projection neurons (eg. histological reconstructions from tracer injections) or low resolution descriptions of bulk white matter tracts (eg. dMRI). To overcome these technical limitations and bridge the gap between whole-brain and single-neuron imaging modalities, we built a pipeline to reconstruct individual axons traveling through dense white matter tracts in nonhuman primate and human brains.
Whole brains from each species are fixed, blocked into 1cm slabs, and serially sectioned at 100-250um. Each section is antibody labeled to visualize projection axons and fiducial markers such as cell bodies and vasculature, hydrogel embedded and expanded to segregate single axons within cabled white matter tracts and imaged with a custom light sheet microscope enabling fast acquisition of large tissue volumes. Image data from each section is reconstructed into a 3D volume and labeled axons are automatically segmented with machine learning. Identified axons are manually proofread to correct misaligned or disconnected segments, yielding accurate reconstructions of individual axons within each imaged volume. Pilot data from this pipeline explores axon trajectories between V1 and V2 in macaque monkeys and humans. Future studies will combine fMRI, dMRI and multiphoton imaging with axonal connectomics in individual brain areas, and scale to multimodal atlases of whole brains in nonhuman primates and humans.

Nanosymposium

349. On Neural Connectomics

Location: SDCC 25

Time: Monday, November 14, 2022, 1:00 PM - 3:15 PM

Presentation Number: 349.02

Topic: I.03. Anatomical Methods

Title: Functional organisation of a complete male adult Drosophila nerve cord connectome

Authors: *E. C. MARIN[1], B. J. MORRIS[1], D. KRZEMINSKI[1], T. STUERNER[2], A. CHAMPION[1], I. F. M. TAMIMI[1], G. BADALAMENTE[1], M. GKANTIA[1], C. R. DUNNE[1], P. SCHLEGEL[1], K. EICHLER[1], S. FANG[1], S.-Y. TAKEMURA[3], F. LI[3], G. M. RUBIN[3], S. BERG[3], G. CARD[3], M. M. COSTA[1], D. SHEPHERD[4], G. S. X. E. JEFFERIS[2];
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Abstract: The past few years have seen huge advances in the field of connectomics and in particular the production and analysis of several large-scale electron microscopy datasets from *Drosophila melanogaster*. These have yielded enormous amounts of data and numerous novel biological insights but are limited either in the proportion of neurons and synapses so far reconstructed (e.g., the Full Adult Fly Brain/FAFB) or in the completeness of the volume (e.g., the hemibrain, which recovers roughly 25% of neurons in the central brain). Now, improved imaging and automated segmentation protocols combined with human proofreading and expert annotation have been leveraged to produce a complete connectome of the ventral nerve cord (equivalent to the vertebrate spinal cord) of a male adult fly. Thus far we have reconstructed and annotated 6693 sensory neurons, 1328 descending neurons, 1863 ascending neurons, 753 motor neurons, and 13,065 intrinsic neurons in this volume. Here we focus on developmental origin as an organising factor. Each neuron arises from stereotyped, repeating arrays of neuroblasts in the developing nerve cord, forming clonally related groups termed hemilineages. We find that the neurons of each hemilineage generally express a single fast-acting neurotransmitter, as expected from published light-level data, but that the earliest born neurons do not necessarily conform. We describe diverse morphological and connectivity subtypes within hemilineages that we validate across hemispheres. We also discover homologous cell types across segments that reveal serially repeating neural circuits. Finally, we validate and refine specific hemilineage-based sensorimotor circuit motifs first proposed in studies of the late larval CNS and later supported by functional studies in the adult.

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Nanosymposium

349. On Neural Connectomics

Location: SDCC 25

Time: Monday, November 14, 2022, 1:00 PM - 3:15 PM

Presentation Number: 349.03

Topic: I.03. Anatomical Methods

Title: Imaging 3D brain organoid architecture from meso- to nanoscale across development

Authors: *J. E. RODRIGUEZ GATICA[1], V. IEFREMOVA[2], S. AU YEUNG[2], Y. BREITKREUZ[3], O. BRUSTLE[2,4], M. K. SCHWARZ[5], U. KUBITSCHECK[1];
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Abstract: Organoids are human stem cell-derived three-dimensional cultures offering a new avenue to model human brain development and disease processes. Brain organoids display complex structures that recapitulate several aspects of early neurogenesis, including the formation of an apical and basal surface, polarized neuroepithelium, neurogenic ventricular and outer radial glia (oRG), the formation of layered, cortex-like architectures, and maturation to the level of synapse formation (Qian, Song & Ming 2019). Thus, these allow us to study various aspects of human brain development in fine details in vitro in a tissue-like context.
However, spatial relationships of subcellular structures such as synaptic contacts between distant neurons are hardly accessible by conventional light microscopy because the critical details of neuronal connectivity occur on length scales of about 100 nm. This limitation can be overcome by systems that quickly image the entire organoid in three dimensions and super-resolution.
To that end, we have developed a setup combining tissue expansion and light-sheet fluorescence microscopy for imaging and quantifying diverse spatial parameters during organoid development. Light sheet fluorescence expansion microscopy (LSFEM) technique enables zooming from a mesoscopic perspective into super-resolution within a single imaging session, thus revealing cellular and subcellular structural details in three spatial dimensions (Bürgers et al. 2019; Rodriguez-Gatica et al. 2021).
Here we present a novel brain-organoid analysis pipeline, which employs LSFEM to image entire brain organoids during different developmental stages in 3D. We demonstrate the detection of oRG within extended organoids, the unequivocal delineation of mitotic cleavage planes, dendritic spine formation and the spatial colocalization of pre- and postsynaptic proteins.

We expect LSFEM to facilitate qualitative and quantitative assessment of organoids in developmental and disease-related studies.

BÜRGERS, J. et al., 2019. Light-sheet fluorescence expansion microscopy: fast mapping of neural circuits at super resolution. Neurophotonics, 6(1).
QIAN, X., SONG, H., and MING, G.-L., 2019. Brain organoids: advances, applications and challenges. Development (Cambridge, England), 146(8).
RODRIGUEZ-GATICA, J. E. et al., 2021. Imaging Three-Dimensional Brain Organoid Architecture from Meso- to Nanoscale across Development. BioRxiv.

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Nanosymposium

349. On Neural Connectomics

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Time: Monday, November 14, 2022, 1:00 PM - 3:15 PM

Presentation Number: 349.04

Topic: I.03. Anatomical Methods

Support: Wellcome Trust Collaborative Award 220343/Z/20/Z

Title: Automatic cell typing of Drosophila male ventral nervous system based on connectivity and morphology

Authors: *D. KRZEMINSKI[1], A. CHAMPION[1], E. MARIN[1], D. SHEPHERD[2], S.-Y. TAKEMURA[3], S. BERG[3], M. COSTA[1], G. JEFFERIS[1];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Bangor Univ., Bangor Univ., Bangor, United Kingdom; [3]Janelia Res. Campus, Ashburn, VA

Abstract: Thanks to critical technological and methodological advances, several large-scale connectomes of whole or partial nervous systems have been reconstructed recently. For example, the milestone Hemibrain dataset traces 20 million synapses and around 26,000 neurons. To allow systematic analysis and comparison, the neurons need to be grouped into morphological, neurodevelopmental and functional subpopulations. However, defining a single typing scheme for an entire connectome requires balance between competing dimensions, e.g., synaptic and functional factors, each suited to a particular research goal.
In this study, we use the Male Adult Ventral Nerve Cord (VNC) electron microscopy volume produced and imaged at Janelia Research Campus. Segmentation with the flood-filling neural network's algorithm and human proofreading resulted in 23,921 reconstructed neuron meshes, with synaptic contacts estimated using a 3D U-Net model. These predictions allowed us to infer local circuit connectivity graphs with high accuracy.
Here, we present a hierarchical cell typing procedure for the VNC, incorporating neuroanatomy, connectivity, inferred developmental origin, and ultrastructure-predicted neurotransmitter expression as criteria. We first identified broader neuronal classes, depending on their axonal

processes: ascending or descending (through the neck), sensory or motor (from or to peripheral nerves), or local interneurons (restricted to VNC). Next, using the topological NBLAST algorithm, we assigned developmental origin to VNC neurons, based on their morphology and location. Then, we used a linear support vector machine classifier to discriminate between early-born primary and later-born secondary neurons. To identify serially homologous neurons within the repeated segmental organisation of the VNC, we employed an iterative procedure based on a "seeded" version of the Fast Approximate Quadratic Assignment algorithm, which aims to minimise the number of edge disagreements between the two graphs. Finally, we fit a series of hierarchical clustering models to find the optimal grouping between the types of neurons. Such a procedure preserves symmetries including lateral and serial homology while providing tools for researchers to dynamically correct the type clusters in response to analysis.

In total, we annotated over 17,000 VNC neurons. Our pipeline, tool suite, and resulting cell typing furthers the usefulness of connectome as a common index to integrate multi-modal information on neural circuit composition and function, particularly as multiple other VNC and central nervous system datasets are generated.

Nanosymposium

349. On Neural Connectomics

Location: SDCC 25

Time: Monday, November 14, 2022, 1:00 PM - 3:15 PM

Presentation Number: 349.05

Topic: I.03. Anatomical Methods

Title: Ultrastructural readout of in vivo synaptic activity for functional connectomics

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Abstract: Large-volume ultrastructural mapping approaches offer high-resolution data on structural connectivity in neural circuits, but lack an integrated synaptic activity readout which is essential for the functional interpretation of the connectome. To resolve this limitation we developed a FIBSEM-based method for measuring presynaptic activity and release probability along with circuit connectivity at nanoscale resolution in awake behaving animals. To investigate

ultrastructure-function relationships in synapses activated by sensory input in primary visual cortex in awake head-fixed mice we used FM 1-43FX labelling and dye photoconversion as a marker of functionally recycled vesicles *in vivo*. This permits active terminals to be visualized using electron microscopy-based connectomics approaches. Synapses were labelled by intracranial infusion of the FM dye in layer 2/3 visual cortex circuitry via an access port embedded in a cranial window while the animal was presented with a visual stimulus. After transcardial perfusion fixation, the brain was postfixed overnight. Vibratome sections containing the dye-loaded presynaptic boutons were photoconverted and processed for electron microscopy. A Zeiss NVision 40 FIBSEM was used to acquire high-resolution 3D data at near-isotropic voxel size (6.2 x 6.2 x 9.3 nm^3 voxels), allowing each synaptic vesicle to appear on 5-6 consecutive images. Two distinct vesicle populations were found in presynaptic terminals: dye-loaded, photoconverted (PC+) active vesicles with a dark lumen, and unloaded (PC-) vesicles with a clear lumen. For visualization and analysis, we used convolutional neural networks to automatically segment neurons, active zones, mitochondria and vesicles. For the vesicles, we used a 3D U-Net to predict, for each voxel, whether it was part of a vesicle, and if so, whether it was photoconverted or not. Those predictions were postprocessed to find the most likely positions of PC+ and PC- vesicles, which we then counted within the segmented axon volumes and assigned to individual boutons. We find that the number of PC+ vesicles scales with the total number of vesicles on average, yet the fraction of PC+ vesicles in the total vesicle pool varies greatly between individual synapses. The numbers of PC+ and PC- vesicles both approximate to lognormal distributions across a large number of synapses. Finally, we demonstrate that neighbouring boutons of the same axon, which share the same spiking activity, can differ greatly in their presynaptic release probability in vivo. In summary, this approach adds key functional information - the activity and strength of synapses - to the connectome.

Disclosures: A. Simon: None. **A. Roth:** None. **A. Sheridan:** None. **M. Fisek:** None. **V. Marra:** None. **C. Racca:** None. **J. Funke:** None. **K. Staras:** None. **M. Hausser:** None.

Nanosymposium

349. On Neural Connectomics

Location: SDCC 25

Time: Monday, November 14, 2022, 1:00 PM - 3:15 PM

Presentation Number: 349.06

Topic: I.03. Anatomical Methods

Title: Serial section electron tomography for whole-brain mammalian connectomics

Authors: *A. T. KUAN[1], S. PHAN[2], M. KIM[1], M. H. ELLISMAN[3], W.-C. A. LEE[4];
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Abstract: Recent advances in high-throughput electron microscopy (EM) have enabled connectomic datasets of uprecedented scale, including complete insect nervous systems and microcircuits in the mouse brain. However, scaling connectomic imaging to whole mouse brain

volumes (~500 mm³) faces critical sample preparation and scaling challenges. In particular, sectioning an entire mouse brain into thin-sections (< 50 nm thick) is very difficult, and imaging such a large sample in a reasonable amount of time (< 10 years) is not yet possible. Here, we present a large-scale TEM tomography approach that circumvents the need for ultra-thin sectioning and can increase effective imaging rates. Using intermediate voltage EM tomography, we show that semi-thick brain sections (0.5-2.0 μm) can be reconstructed at synapse resolution from a moderate number of projections (~15-30), and that successive sections can be stitched together to form contiguous volumes. Furthermore, we show that the same samples can be imaged again at higher resolution to confirm fine structures such as gap junctions. We examine the tradeoffs between imaging speed and quality, showing that TEM tomography provides a powerful and flexible imaging approach that is competitive with current state-of-the-art high-throughput EM approaches. We propose that optimizing this approach for high-throughput imaging by integrating electron tomography with tape-based sample handling can provide an end-to-end imaging solution for whole-brain mouse connectomics.

Disclosures: A.T. Kuan: None. **S. Phan:** None. **M. Kim:** None. **M.H. Ellisman:** None. **W.A. Lee:** None.

Nanosymposium

349. On Neural Connectomics

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Time: Monday, November 14, 2022, 1:00 PM - 3:15 PM

Presentation Number: 349.07

Topic: I.03. Anatomical Methods

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NSTI 2021ZD0203900

Title: An integrated resource for marmoset brain functional and structural connectivity

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Abstract: Mapping non-human primates (NHP) brain architecture by integrating structural and functional connectivity in both invasive and non-invasive ways is necessary and timely for advancing our understanding of our human brain organization. Here, we developed a standardized awake imaging protocol for marmosets to acquire the largest awake NHP resting-state functional MRI dataset to date across two research institutions with different scanners (7T and 9.4T), National Institutes of Health, USA, and the Institute of Neuroscience, China (26 and 13 monkeys in the same age group; 12117 mins in total). We scanned test-retest runs for each

animal, resulting in a similar data quantity of two institutes (364 and 346 runs) and included two "flagship" with many runs (64 and 40 runs). Besides the same data quantity, the data quality is also highly harmonized. Then, we registered the extensive collection of marmoset neuronal tracing data (52 marmosets; 143 injections) onto the same MRI space at the voxel or vertex level, and we also included extra high-resolution *ex-vivo* diffusion MRI (dMRI, 80 µm isotropic) and *in-vivo* dMRI data (250 µm isotropic) to enhance the capacity of our resource. Ultimately, an extensive resource combining structural and functional connectivity was created. Based on the awake NHP resting-state functional MRI dataset, we first created useful maps of resting-state brain networks, including 15 cortical networks. Then we developed a fine-grained cortical parcellation containing 96 functional parcels per hemisphere. To reflect individual characteristics, we also developed a deep-learning-based method to map such functional parcellation map onto every individual brain and demonstrated their good reliability in the test-retest unique dataset and the successful applications of task-fMRI activation. Thus, our functional parcellation not only preserves the topographical organization of functional connectivity but also reflects individual variabilities. Lastly, based on the structural connectivity from diffusion tractography and neuronal tracing, we adopted whole-brain computational modeling to link the structural and functional connectivity to investigate the structural basis behind our functional parcellation. The dataset and associated supporting tools (interactive online viewer for functional and structural connectivity comparison, computational modeling, etc.) are publicly available via the Marmoset Brain Mapping Project (https://marmosetbrainmapping.org). This resource will enable modeling structure-function relationships and facilitate future comparative and translational studies of primate brains.

Disclosures: X. Tian: None. **G. Deco:** None. **M. Rosa:** None. **C. Liu:** None. **A.C. Silva:** None.

Nanosymposium

349. On Neural Connectomics

Location: SDCC 25

Time: Monday, November 14, 2022, 1:00 PM - 3:15 PM

Presentation Number: 349.08

Topic: I.03. Anatomical Methods

Title: The Virtual Fly Brain -an interactive tool for neurobiologists

Authors: *J. ARMSTRONG[1], N. BROWN[2], R. C. COURT[1], M. COSTA[3], G. JEFFERIS[3], A. LARKIN[2], N. MATENTZOGLU[4], G. MILLBURN[2], A. MCLACHLAN[2], C. J. O'KANE[5], D. OSUMI-SUTHERLAND[4], H. PARKINSON[4], C. PILGRIM[2];
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Abstract: *Drosophila* is the only model organism with complex adaptive behaviours for which we also have a powerful genetic toolkit and extensive connectomic and transcriptomic coverage of the nervous system. This offers unparalleled opportunities to reveal the mechanisms by which brains control complex behaviour. Virtual Fly Brain (VFB) virtualflybrain.org provides a uniquely integrated, queryable view of *Drosophila* neuroscience data. Established pipelines with all major data providers enable rapid integration of new bulk data. VFB provides an essential system ensuring key data is released openly and rapidly following FAIR data standards. Through close collaboration with international data providers and our own curation efforts we provide a single integrative platform to query across the most extensive set of *Drosophila* neuroscience data available. We currently maintain information directly curated from over 1000 primary publications describing over 13,000 neuro-anatomical references and a comprehensive neuroanatomical ontology for the *Drosophila* nervous system. Linked through the ontology annotation, as well as through alignment to reference brain volumes, VFB contains almost 70,000 single-neuron images spanning over 5000 neuron types. We also integrate public connectomics datasets currently with 28,000 individual neurons and their potential connections. To unlock the potential of these anatomical datasets we also integrate over 1500 transgenic strains so users can find and select appropriate tools to test circuit hypotheses. The integration of brain scRNAseq data is ongoing. A custom designed suite of 3D graphical and text-based query tools provide a powerful set of methods to search for, visualise and download relevant datasets. In all cases the provenance of the data is retained to clearly indicate the original source of each dataset as well as any annotation that has been added since its public release. More recently we have developed an API that enables batch processing of queries and data analytics using either Python or R platforms. In addition to the resource itself all the software underpinning the website, the databases and the APIs are also released open-source. A brief demo will be available at the conference.

Disclosures: J. Armstrong: None. **N. Brown:** None. **R.C. Court:** None. **M. Costa:** None. **G. Jefferis:** None. **A. Larkin:** None. **N. Matentzoglu:** None. **G. Millburn:** None. **A. McLachlan:** None. **C.J. O'Kane:** None. **D. Osumi-Sutherland:** None. **H. Parkinson:** None. **C. Pilgrim:** None.

Nanosymposium

424. Brain Oscillations: From Health to Disease

Location: SDCC 1

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 424.01

Topic: B.07. Network Interactions

Title: Distinguishing network deficits in rodent models of psychiatric disorders

Authors: J. C. RODRIGUEZ DIAZ[1], D. L. PRITCHETT[3], **K. JONES**[2];
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Abstract: Oscillations play crucial roles in many cognitive processes such as memory formation and attention. GABAergic interneurons are crucial for synchronizing neuronal activity leading to gamma oscillations (30-60 Hz). Abnormal oscillatory activity in the hippocampus has been implicated in the pathology of some mental disorders including schizophrenia, however the neurobiological mechanism underlying these abnormal oscillations are not yet fully understood. We set out to develop an assay that would allow for the study of gamma oscillations in hippocampal sections using microelectrode arrays. Extracellular electrophysiological recordings were performed using 60- channel perforated micro electrode arrays (pMEAs). Oscillatory activity in the gamma band was induced pharmacologically with kainate application. Established oscillations were inhibited by bath application of the GABAA receptor antagonist bicuculline. Bath application of kainate induced and maintained oscillatory activity in the gamma band in both CA1 and CA3 regions of the hippocampus. CA1 oscillations had a narrow band with a peak at around 30 Hz while in CA3 the oscillations were broader. Kainate-induced oscillations in CA1 and CA3 were abolished by application of the GABAA receptor antagonist bicuculline. These studies suggest that kainate-induced oscillatory activity can serve as a model of *in vivo* GABA-dependent gamma oscillations in the hippocampus. Furthermore, pMEAs provide a tool to study oscillations in nearby regions during chemically induced oscillations. Future studies will focus determining the oscillations sensitivity to glutamate receptor antagonists and comparing these with oscillations induced in an animal model of schizophrenia.

Disclosures: J.C. Rodriguez Diaz: None. **D.L. Pritchett:** None. **K. Jones:** None.

Nanosymposium

424. Brain Oscillations: From Health to Disease

Location: SDCC 1

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 424.02

Topic: B.07. Network Interactions

Support: Research stipend to Marie-Elisabeth Burkart from the Medical Faculty, Leipzig University
Research stipend to Josephine Kurzke from the Medical Faculty, Leipzig University

Title: DEND mutation disrupts hippocampal network activity and nocturnal gamma shifts

Authors: M.-E. BURKART[1], J. KURZKE[1], J. VERA[2], F. M. ASHCROFT[3], J. EILERS[1], *K. LIPPMANN[1];
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Abstract: ATP-sensitive potassium (K_{ATP}) channels mediate cell metabolism and electrical activity by coupling intracellular ATP levels to membrane K^+-conductance. In humans, the activating mutation V59M in the $K_{ir}6.2$ subunit of K_{ATP} channels causes developmental **d**elay and **e**pilepsy with **n**eonatal **d**iabetes, i.e., DEND syndrome. While the origin of neonatal diabetes is well understood, the pathophysiology of the neurological symptoms remains unclear. Inhibitory parvalbumin-positive interneurons (PV-INs) are key players in generating cognition-associated hippocampal sharp-waves and gamma oscillations, while their dysfunction results in epilepsy. Therefore, we asked whether expressing the V59M mutation selectively in PV-INs would be sufficient to induce the neurological symptoms of DEND syndrome. Indeed, acute hippocampal slices of heterozygous PV-V59M mice showed disturbed sharp-waves and gamma oscillations. Moreover, V59M-mutated PV-INs were characterized by a reduced power of intrinsic gamma oscillations, a reduced gamma resonance behaviour and a reduced synaptic release of GABA. PV-V59M mice showed seizures and, unexpectedly, reduced nocturnal gamma shifts *in vivo*. Our findings provide evidence that the K_{ATP} channel mutation $K_{ir}6.2$-V59M, expressed only in PV-INs, leads to disturbed network oscillations and seizures potentially underlying the neurological symptoms of DEND syndrome.

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Nanosymposium

424. Brain Oscillations: From Health to Disease

Location: SDCC 1

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 424.03

Topic: B.07. Network Interactions

Title: Closed-loop phase-dependent optogenetic modulation of motor cortical theta oscillations

Authors: *J. MYATT, R. TOTH, Y. PENG, J. VAN RHEEDE, N. BERRY, C. MCNAMARA, C. J. STAGG, A. SHAROTT;
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Abstract: Neuronal oscillations are a prominent feature of motor cortical local field potentials (LFPs) and abnormalities in oscillatory activity have been linked to several disorders. Theta-modulated gamma frequency alternating current stimulation modulates human motor learning, however, it is unclear how these frequencies modulate motor cortical activity at the microcircuit level. Here, we aimed to develop a method of bidirectionally modulating theta and gamma coupled oscillations in the motor cortex using closed-loop optogenetic stimulation of

parvalbumin-expressing (PV) interneurons. Motor cortical LFPs and single units were recorded in PV-Cre mice in which PV-interneurons were transfected with Channelrhodopsin-2, allowing modulation of ongoing spiking activity with blue light. Using our recently developed phase-tracking system, pulses of blue-light were delivered at four target phases of the ongoing theta oscillation in the motor cortical LFP. Light was delivered over a quarter of the theta cycle, either as a continuous pulse or a burst of three pulses at gamma frequency. Both a continuous pulse and gamma frequency stimulation modulated theta power in a phase-dependent manner, with gamma frequency stimulation demonstrating stronger modulation. Stimulation targeted to the ascending and descending phases suppressing and amplifying theta power respectively. Preliminary data suggests that stimulating pyramidal neurons also results in phase-dependent amplification and suppression, with a phase offset of 90° as compared to PV-interneurons. These findings demonstrate that the direction and magnitude of changes in theta power mediated by PV-expressing interneurons are sensitive to the phase of the ongoing oscillation. This approach can be used to uncover the mechanism through which oscillatory manipulations modulate cortical activity in humans and aid their future development.

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Nanosymposium

424. Brain Oscillations: From Health to Disease

Location: SDCC 1

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 424.04

Topic: B.07. Network Interactions

Support: NS120723

Title: Long-range inhibitory neurons coordinate state-dependent cortical network synchronization

Authors: *J. RATLIFF[1], G. TERRAL[1], C. RAMAKRISHNAN[2], L. E. FENNO[2], K. DEISSEROTH[2], R. BATISTA-BRITO[1];
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Abstract: Cortical brain states are typically associated with changes in network synchronization and are key determinants for behavioral responses, such as sleep and wake. Though the mechanisms enabling these distinct modes of cortical operation remain largely unknown, inhibitory neurons (INs) have been suggested repeatedly as a regulator of behavioral-state dependent neocortical activity. Here we investigate how a unique subpopulation of long-range INs impact cortical states. These cells are defined by the co-expression of somatostatin (SST) and neuronal nitric oxide synthase (nNOS), namely SST/nNOS cells. Although they constitute a very small minority of neocortical INs, SST/nNOS cells are evolutionarily old and conserved

from amphibians to humans. Further, they have unique anatomical characteristic for neocortical INs, with local and long-range projections that span millimeters across cortical areas. Their remarkably distinctive features and deep evolutionary conservation suggest that SST/nNOS cells play an important role in neocortical activity coordination. Until recently, the genetic targeting of SST/nNOS cells has been difficult. We have used intersectional genetic tools to manipulate neocortical SST/nNOS cells in mice to interrogate their *in vivo* functional roles. Using 2-photon calcium imaging with high precision state monitoring, we find that SST/nNOS cells are specifically active during low-arousal states characterized by low movement and synchronized local field. Using optogenetic manipulation of SST/nNOS cells in combination with *in vivo* extracellular recordings, we show that the activity of SST/nNOS cells is sufficient to induce a synchronized network state, with both increases in low-frequency LFP power and increases in spiking entrainment to these low-frequencies. These observations are specific to SST/nNOS cells as optogenetic activation of SST+/nNOS- cells leads to reduced neocortical network synchrony. Taken together, our data suggests that SST/nNOS cells are specifically active during low arousal states and during slow wave sleep states when cortical cholinergic tone is low. However, during movement and during REM sleep, when cholinergic tone is high, these cells are remarkably silent. We have begun to investigate how cholinergic modulation of SST/nNOS cells through the Gi coupled type-2 muscarinic acetylcholine receptor may play a role in shaping the state dependent activity of these cells.

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Nanosymposium

424. Brain Oscillations: From Health to Disease

Location: SDCC 1

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 424.05

Topic: B.07. Network Interactions

Support: Marie Curie Grant 707404

Title: Ultradian timing of excitation-inhibition balanced temporal dynamics in hippocampus

**Authors: **D. KIM[1], E. CHARLETT-GREEN[2], J. ROHLING[3], S. MICHEL[3], S. ATON[1], B. O. WATSON[1], *****N. OGNJANOVSKI[1];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Oxford Univ., Oxford, United Kingdom; [3]Leiden Univ. Med. Ctr., Leiden, Netherlands

Abstract: Twenty-four-hour rhythmicity in physiology and behavior is driven by changes in neuronal activity that varies across the light-dark cycle. This circadian rhythmicity is most prominent in neurons of the brain's central pacemaker the suprachiasmatic nucleus but is also found in other brain regions. This includes the hippocampus, which shows circadian rhythmicity in both clock-gene expression and synaptic plasticity. However, it is currently unknown whether

electrical properties of the hippocampus are modulated by the light-dark cycle. Since new experimental data is converging towards a critical role of oscillations in connecting activity to sensory processes- such as light perception, we implanted tetrodes into CA1 of C57BL/6J mice to identify circadian patterns in electrical activity in the hippocampus across the day. We recorded individual cells' firing rates, local field potentials, and assessed connectivity and E/I balance for 24hr across a 12:12 cycle. We then quantified dynamics on an hour-by-hour basis to report the following findings:

1. Fast-spiking inhibitory interneurons show ultradian rhythmic activity across the day, with peaks in firing at light transitions, while excitatory principal cells' firings remain relatively static.

2. Sleep-associated NREM delta (0.5-3Hz) and sharp-wave ripples (SPWRs- 150-250Hz) as well as REM and waking theta (4-10 Hz) are temporally dynamic. Delta and SPWRs were comparatively higher during NREM occurring in the light phase.

3. Measures of connectivity, stability, and E/I balance show that hippocampal network structure is not static across the day but fluctuates with inhibitory cell firing (peak at ZT6).

These results show for the first time the dynamic baseline structure of hippocampal electric activity. Network structure seems to reorganize over time, possibly according to the behavioral demands of the rest-activity cycle. Specifically, stability and E/I balance are shown to be indicative of a critical network state, which may indicate an optimal timing of learning in the hippocampus.

Disclosures: D. Kim: None. **E. Charlett-Green:** None. **J. Rohling:** None. **S. Michel:** None. **S. Aton:** None. **B.O. Watson:** None. **N. Ognjanovski:** None.

Nanosymposium

424. Brain Oscillations: From Health to Disease

Location: SDCC 1

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 424.06

Topic: B.08. Epilepsy

Support: Ontario Brain Institute

Title: Delta - fast ripple coupling suppression: designing a brain-mimetic stimulation paradigm for seizure abolishment

Authors: *U. TUFA[1], A. ZAHRA[2], C. WU[2], L. ZHANG[2], P. L. CARLEN[3], B. L. BARDAKJIAN[1];
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Abstract: Deep brain stimulation can be an effective alternative treatment for patients that are intractable to anticonvulsant drugs and do not meet surgical criteria. Clinical trials have

demonstrated the safety of thalamic stimulation using a high frequency stimulus with limited efficacy. Our group has previously shown, in silico, the success of stimulation with a brain-mimetic therapeutic signal, outperforming mono-rhythmic waveforms. In this study we extend our findings in vivo and investigate a thalamic continuous stimulation paradigm using a brain-mimetic signal, where the amplitude of a high frequency rhythm is modulated by the phase of a low frequency rhythm forming a cross-frequency coupled (CFC) waveform, to suppress seizure-like events (SLEs) in a kindled mouse model. We aim to show that application of our brain-mimetic stimulation is more effective in seizure control than mono-rhythmic stimulation. Bipolar electrodes were implanted in the CA3 of the hippocampus and in the contralateral medial dorsal nucleus of the thalamus, allowing for stimulation and iEEG recordings. Video analysis was used for assessment of animal motor behavior. Mice were kindled daily through unilateral CA3 stimulations reaching evoked convulsive SLEs, then spontaneous recurrent seizures. To test suppression in fully kindled mice, thalamic stimulation using a CFC waveform was applied continuously for 15 minutes, followed by hippocampal stimulation to evoke an SLE. Thalamic stimulation, applied continuously for 24 hours, was also tested in extended kindled mice with spontaneous recurrent seizures. We found a 1Hz-100Hz phase-amplitude CFC waveform to be effective in suppressing SLEs (confirmed by iEEG and video analysis) and increasing kindling threshold. Low frequency and interictal spike suppression following interictal stimulus administration was found as a marker to assess the effective stimulus parameters. Our bi-rhythmic CFC stimulus outperformed mono-rhythmic stimuli in evoked SLEs. These findings are important in the development of novel brain stimulation strategies for epileptic patients.

Disclosures: U. Tufa: None. **A. Zahra:** None. **C. Wu:** None. **L. Zhang:** None. **P.L. Carlen:** None. **B.L. Bardakjian:** None.

Nanosymposium

424. Brain Oscillations: From Health to Disease

Location: SDCC 1

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 424.07

Topic: B.08. Epilepsy

Title: Simultaneous high-frequency oscillations (HFOs) and interictal epileptiform discharges (IEDs) predict the seizure onset zone (SOZ)

Authors: *C. KAPELLER[1], M. MOHAMMADPOUR[2], K. KAMADA[3], M. CHING[2], F. CAO[2], C. GUGER[4], K. MAYR[2];
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Abstract: HFOs can be utilized as a biomarker for the epileptogenic zone. Localizing HFOs supports better delineation of the resection areas in patients with intractable epilepsy and improves surgical outcomes. Identifying HFOs is challenging, and their exact clinical definition

is still unclear. Thus, HFOs can be divided into normal HFOs (nHFO) and pathological HFOs (pHFO), each with different signal characteristics. This study aimed in analyzing resting-state ECoG signals to differentiate nHFO from pHFO, under consideration of IED co-occurrence. Five patients with epilepsy underwent cortical electrode implantation in the Megumino Hospital in Japan. ECoG signals were reviewed to find seizure onset zone (SOZ) and confirmed with video EEG recorded in EMU. An automatic event detector identified HFOs and IEDs in sleep-state ECoG, and compared whether event times overlap. If there is an overlap between IEDs and HFOs, the event is considered pHFO, and HFOs without IEDs are considered nHFO. Specifically, the detector filters the signals (5-60 Hz for IEDs and 60-250 Hz for HFOs detection), applies a Hilbert transform to calculate signal envelop, epochs into 1-minute segments, which are then normalized by robust z-scoring. Events greater than 3-SDs above the median value are considered events of interest (EOIs). Finally, detection criteria (duration>6 ms, minimum period between EOIs>50 ms, amplitude>5 uV, and number of oscillations >4) were set to fulfill HFOs and IEDs detection. After HFO detection, the positive predictive value (PPV) showed the relationship between the n-HFO and pHFOs against SOZ.

In total, 120 HFOs per electrode on average (range 0-40/min) were identified from 689 electrodes in five patients with 157.3 hours of recordings. Thus, 12.8% of the HFOs were identified as pHFO, with average HFO rates of pHFO(SOZ)=3.72/min and pHFO(non-SOZ)=0.28/min. Rates for nHFOs were nHFO(SOZ)=5/min, nHFO(non-SOZ)=3.28/min. The SOZ could be determined with a PPV(nHFO)=0.6, and a PPV(pHFO)=0.92.

From a clinical perspective, pathological and physiological HFOs could exist in all brain structures and occur either in SOZ or healthy areas. Preliminary results show that pHFO, identified by overlapping IEDs and HFOs, could predict SOZ, whereas nHFO is not SOZ specific.

Nanosymposium

424. Brain Oscillations: From Health to Disease

Location: SDCC 1

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 424.08

Topic: B.08. Epilepsy

Title: Dynamic segregation and integration during interictal epileptic discharges revealed by connectome spectrum analyses

Authors: *I. RIGONI[1], J. RUE-QUERALT[2], K. GLOMB[2], M. PRETI[3], S. TOURBIER[2], N. ROEHRI[1], L. SPINELLI[1], M. SEEK[1], D. VAN DE VILLE[3], P. HAGMANN[2], S. VULLIEMOZ[1];

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Abstract: Recent research has uncovered aspects of the coupling between brain activity and underlying structural connections in healthy subjects, but alterations in pathological conditions remain largely unknown. Here we exploit graph signal processing to decompose interictal epileptic discharges (IEDs) -measured via EEG activity- on spatial maps extracted from the structural connectome (SC). The goal of this study is to disentangle the dynamic interplay between processes of segregation and integration characterizing IEDs in patients with temporal lobe epilepsy (TLE). IEDs of 18 TLE patients were source-reconstructed in 118 region of interests (ROI) and decomposed as the sum of SC graph Laplacian eigenvectors, or "network harmonics". The energy spectrum of the transformed signal was split in the low-frequency harmonics (LF, long-range interactions reflecting integration) and the high-frequency ones (HF, short-range interactions reflecting segregation). With the first we reconstructed the part of the signal, in ROI space, mostly coupled to the structure (Xc), with the latter we reconstructed the decoupled one (Xd). Xc and Xd norms were calculated over all ROI and the dynamics of their energy distribution along time were compared with a cluster-based permutation test across patients. To analyze segregation and integration at the regional level, patients were divided according to the lateralization of the epilepsy ($N_{RIGHT}=9$ and $N_{LEFT}=9$). For each ROI, the structural decoupling index (SDI, ratio between Xd and Xc) was calculated. For ROI that were more coupled/decoupled than the best performing surrogate in at least 6 patients per group, the SDI was compared between the significant time windows. The permutation test identified two clusters in time: until the IED onset (C1) the energy of HF harmonics was bigger than that of LF ($p<.001$); around its first peak (C2) the energy of the coupled signal was higher ($p<.05$). At the ROI level, the ipsilateral mesial regions were significantly more coupled to the structural network than in surrogates over the whole epoch. Across patients, ipsilateral hippocampus and entorhinal cortex became more coupled during C2 ($p<.01$). In conclusion, at whole-brain level, segregation seems to give way to integrative processes during the IED, as over C2 smooth spatial maps (long-range couplings) embed most of the energy of the signal. Locally, we observe that are indeed those ROI likely involved in the epileptogenic network (ipsilateral hippocampus and entorhinal cortex) to be the one increasing their reliance on long-range couplings during C2, in line with the interpretation that IED propagation is supported via mean of integrative processes.

Disclosures: I. Rigoni: None. **J. Rue-Queralt:** None. **K. Glomb:** None. **M. Preti:** None. **S. Tourbier:** None. **N. Roehri:** None. **L. Spinelli:** None. **M. Seek:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Epilog NV (Ghent, BE). F. Consulting Fees (e.g., advisory boards); Epilog NV (Ghent, BE). **D. Van de Ville:** None. **P. Hagmann:** None. **S. Vulliemoz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Epilog, NV (Ghent, BE). F. Consulting Fees (e.g., advisory boards); Epilog, NV (Ghent, BE).

Nanosymposium

425. Astrocyte Development and Function

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 425.01

Topic: B.09. Glial Mechanisms

Title: Lipidomic profiling of human reactive astrocytes uncovers inflammatory lipid signature

Authors: *I. V. L. ROSE[1], K. LENG[1], P. GROSJEAN[1], S. WADE[1], N. BLOMBERG[2], L. E. JOHANSEN[3], B. ROONEY[1], R. H. N. VAN DER KANT[3], M. GIERA[2], M. KAMPMANN[1];
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Abstract: Astrocytes are the most numerous cell type in the human brain and are an integral component in both homeostatic processes and disease-relevant responses. In response to central nervous system injury or disease, astrocytes become reactive, adopting context-dependent states and functional outputs. Certain inflammatory insults induce reactive astrocytes that lose homeostatic functions and gain harmful outputs through cellular pathways that are not fully understood. Using CRISPRi screening for regulators of reactive astrocytes, we previously found that autocrine-paracrine IL-6 and interferon signaling downstream of canonical NF-κB activation drove two distinct inflammatory reactive signatures (Leng et al. 2022, *bioRxiv*, doi: 10.1101/2021.08.23.457400). In addition, certain species of saturated lipids, such as long-chain saturated fatty acids, were recently identified as a secreted neurotoxic factor in a rodent *in vitro* model of astrocyte activation (Guttenplan et al. 2021, *Nature*, PMID: 34616039). The mechanisms by which astrocyte reactivity induces production of putative neurotoxic lipids are currently unknown. Further, how neurons respond to neurotoxic signals from reactive astrocytes is not fully characterized, especially in human models, and identifying these mechanisms can provide further evidence for pathways identified from characterizing reactive astrocytes and their conditioned media. Using comprehensive, quantitative lipidomic analysis of iPSC-derived human reactive astrocytes, we identified characteristic signatures associated with the neuroinflammatory reactive transition, which includes increased fully saturated triacylglycerols, and specific species of phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, and phosphatidylserines. Further, transcriptomic characterization of neurons responding to reactive astrocyte-conditioned media indicated potential roles for insulin-like growth factor (IGF) regulation by IGF binding proteins and pappalysins, as well as lipoprotein lipase - a potential response in the neurons to triacylglycerols from reactive astrocytes. Together, these

findings further elucidate mechanisms involved in astrocyte reactivity and form a more complete picture of differential lipid regulation in astrocyte activation and its impact on neurons.

Nanosymposium

425. Astrocyte Development and Function

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 425.02

Topic: B.09. Glial Mechanisms

Title: A non-canonical role for IRE1αlinks ER and mitochondria as key regulators of astrocyte dysfunction: implications methamphetamine use and HIV-associated neurocognitive disorders

Authors: *J. PROULX, K. BORGMANN;
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Abstract: Human immunodeficiency virus 1 (HIV-1) invades the central nervous system (CNS) early during infection and can persist in the CNS for life despite effective antiretroviral treatment. Infection and activation of residential glial cells lead to low viral replication and chronic inflammation, which damage neurons contributing to a spectrum of HIV-associated neurocognitive disorders (HAND). Astrocytes are the most numerous glial cells in the CNS and provide essential support to neurons. During a neuropathological challenge, such as HIV-1 infection, astrocytes can shift their neurotrophic functions to become neurotoxic and even serve as latent reservoirs for HIV-1 infection. Notably, substance use disorders, including methamphetamine (METH) are disproportionately elevated among people living with HIV-1. METH use can induce neurotoxic and neurodegenerative consequences, which can increase one's risk and severity of HAND. A better understanding of HIV-1 infection and METH

exposure both alone and in combination on astrocyte function could help identify key cellular or molecular targets that can regulate astrocyte neuroprotective versus neurotoxic phenotypes to optimize neuronal fitness and combat CNS pathology. Mitochondria are essential organelles for regulating metabolic, antioxidant, and inflammatory profiles. Moreover, endoplasmic reticulum (ER)-associated signaling pathways, such as calcium and the unfolded protein response (UPR), are important messengers for cellular fate and function, including inflammation and mitochondrial homeostasis. Our previous studies in primary human astrocytes demonstrated increased UPR mediator protein expression following HIV-1 infection or chronic METH exposure, of which, inositol-requiring enzyme 1 alpha (IRE1α) was most prominently elevated. Interestingly, pharmacological inhibition of the three UPR arms, illuminated that IRE1α is a potential regulator of astrocyte mitochondrial respiration. Here, we further delve into the functional role of IRE1α in primary human astrocytes using an IRE1α overexpression plasmid followed by astrocyte activation with the proinflammatory cytokine, interleukin 1β (IL-1β). Our findings confirm IRE1α modulates astrocyte metabolic function, morphological activation, cytokine secretion, and glutamate clearance, highlighting a novel target for regulating astrocyte metabolic and inflammatory phenotypes. Therapeutic targeting of astrocyte IRE1α could help combat astrocyte dysfunction and potentially promote a more neuroprotective phenotype during CNS pathologies.

Disclosures: J. Proulx: None. **K. Borgmann:** None.

Nanosymposium

425. Astrocyte Development and Function

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Topic: B.09. Glial Mechanisms

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Title: Involvement of the elastin synthesis pathway in reactive astrocytosis in primary optic nerve head astrocytes

Authors: *S. KAJA[1,2], H. N. HARIANI[1], V. R. RAO[1], A. K. GHOSH[2];
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Abstract: Glaucoma is a progressive optic neuropathy that manifests with a characteristic sequence of pathological events, including optic nerve head remodeling. Optic nerve head

astrocytes (ONHA) are the primary cell type in the optic nerve head. Noxious stimuli trigger reactive astrocytosis (RA), a morphological and structural remodeling associated with increased expression of glial fibrillary acidic protein (GFAP), proliferation and migration, retraction of processes and reduced stellation, and changes in actin cytoskeleton and secretion of extracellular matrix proteins. RA is an early pathological process in glaucoma, underlying the characteristic optic nerve head remodeling. The molecular mechanisms underlying RA remain largely unknown. The objective of the present study was to further elucidate the molecular and functional effects of mechanical strain on elastin synthesis pathways. RA was induced by exposure of ONHA to mechanical strain (10% equibiaxial stretch for 16 h) using a Flexcell® FX-5000 Tension System (Flexcell International). Induction of RA was confirmed by increased GFAP immunoreactivity. Induction of RA resulted in a significant decrease in lysyl oxidase like 1 (Loxl1) gene expression and protein levels. Concomitantly, elastin levels were significantly decreased, consistent with the role of Loxl1 as a cross-linking matrix enzyme required for normal elastic fiber formation and stabilization. Intriguingly, expression of enzymes upstream of Loxl1 in the elastin synthesis pathway, fibrillin-1 and fibulin-5, was not altered. To confirm a possible involvement of Loxl1 in RA, we used siRNA-mediated knockdown (KD). Partial (~50%) KD of Loxl1 resulted in increased GFAP expression as well as actin cytoskeletal remodeling suggestive of RA. Elastin expression was reduced by approximately 30%. To further elucidate the functional consequences of reduced Loxl1 expression, we transduced ONHA with lentivirus expressing Loxl1 shRNA and generated a stable ONHA line. Exosomes were purified and the functional effects of exosomes on neurite outgrowth were tested by adding exosomes from control (scramble) and Loxl1-KD ONHA to cortical neuronal cultures. Control ONHA exosomes increased neurite outgrowth by 20% compared with media alone, while exosomes from Loxl1-KD ONHA prevented increases in neurite outgrowth. Our data suggest that glaucomatous insults can affect elastin synthesis in ONHA through modulation of Loxl1. Impaired Loxl1 function is sufficient to induce molecular signatures of RA in ONHA. Impaired elastin synthesis in glaucoma may be responsible, in part, for impaired neuron-glia signaling in glaucoma.

Disclosures: S. Kaja: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Experimentica Ltd., K&P Scientific LLC, AcuiSee, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); K&P Scientific LLC, Experimentica Ltd., eyeNOS, Inc.. F. Consulting Fees (e.g., advisory boards); Experimentica Ltd.. **H.N. Hariani:** None. **V.R. Rao:** None. **A.K. Ghosh:** A. Employment/Salary (full or part-time):; Experimentica Ltd.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); eyeNOS, Inc..

Nanosymposium

425. Astrocyte Development and Function

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 425.04

Topic: B.09. Glial Mechanisms

Title: Use of Toxoplasma infection and a model of methamphetamine abuse reveals reactive astrocyte heterogeneity during chronic inflammation

Authors: *Z. FIGUEROA[1], W. AGNEW-SVOBODA[1], M. RICCOMAGNO[2], T. A. FIACCO[2], E. WILSON[3];
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Abstract: Neuroinflammation is a feature common to neurodegenerative disease, infection, and brain injury, resulting in immune responses characterized in part by changes in astrocytes. Astrocytes ordinarily provide critical support for neurons but become reactive in response to inflammation. Reactive astrocytes (RAs) are defined by their proliferation, enlarged cell bodies and processes, and change in function. A longstanding and unresolved issue is whether RAs contribute to or help alleviate disease progression. Chronic abuse of methamphetamine (meth) is a leading cause of overdose deaths in California and is a public health problem worldwide. Toxoplasma gondii is a highly successful neurotropic parasite that causes persistent subclinical neuroinflammation due to cyst formation in neurons that last for the lifetime of the host. Using these two contrasting models of chronic inflammation, we will investigate the development and function of reactive astrocytes to determine if unique subsets exist during chronic stimulation. Using flow cytometry to analyze integrin expression, we determined there is astrocyte heterogeneity throughout T. gondii infection and meth exposure, based on astrocytic expression of CD51, CD63, and CD71. Furthermore, we have characterized previously undescribed subsets of astrocytes conserved during both models of chronic inflammation. To further analyze these RA subsets, single cell RNA sequencing experiments were conducted to investigate the transcriptional profile of these astrocyte populations. Considerable cohesiveness in the kinetics and subsets were observed between protein and transcriptional data. Using a novel inducible Cre knock-in mouse line driven by the lipocalin 2 promoter, we have isolated and tracked RA populations to determine the resolution of astrocyte reactivity during and throughout chronic inflammation.

Disclosures: Z. Figueroa: None. **W. Agnew-Svoboda:** None. **M. Riccomagno:** None. **T.A. Fiacco:** None. **E. Wilson:** None.

Nanosymposium

425. Astrocyte Development and Function

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 425.05

Topic: B.09. Glial Mechanisms

Title: Mitochondrial dynamics and astrocyte contribution to development of Rett pathophysiology in human ESC derived cerebral organoids

Authors: *D. TOMASELLO[1], **I. BARRASA**[1], **R. JAENISCH**[1,2];
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Abstract: Rett syndrome (RTT) is a postnatal neurodevelopmental disorder, largely due to mutation of the Methyl CpG-binding Protein 2 gene (MECP2), and is associated with severe mental disability and autism-like syndromes that manifests during early childhood. An important aspect of neurodevelopmental disorders are the cell non-autonomous functions that are sparsely understood. Astrocytes provide structural and molecular support for neurons that are essential for proper development and maturation. We found human embryonic stem cell (ESC) derived RTT astrocytes have impaired glutamate uptake, a key function that prevents excitotoxicity. We have identified RTT astrocyte mitochondrial function and glycolytic capacity are severely diminished. Interestingly, RTT astrocytes revealed increased mitochondrial mass, a possible mechanism to compensate for limited mitochondrial function. When mitochondrial activity was stimulated, RTT astrocytes maintain a threshold of activity irrespective of glycolytic shift. Metabolomic analysis defined metabolic disruption in energy balance and amino acid supply. RTT cerebral organoids revealed developmental delay in maturation of both neurons and astrocytes. Additionally, we observed mitochondrial transplantation from RTT astrocytes to RTT neurons, indicating dysfunctional mitochondria in astrocytes may amplify disease pathology. These findings highlight cell non-autonomous contribution to RTT, impacting neuronal bioenergetics and function.

Disclosures: D. Tomasello: None. **I. Barrasa:** None. **R. Jaenisch:** None.

Nanosymposium

425. Astrocyte Development and Function

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Topic: B.09. Glial Mechanisms

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Title: Induced pluripotent stem cell-derived astrocytes from patients with schizophrenia exhibit an inflammatory phenotype that affects vascularization

Authors: *P. TRINDADE[1], J. M. NASCIMENTO[2], B. S. CASAS[3], T. MONTEVERDE[3], J. GASPAROTTO[4], C. T. RIBEIRO[5], S. DEVALLE[6], D. SAUMA[3], J. F. MOREIRA[5], D. P. GELAIN[5], V. PALMA[3], D. MARTINS-DE-SOUZA[7], S. K. REHEN[8], L. O. PORCIÚNCULA[5]; [1]Federal Univ. of Rio de Janeiro (UFRJ), Federal Univ. of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil; [2]Biochem. and Tissue Biol., Univ. of Campinas (UNICAMP) / IDOR, Campinas - SP, Brazil; [3]Dept. de Biología, Univ. de Chile, Santiago, Chile; [4]Inst. de Ciências Biomédicas, Univ. Federal de Alfenas, Alfenas - MG, Brazil; [5]Dept. de Bioquímica, UFRGS, Porto Alegre - RS, Brazil; [6]IDOR, Rio de Janeiro, Brazil; [7]Biochem. and Tissue Biol., Univ. of Campinas (UNICAMP), Campinas - SP, Brazil; [8]IDOR, UFRJ / IDOR, Rio de Janeiro, Brazil

Abstract: Molecular and functional abnormalities of astrocytes have been implicated in the etiology and pathogenesis of schizophrenia (SCZ). In this study, we examined the proteome, inflammatory responses, and secretome effects on vascularization of human induced pluripotent stem cell (hiPSC)-derived astrocytes obtained from three patients with SCZ and four control subjects. Proteomic analysis revealed alterations in several proteins related to immune function and vascularization in SCZ astrocytes, while no changes were detected in typical astroglial markers like GFAP and Vimentin. Protein-protein interaction network of both up- and down-regulated inflammation-related proteins pointed nuclear factor kappa B components (NF-κB - RELA/p65 and NFKB1/p50) as important signaling hubs. Corroborating the previous result, RT-qPCR experiments indicate a 50% decrease in NF-κB p65 expression in SCZ astrocytes compared to control cells. We also found a 20% reduction of NF-κB immunolabeling in SCZ astrocytes. SCZ-patient-derived–astrocyte conditioned medium (AsczCM) exhibited significantly elevated levels of cytokines such as interleukin (IL)-2, IL-4, IL-6 and IL-8. Interestingly, when SCZ astrocytes were challenged with tumor necrosis factor alpha (TNF-α) exposure, no incremental secretion of cytokines was found in the SCZ conditioned media after stimulation, except for IL-6 which nearly doubled. Proteomic evaluation of AsczCM has shown that differentially expressed proteins in SCZ are mainly related to activation of angiogenesis and development of vasculature, among other biological functions. Then, we further evaluated the biological potential of the astrocyte conditioned media to modulate *in vivo* vascularization by using the chicken chorioallantoic membrane (CAM) assay. AsczCM reduced the diameter and increased the number of newly grown vessels. This effect could be mimicked with exogenous addition of IL-8, in similar concentrations to what was found in AsczCM. Taken together, our results suggest that SCZ astrocytes are immunologically dysfunctional and may consequently affect vascularization through secreted factors. Likewise, IL-8 emerges as a player in schizophrenia-mediated vascular changes. The astroglial changes observed in this study may potentially contribute to developmental aspects of schizophrenia brain.

Disclosures: P. Trindade: None. **J.M. Nascimento:** None. **B.S. Casas:** None. **T. Monteverde:** None. **J. Gasparotto:** None. **C.T. Ribeiro:** None. **S. Devalle:** None. **D. Sauma:** None. **J.F. Moreira:** None. **D.P. Gelain:** None. **V. Palma:** None. **D. Martins-de-Souza:** None. **S.K. Rehen:** None. **L.O. Porciúncula:** None.

Nanosymposium

425. Astrocyte Development and Function

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 425.07

Topic: B.09. Glial Mechanisms

Title: Akt2 modulates astrocytic nicotine responses in vivo

Authors: *A. LOMBARDI, R. MILSTEAD, E. SCHMITT, C. BORSKI, C. HOEFFER;
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Abstract: A better understanding of nicotine neurobiology is needed to reduce or prevent chronic addiction, the detrimental effects of nicotine withdrawal, and increase successful cessation of use. Nicotine binds and activates two astrocytically expressed nicotinic acetylcholine receptors (nAChRs), α4β2 and α7. We recently found that *Protein kinase B-β* (*Pkb-β* or *Akt2*) expression is restricted to astrocytes in mice and humans. AKT2 may play a role in astrocytic nicotinic responses. We generated astrocyte-specific *Akt2* conditional knockout (cKO) and complete *Akt2* KO mice for *in vivo* and *in vitro* experimental preparations. For *in vivo* studies, we examined mice exposed to chronic nicotine for two weeks in drinking water (200 μg/mL) and following acute nicotine challenge (0.09, 0.2 mg/kg) after 24 hrs. Our *in vitro* studies used cultured mouse astrocytes to measure nicotine-dependent astrogliotic responses. We validated our approaches using lipopolysaccharide (LPS) exposure. Sholl analysis was used to measure glial fibrillary acidic protein responses in astrocytes. Our data show that wild-type (WT) mice exhibit activated AKT2 and increased astrocyte morphological complexity following acute nicotine but decreasing complexity following chronic nicotine use. Conversely, *Akt2* cKO mice showed increased astrocyte morphology compared to controls following chronic in vivo nicotine treatment. Additionally, we performed conditioned place preference (CPP) on WT and *Akt2* cKO mice to investigate the role of AKT2 in the motivational effects of nicotine. In culture, we found that 100μM nicotine was sufficient for inducing morphological changes and blocking α7 nAChRs prevented observed increases in morphology. These findings show the importance of nAChRs and *Akt2* signaling in the astrocytic response to nicotine.

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Nanosymposium

425. Astrocyte Development and Function

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Presentation Number: 425.08

Topic: B.09. Glial Mechanisms

Title: Macrophage-derived amphiregulin modulates astrocyte network size through cx43 gap junctions and interastrocyte tunneling naonotubes

Authors: *M. COOPER[1], M. SELLES[2], S. A. LIDDELOW[3], M. V. CHAO[4];
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Abstract: Early in neurodegenerative disease, astrocytes can expand their gap junctional networks to redistribute resources and maintain neuronal function. How is this beneficial form of astrocyte reactivity induced? To answer this, we looked at similar mechanisms in other organs and applied them to the nervous system. In the heart, cardiac macrophages induce gap-junctional network expansion in myocytes during arrhythmia. There, macrophage-derived amphiregulin (AREG) signals upon epidermal growth factor receptor (EGFR) to induce phosphorylation of connexin 43 (Cx43), recruiting it to the cell membrane to form additional gap junctions and rescue the tissue. Here, we test this mechanism in the resident macrophages of the brain (microglia) and the resident Cx43 network mediators (astrocytes). Like cardiac macrophages, cultured primary microglia (serum-free; n = 3 isolations) release AREG in response to stress (LPS, 1ug/ml; p < 0.001 vs baseline). Cultured primary astrocytes (serum-free; mRNA n = 3 isolations; protein n = 5 isolations) respond to AREG (100ng/mL) like cardiac myocytes: they phosphorylate Cx43, with a 3-fold increase 9 min after AREG administration (p < 0.001). Phosphorylation returns to baseline within 90 min. Three min after AREG administration, Cx43 mRNA increases 2-fold; protein levels increase 2-fold by 11 min (mRNA p = 0.007; protein p ≤ 0.01). Although astrocytes constitutively express little dimerized EGFR, AREG administration increases membrane-localized EGFR 2-fold within a min (p = 0.006) and 3-fold in 7 minutes (p < 0.001). To measure the change in functional gap-junction network size induced by AREG, astrocytes were cut-loaded with biotin (244Da) which traverses gap junctions, and dextran (10kDa) which is constrained within cells and thus only marks initially loaded astrocytes. We found a 3-fold increase in astrocyte connectivity in as little as 10 min after AREG (p < 0.001). Intriguingly, higher levels of AREG (1000 ng/mL) have the opposite effect - Cx43 mRNA is reduced 2-fold in 3 min (p = 0.02), and Cx43 protein declines 2-fold within 30 min (p = 0.01; n = 3 isolations). Unlike published data in the heart, astrocytes also respond to 100ng/mL AREG by extending tunneling nanotubes within 60 seconds of administration (n = 3 isolations). Higher AREG concentrations (1000 ng/mL) result in astrocyte process retraction within 15 min of administration (n = 3 isolations). This differential response to EGFR activation may dictate whether astrocytes enlarge or contract their network to respond to a stressor through beneficial or harmful forms of reactivity.

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Topic: B.09. Glial Mechanisms

Support: NIH NCI R35CA232103

Title: A transgenic mouse model to study astrocyte-derived extracellular vesicles

Authors: *L. NIELAND[1,3], E. ABU HAIDAR[1], S. DAS[2], S. GOULD[5], M. L. D. BROEKMAN[3,6,4], X. O. BREAKEFIELD[1], E. ABELS[4];
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Abstract: Astrocytes, the most abundant glial cells of the brain, regulate glutamate and ion homeostasis, metabolism, respond to environmental factors, and become activated neural stress. Interestingly, astrocytes interact with many different cell types in their surroundings. One route of interaction is through signaling mediated by extracellular vesicles (EVs). However, appropriate models to explore astrocyte communication mediated by EVs *in vivo* are lacking. Here we show the characterization of a new mouse model to track astrocyte-derived EVs. To this aim a Cre reporter mouse under the astrocyte specific GFAP promoter was crossed with a Cre-dependent reporter mouse with a loxP-floxed stop codon upstream of the human CD81 tagged with mNeonGreen under a CAG promoter. Here we show that in this new transgenic mouse only astrocytes express mNeonGreen. Astrocytes isolated from neonatal brains co-express GFAP with mNeonGreen, and EVs derived from these cultured astrocytes display mNeonGreen. In contrast, microglia isolated from these neonatal brains do not show mNeonGreen expression. We found that this model can be used to track astrocyte-derived EVs *in vivo* and in *vitro*. Ultimately, allowing us to study the interaction of astrocytes with neighboring cells via EVs. We anticipate that this new model can be used to fully understand how astrocytes use EVs to communicate with their surroundings both during homeostasis, and normal development, but also in a neoplasm or neuropathological setting.

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Nanosymposium

425. Astrocyte Development and Function

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Presentation Number: 425.10

Topic: B.09. Glial Mechanisms

Title: Sonic hedgehog-dependent recruitment of GABAergic interneurons into developing vLGN

Authors: *K. STEBBINS[1,6,7], R. SOMAIYA[1,6], H. XIE[2,3,4,9], A. GARCIA[10,11], M. A. FOX[5,6,4,8];
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Abstract: Retinal ganglion cell (RGC) axons play critical roles in the development of visual thalamus, a set of diencephalic brain structures critical for the processing of both image-forming and non-image-forming visual information. Specifically, early-arriving RGC axons regulate the timing of non-retinal innervation of visual thalamus, contribute to the morphological development of retino-recipient thalamic relay cells, and direct the migration of local GABAergic interneurons into two specific nuclei of visual thalamus, the dorsal lateral geniculate nucleus (dLGN) and ventral lateral geniculate nucleus (vLGN). We previously reported that RGC axons signal to thalamic astrocytes to generate Fibroblast Growth Factor 15 (FGF15), a motogen required for the recruitment of interneurons into dLGN and vLGN. However, the mechanism by which retinal inputs induce astrocytic expression of FGF15 remains unclear. We hypothesized that either retinal activity or molecular cues secreted by early arriving RGC axons could influence astrocytic-expression of FGF15 and, therefore, interneuron migration. Results presented here demonstrate that impairing RGC activity had no impact on interneuron recruitment into mouse visual thalamus; instead, our data show that retinal-derived Sonic Hedgehog (SHH) is essential for interneuron recruitment. Specifically, using transcriptomics, in situ hybridization, and reporter lines, we observed that RGCs express SHH and that astrocytes in developing visual thalamus express downstream signaling components of this pathway. These results show there is a significant reduction in Fgf15 expression and in thalamic GABAergic interneurons in conditional mouse mutants that lack RGC-derived SHH. Our findings not only identify thalamic roles for SHH signaling but also a novel way through which axo-glia interaction influences the development of inhibitory circuits in mammalian vLGN.

Disclosures: K. Stebbins: None. **R. Somaiya:** None. **H. Xie:** None. **A. Garcia:** None. **M.A. Fox:** None.

Nanosymposium

425. Astrocyte Development and Function

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 425.11

Topic: B.09. Glial Mechanisms

Title: Fibroblast Growth Factor Receptor 1 Signaling in Hypothalamic Tanycyte Development-Interactions with Diet and Exercise

Authors: *J. A. STAGRAY, A. ESTEVE, G. NORA, J. RICHARD, K. M. SMITH;
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Abstract: Fibroblast Growth Factor Receptor 1 (FGFR$_1$) is a tyrosine kinase receptor that is expressed within Tanycytes of the hypothalamus, and responds to secreted signaling factors such as fibroblast growth factors (FGF) and cell adhesion molecules. FGF-21 is a neuroendocrine signal produced by liver cells to modulate feeding behaviors. FGF-21 is involved in lipo-degradation pathways through MAPK, STAT 1/3/5, PLC γ, and PKC, and in suppression of feeding. Tanycytes are specialized radial glial like astrocytes that line the 3rd ventricle (3V) region abutting the hypothalamus. In adult mice, daughter cells of tanycytes, including neurons, detach and integrate into hypothalamic nuclei involved in feeding circuits, such as the Arcuate Nucleus (ARC). There are two primary subdivisions of Tanycytes, the α Tanycytes along the medial aspect of the 3V wall and β Tanycytes in the ventral wall of the 3V. Tanycytes send long processes deep into adjacent parenchymal hypothalamic nuclei. Tanycyte cell bodies interact with the 3V and CSF. The processes of β Tanycytes also directly interact with fenestrated capillaries of the medial eminence (ME) assisting in the transport of endocrine cues. Tanycytes demonstrate an ability to interact in glucose homeostatic roles and their morphology potentially governs feeding behavior pathways and cellular mechanisms. Our study model, Nestin-Cre mediated inactivation of Fgfr1, has altered Tanycyte morphologies compared to Cre-control mice in both males and females. β Tanycyte process length are found significantly decreased in FGFR1 KOs. These mice also displayed impaired glucose tolerance compared to controls after a high-fat high-sucrose diet (HFD). HFD induced decreased tanycyte radial process length in both α and β tanycytes of FGFR1 KOs. We determined if altered process lengths occur at P0. This allows us to determine if this phenotype is arising in the prenatal development of the hypothalamus, or during postnatal development. FGFR$_1$ is also able to form hetero dimers with membrane embedded proteins, such as cellular adhesion molecules. We will investigate potential roles of cellular attachments, and cadherin interactions, between FGFR$_1$ molecules in Tanycytes. Furthermore, after HFD, a decrease in SOX2+ stem cells was observed along the 3V in FGFR1 KO mice. Future studies will examine if postnatal neurogenesis is reduced in FGFR1 KO mice, as well as the roles of diet reversal and exercise upon the loss of stem cells and tanycyte length after HFD.

Disclosures: J.A. Stagray: None. **A. Esteve:** None. **G. Nora:** None. **J. Richard:** None. **K.M. Smith:** None.

Nanosymposium

425. Astrocyte Development and Function

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 425.12

Topic: B.09. Glial Mechanisms

Support: Dr. Miriam and Sheldon G Adelson Medical Research Foundation

Title: A toolbox of astrocyte-specific AAV using microRNA targeting sequences

Authors: *A. J. GLEICHMAN[1], R. KAWAGUCHI[2], M. V. SOFRONIEW[3], S. CARMICHAEL[1];
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Abstract: Cell type-specific genetic manipulation is a critical tool in biomedical science. While transgenic animals are one way to obtain this specificity, they can be slow and costly to generate. Viral manipulation is a faster, cheaper alternative. Adeno-associated viruses (AAV) are widely used due to their safety profile, limited toxicity, and the natural occurrence of serotypes with different tropism. Furthermore, AAV capsids have been developed which confer new properties on the virus, including systemically deliverable AAV which can be injected into mice intravenously (IV), cross the blood-brain barrier, and infect cells throughout the CNS. These designer capsids, such as PHP.eB, make it feasible to theoretically modify essentially all infectable cells in the CNS with one injection. One issue in implementing this in astrocytes has been that AAV with astrocyte-specific promoters have shown significant expression in other cell types. This expression is variable depending on the capsid and the cargo expressed, but can be quite extensive particularly when expressing Cre and is primarily seen in neurons and sparsely in endothelial cells. To generate astrocyte-specific Cre viruses, we added a previously reported targeting sequence for miR124 (miR124-T) to the plasmid. miR124 expression is high in neurons but very low in astrocytes; thus, the inclusion of miR124-T should lead to miR-mediated degradation of the transgene in neurons but not in astrocytes. This reduced, but did not eliminate neuronal expression, nor did it affect endothelial expression. We therefore generated a series of miR targeting viruses with expression patterns high in neurons or endothelial cells and low in astrocytes and ultimately developed a single unified cassette of 6 miR targeting sequences with 4 copies of each. In Cre-dependent reporter animals, either intracortical or IV delivery of this Cre-miR cassette virus leads to highly astrocyte-specific expression patterns that are stable for at least 8 weeks and show similar specificity when delivered across the mouse lifespan (postnatal day 1 to 29 months). We have generated multiple viruses using the miR cassette for astrocyte-specific expression, including both light- and tamoxifen-inducible Cre viruses; spaghetti monster reporter viruses; and Cre-dependent reporter viruses with reduced Cre-independent neuronal leak. We hope these viruses will be a useful resource for the community and an easy, accessible way to specifically manipulate astrocytes either in targeted brain regions or CNS-wide.

Disclosures: A.J. Gleichman: None. **R. Kawaguchi:** None. **M.V. Sofroniew:** None. **S. Carmichael:** None.

Nanosymposium

425. Astrocyte Development and Function

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 425.13

Topic: B.09. Glial Mechanisms

Support: NDSEG Fellowship

Title: CRISPRi-based genetic screens in iPSC-derived 3D neuron-astrocyte-microglia iAssembloids

Authors: *E. LI, M. KOONTZ, C. BENITEZ, S. BOGGESS, O. TETER, A. SAMELSON, I. V. L. ROSE, E. ULLIAN, M. KAMPMANN;
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Abstract: The interactions between glial and neuronal cells play a large role in brain function and dysfunction. For example, microglia and astrocytes can become reactive, leading to a response that may be detrimental to neuronal survival in neurodegenerative diseases. Many studies have investigated how certain glial cell types may independently contribute to disease through either gain of toxic function or loss of normal function, how neuron-neuron and neuron-glia interactions make neurons more vulnerable to disease is less understood.
Therefore, we generated 3D iPSC-derived neuron-astrocyte-microglia assembloids (iAssembloids) to model how glial and neuronal cells can drive neuronal death. We have found that iPSC-derived neurons cultured in iAssembloids have higher expression of axon guidance-related and glutamatergic receptor subunit genes and are much more electrophysiologically active than standard monocultured iPSC-derived NGN2 neurons. Using transcription factor-based neuronal and microglial differentiation protocols, iAssembloids are reproducible and easy to generate in a high-throughput manner, making them suitable for large-scale screening applications.
We then performed a large-scale pooled CRISPRi-based functional genomics screens (targeting over 2,000 genes) in the iPSC-derived neurons within iAssembloids. These screens identified pathways that affect neuronal survival in the iAssembloid context, but not in monoculture. We found that knockdown of GSK3B, which did not affect survival of monocultured neurons, strongly improved neuronal survival in iAssembloids, phenocopying studies in in vivo systems. We combined our functional genomics platform with single-cell transcriptomics (CROP-seq) and identified perturbations that caused distinct changes in neurons within iAssembloid compared to monocultured neurons. We found that knockdown of GSK3B upregulated the expression of NRF2 targets, a known response to oxidative stress, specifically in iAssembloids. This tractable system allows us to elucidate a mechanism by which neuronal activity and oxidative stress can interact through GSK3B.
We aim to expand CRISPRi-based screens in iAssembloids to compare neuronal phenotypes when co-cultured in different isogenic, disease-related glial environments such as in APOE3 and APOE4 expressing astrocytes. By performing CRISPRi-based screens in assembloids derived from patient iPSCs with genetic risk variants, we can start to decipher the processes in which glial cells are hypothesized to contribute to disease progression.

Nanosymposium

426. Alzheimer's Disease Genomics

Location: SDCC 7

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 426.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Molecular Atlas of Alzheimer's Disease Vulnerable Entorhinal Cortex

Authors: A. MORELLO MEGIAS[1], H. KAHVECIOGLU[1], S. JOSEPH[1], I. SALAS-ALLENDE[2], P. RODRIGUEZ RODRIGUEZ[3], W. WANG[2], ***J.-P. ROUSSARIE**[1];
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Abstract: A major obstacle in our understanding of early stages of Alzheimer's disease (AD), is our limited knowledge of the very first neurons to die in the disease, and of their surrounding cellular environment. In most patients with amnestic AD, neurons from the layer II of the entorhinal cortex (ECII) are the first neurons to form neurofibrillary tangles and degenerate. We previously profiled neurons from the entire ECII in the mouse using cell-type specific ribosomal profiling and demonstrated that a gene module involved in axonal plasticity underlies their vulnerability to neurofibrillary degeneration (Roussarie et al., Neuron, 2020). This proved that molecular characteristics of ECII neurons could predispose them to the formation of pathological lesions in AD. A large literature describes the heterogeneity in connectivity, cytoarchitectonics and function of this region in mice and primates. In addition, the transentorhinal subdivision of the EC shows strikingly higher vulnerability than the rest of the ECII. Here we propose a detailed molecular atlas of the EC, and of layer II in particular. Using single-nucleus RNA-sequencing and *in situ* hybridization in the mouse, we 1) delineate molecular subpopulations of EC - some discrete, some in transcriptional gradients, in a data-driven way, 2) identify markers for these anatomically distinct populations of ECII neurons, as well as for specific cell states within these populations, 3) identify subpopulation-specific signal in our whole ECII ribosomal profiling data. Overall this study is an indispensable first step in the characterization of

transentorhinal neurons, and in the precise molecular dissection of the neuronal pathological cascade taking place during prodromal AD.

Nanosymposium

426. Alzheimer's Disease Genomics

Location: SDCC 7

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 426.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Single-cell transcriptional hallmarks and individual subtyping for Alzheimer's Disease across 430 participants

Authors: *Y. TANIGAWA[1,2], N. SUN[1,2], W. F. LI[1,2], D. VON MAYDELL[1,2], C. A. BOIX[1,2], L. A. AKAY[1,2], K. GALANI[1,2], H. MATHYS[1,2,3], D. A. BENNETT[4], L.-H. TSAI[1,2], M. KELLIS[1,2];
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Abstract: Alzheimer's Disease (AD) is multifaceted, with many implicated biological pathways, across diverse cell types. The heterogeneous phenotypic manifestation, beyond the common characteristic signature of Amyloid beta plaque, across cognition, pathology, and treatment response is well recognized, but the molecular and cellular heterogeneity of AD remains uncharacterized at genomic and cellular resolution.
Here, we use single-cell RNA-seq profiling of 1.9 million cells from 430 human dorsolateral prefrontal cortex post-mortem brain samples across age-matched AD and non-AD individuals spanning all stages of AD progression. To assess heterogeneous molecular manifestation of the key biological processes, we develop a regularized multivariate differential expression analysis framework and identify 1621 gene expression patterns across 6 major cell types (implicating 1391 unique genes) associated with AD, which we cluster into 30 transcriptional hallmarks (Tx1-Tx30).
Our 30 transcriptional hallmarks capture several known cellular and pathological signatures in

AD, pinpointing their candidate driver genes and cell types of action, and are associated with distinct phenotypic enrichments. For example, cytoplasmic translation in oligodendrocytes (Tx12) was most associated with early AD (p-value=5.3×10^{-7}) but not late AD (p>0.9) changes; cytoplasmic translation in oligodendrocyte precursor cells (Tx23) instead showed the strongest association (p=1.0×10^{-9}) with amyloid level in the cortex; and response to zinc ion in astrocytes (Tx16) was most associated with neuritic plaque burden (p=2.6×10^{-9}).

Using combinations of hallmark burdens, we classify our 430 donors into 12 AD and non-AD subtypes. Four and three of those groups were enriched in AD cases and non-AD individuals, respectively, and the other five were balanced between AD cases and controls. One of the AD groups driven by oligodendrocyte-associated hallmarks was preferentially enriched in neuritic plaque burden, which is more directly indicative of neuronal damage.

Focusing on genetic variants associated with single-cell gene expression (sc-eQTLs) across our 430 samples, we found the genetic basis for some hallmarks (heritability ~= 2%), indicating the ability to predict dysregulated AD hallmarks, risk groups, and subtypes across individuals decades before symptoms occur.

Overall, our results pave the way towards pathway-level therapeutic development, personalized prognosis and treatment tuning in AD, and more generally towards genetics-based prognosis, transcriptional subtyping, and clinical trial design in complex and heterogeneous traits even in inaccessible tissues.

Nanosymposium

426. Alzheimer's Disease Genomics

Location: SDCC 7

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 426.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Delayed and brain region specific deletion of the NMDA receptor subunit GluN3A causes AD-like functional and behavioral changes in mice

Authors: *M. JIANG[1], J. FIDLER[2], K. BERGLUND[1], X. GU[1], A. WU[1], T. ESTABA[1], J. PATEL[1], L. WEI[1], S. YU[1];
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Abstract: Alzheimer's disease (AD) and related dementia are serious neurodegenerative disorders among aging populations. The Ca2+ hypothesis for AD proposes that Ca2+

dyshomeostasis in the brain is a pathogenic mechanism and prolonged process of AD. Hyperactivity of NMDARs has been implicated in AD as both a potential cause and outcome of other known AD-related pathologies. However, the instigating factors such as the time and trigger that initiate Ca2+ dysregulation, pathophysiology, and AD pathology have remained obscure. NMDAR overactivation is controlled by inhibitory GluN3 subunits. Expression of GluN3A in the receptor complex reduces NMDAR currents, while deletion of GluN3A causes larger NMDA currents and elevated intracellular Ca2+. We hypothesized that GluN3A is a critical endogenous NMDAR regulator that is constantly required for Ca2+ homeostasis and its deficiency results in slowly evolved "degenerative excitotoxicity". Our recent work disclosed that mice deficient in GluN3A in the whole brain from the embryonic stage (GluN3A KO mice) developed age-dependent AD-like pathophysiology, functional and behavioral deficits including cognitive decline, and Aβ/tau pathology during aging process. The current work aims to determine the critical time window and brain region specificity of GluN3A deficiency in sporadic AD development. Here we examine whether delayed GluN3A deficiency achieved by CRISPR knockout at young adult ages can lead to neurodegenerative changes and AD-related dementia. Using an AAV packaged CRISPR targeting GluN3A, adult wild-type (WT) mice (3-month old) were subjected to GluN3A selective knockout (sKO) from the hippocampus and cortex via stereotaxic injection. Examinations at 3 months after sKO demonstrated that the delayed deletion of GluN3A in these two brain regions caused similar disease progress as in the conventional GluN3A KO mice. These delayed sKO mice exhibited age-dependent olfactory dysfunction in the olfactory discrimination test, followed by progression of psychological/cognitive declines. Learning and memory deficits were identified in the Y-maze test, fear conditioning test, and Morris water maze test. Delayed GluN3A sKO animals also displayed anxiety-like behavior in the open field test as well as sociability abnormalities in the social novelty test. The result indicates that delayed and selective deficiency of the GluN3A subunit in the brain region important for cognitive function induces adult mice to develop AD-related functional and behavioral impairments.

Disclosures: M. Jiang: None. **J. Fidler:** None. **K. Berglund:** None. **X. Gu:** None. **A. Wu:** None. **T. Estaba:** None. **J. Patel:** None. **L. Wei:** None. **S. Yu:** None.

Nanosymposium

426. Alzheimer's Disease Genomics

Location: SDCC 7

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 426.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Alzheimer's disease related vascular molecular alterations in human brain and blood samples

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Abstract: Inter-cellular communication within the gliovascular unit (GVU) is critical for cerebral blood flow regulation, and maintenance of the blood-brain-barrier (BBB). Although numerous studies have demonstrated individual cellular contributions within the GVU to BBB breakdown in Alzheimer's disease (AD), the precise molecular changes contributing to its pathophysiology are unclear. Further, the extent of preservation of these brain GVU molecular perturbations in blood of living elderly individuals is unclear. In this study, our main goal is to identify vascular transcriptional alterations in human brain tissue in AD and to determine whether these alterations are preserved in blood of longitudinally followed older participants. We performed single nucleus RNA sequencing (snRNAseq) of temporal cortex tissue in 24 AD and control brains to uncover the transcriptional changes within the cells of the GVU. To determine whether GVU transcriptional alterations detected in brain are preserved in blood, we analyzed existing blood expression, genetic and imaging data from two longitudinal antemortem cohorts, MCSA and ADNI. We acquired the snRNAseq profile of 79,751 total brain cells that include 6,541 astrocytes and 2,210 cerebrovascular cells, the main cell types of the BBB GVU. We identified differentially expressed genes and their enriched pathways in these clusters and detected the most transcriptional changes within activated pericytes. The cerebrovascular transcriptional alterations in activated pericytes are strongly correlated with increasing AD pathology and age. Using our snRNAseq data and a knowledge-based predictive algorithm, we discovered and prioritized molecular interactions between cerebrovascular and astrocyte clusters. In two complementary longitudinal datasets we discovered genetic variants influence the blood expression levels of some of the prioritized GVU genes, among which we also observed associations with brain vascular disease neuroimaging burden, and $A\beta$ deposition and cortical thickness. In summary, we identified novel brain GVU expression changes at a single nucleus

level and prioritized the vascular-astrocytic molecular interactions that may deteriorate in AD thereby leading to breakdown of the BBB and propagation of AD neuropathology. Our findings of perturbed pericytic transcriptional alterations in brain that are preserved in blood constitute proof-of-principle evidence that brain AD-related expression changes may be detected peripherally in living patients. This has implications for future translation of these findings to the clinical setting as centrally-linked peripheral molecular biomarkers of AD.

Nanosymposium

426. Alzheimer's Disease Genomics

Location: SDCC 7

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 426.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Autophagy and Herpesvirus: A collaboration contributing to Alzheimer's disease

Authors: *M. RANJBAR[1], A. RAEISI NAFCHI[2], M. ESMAEILI[2], O. MYERS[1], E. L. BEARER[3];
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Abstract: Since the 1960s viral pathogenesis researchers have considered herpesviruses as an underlying factor for Alzheimer's disease (AD). We reported molecular interactions between herpes simplex virus type 1 (HSV-1) and the amyloid precursor protein (APP). Furthermore, others report biochemical interactions between HSV-1 and autophagy, using several brain banks for specimens of four brain regions in post-mortems of individuals with and without cognitive impairment before death. Readhead et al. 2018 found molecular-genetic evidence linking the activity of 6 different human herpesviruses to AD, including HSV-1, HSV-2, HHN6, HHN7, VZV, and CMV. Of these, HHN6, a common virus causing a minor childhood illness thought to be a nuisance, emerged as the most significant. Using a quantitative trait loci (QTL) approach, a network of candidate AD-associated genes was found that correlated with viral load and activity. These ontology networks did not specifically consider autophagy genes (ATG). We hypothesize that viral replication and egress highjack cellular membrane systems and thereby alter autophagic function. Those individuals carrying genetic variations that protect against this dynamic would be less vulnerable to cognitive impairment despite the viral load, or viral load would be

diminished. First, we prepared lists of ATG including 180 we uniquely identified through machine learning, as well as curated lists from publications and websites. We applied software developed by Readhead et al. 2018, available through Synapse.com, to sequence data from post-mortem brains obtained from publications and public sites hosted by Alzheimer's Center brain banks. Next, we searched the list of QTL that correlated with increased viral load and activity for ATG from 300+ brains in the Nun's Study and the Mount Sinai Brain Banks using custom Python scripts. Lastly, we correlated those ATG-associated QTL with expression levels of these ATG in control and preclinical AD. We identified ATG expression levels correlating with either non-AD and no dementia, or pre-clinical AD from published data. Virtually all ATG were downregulated in pre-clinical compared to non-AD controls. We found that decreased expression of some ATG in AD correlated with single nucleotide polymorphisms associated with increased viral load. This study suggests autophagy as a novel mechanism linking herpesvirus to AD, which may aid in finding new diagnostic and therapeutic targets. Since HHV6 is a common infection in childhood, infecting more than 80% of humans, identifying genetic vulnerabilities to persistence and progression of herpesvirus may be critically important for future prevention of adult AD.

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Nanosymposium

426. Alzheimer's Disease Genomics

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Presentation Number: 426.06

Topic: C.02. Alzheimer's Disease and Other Dementias

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P30AG072976

Title: Human microRNA-298 (miR-298) in biochemical cascades leading to Alzheimer's disease (AD)

Authors: *R. WANG[1], N. CHOPRA[3], B. MALONEY[1], B. T. LAMB[4], A. J. SAYKIN[2], K. SAMBAMURTI[5], S. E. COUNTS[6], D. K. LAHIRI[1];
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Abstract: Background: Alzheimer's disease (AD) is marked by neurofibrillary tangles mainly comprising hyperphosphorylated tau (or MAPT) protein; senile plaques comprising amyloid β (Aβ) peptides; and neuroinflammation. Our goal is to understand how these pathological changes are regulated. Genetic, epigenetic, and environmental factors play important roles in disease progression. We hypothesized that members of the small non-coding microRNA (miRNA) group regulate multiple biological and pathological processes, and that their disruption could contribute to AD pathogenesis. We recently reported that human miR-298 reduced the expression of APP, BACE1, and a specific tau isoform (Chopra et al, 2021; Wang et al, 2022). Herein we report miR-298's role in neuroinflammation. **Methods:** We transfected the human astrocyte cell line U373 MG with either mock, miR-298 mimic, its antagomiR, a combination of both, or negative control mimic for 72 hours. We prepared total RNA and protein from lysates and used conditioned media to measure levels of secreted cytokines. RNA was subjected to RNA sequencing analysis (RNAseq). We performed ELISA and real-time quantitative PCR (qPCR) assays to determine cytokine profiles and APP and tau levels. We also obtained well-characterized autopsy brain tissues from non-cognitively impaired (NCI) and AD subjects. We measured levels of mRNA, miR-298, and AD-related proteins in cortical samples from these subjects. **Results:** RNAseq revealed that miR-298 transfection reduced AD-related gene cluster mRNA expression, including APP, BACE1, MAPT, and the tau-related kinase GKS3β. miR-298 stimulated several pro-inflammatory cytokines and chemokines: IL-6, IL-15, and TNF-α. Additionally, an increase in caspase-3 pointed toward induction of apoptosis. Notably, miR-298 induced IL-6 secretion while reducing tau, APP, and BACE1 proteins. Human brain data additionally associated elevated miR-298 with an increased probability of late-stage AD. **Conclusion:** miR-298 may be a "master switch" regulating AD and inflammation-related genes. The induction of multiple pro-inflammatory cytokines may be due to the combined regulation of miRNA activity and upstream signaling pathways. We suggest that miR-298 could be therapeutic, such as when high pro-inflammatory cytokines would be necessary for neuroprotection along with a simultaneous reduction of levels of tau, APP, and BACE1 proteins. Moreover, at this very early stage, pruning out damaged neurons would be of particular usefulness while the reserve of healthy neurons is still sufficient to maintain overall function.

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Nanosymposium

426. Alzheimer's Disease Genomics

Location: SDCC 7

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 426.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Human microRNA-101-3p (miR101) plays a critical role in regulation that may underlie neurodegenerative diseases

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Abstract: Background: MicroRNA (miRNA) is a group of small non-coding RNA that play a vital role in regulating various biological processes. Aberrations of miRNA may lead to neurodegenerative diseases such as Alzheimer's, disease (AD), Parkinson's disease (PD), and head trauma. Major hallmarks of AD include brain deposition of neuritic plaques consisting of amyloid-β peptides (Aβ), and neurofibrillary tangles comprising hyperphosphorylated tau. In addition to AD, tau may involve in the pathology of concussion / TBI, and α-synuclein aggregation in PD. Specific miRNAs such as miR-101-3p (miR-101) regulate the expression of Aβ precursor protein (APP) and other proteins. miR-101 may protect against cerebral ischemia/reperfusion injury. APP metabolites are critical in neurodevelopmental disorders, e.g., autism spectrum. We report how miR-101 regulates levels of critical proteins, e.g., APP, ECE1, GSK3β, and cytokines, each implicated in several brain disorders.
Methods: Multiple cell lines, including human microglia HMC3 and differentiated neuroblastoma SK-N-SH cells were transfected with miR-101 for 72 hr. Total cellular RNA, protein in cell lysates, and secreted proteins in conditioned media were extracted. We tested target specificity of miR-101 using dual reporter clones containing APP-, ECE-, or GSK3β- 3'-UTR, derived from respective mRNAs. We transfected neuronal cultures with miR-101, and measured protein levels of APP, ECE1, GSK3β, and tau by ELISA. We obtained samples from temporal lobes, cerebella, and posterior cingulate cortices from non-cognitively impaired and AD individuals. We measured miR-101 levels by quantitative RT-PCR.
Results: Treatment of miR-101 significantly reduced APP, ECE1 and GSK3β mRNA 3'-UTR activities in reporter clones. miR-101 reduced APP, ECE1 and GSK3β protein levels in cell cultures. Further, miR-101 transfection stimulated secretion of the pro-inflammatory cytokines, IL-1α, IL-1β, IL-6, and TNF-α in HMC3 cells. We could detect levels of miR-101 and target mRNAs in brain regions of AD subjects.

Conclusion: We propose that miR-101 represents a vital regulator of AD-related proteins as it targeted the 3'-UTRs of APP, ECE1 and GSK3β and reduced their expression. miR-101 transfection reduced GSK3β in multiple cells and cross-species, including human differentiated neurons, neural stem cells, microglia, and astrocytic, and mouse cells. miR-101 transfection induced secretion of multiple pro-inflammatory cytokines. Altogether, our results suggest that miR-101 plays a critical role in regulating multiple disease-related proteins across cell types, which is relevant to understanding AD and other brain disorders.

Disclosures: D.K. Lahiri: None. **R. Wang:** None. **B. Maloney:** None. **K.H. Nudelman:** None. **F.A. White:** None. **J.M. Long:** None. **N.H. Greig:** None. **A.G. Kanthasamy:** None. **S.E. Counts:** None.

Nanosymposium

426. Alzheimer's Disease Genomics

Location: SDCC 7

Time: Tuesday, November 15, 2022, 8:00 AM - 10:00 AM

Presentation Number: 426.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R56AG071152

Title: Modeling behavior, functional connectomics, and spatial proteomics in a mouse model of Alzheimer's Disease

Authors: *L. FADEL[1], E. HIPSKIND[1], N. RUGGIERO[2], C. ORTIZ[2], J. ROMERO[2], A. SAMEE[2], R. PAUTLER[2];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by accumulation of amyloid beta (Aβ) plaques and neurofibrillary tau tangles. Currently, the only definitive way to diagnose AD is through post-mortem assessment of brain tissue. In living patients, a probable AD diagnosis can be determined with cognitive assessments, blood tests for plasma Aβ , and PET imaging. The combination of these tools provides an effective diagnostic model; however, they are not capable of early detection of AD. Advancements in neuroimaging techniques that can detect deficits prior to significant accumulation of pathological features and cognitive decline would aid early detection, diagnosis, and potential therapeutic intervention. In both AD patients and mouse models, rs-fMRI has revealed deficits in functional connectivity in brain regions that are involved in memory functions. These deficits precede cognitive dysfunction, making rs-fMRI a promising clinical marker of AD. We are correlating functional connectivity with proteomic changes and behavioral readouts in the same AD Cg-Tg(APPswe,PSEN1dE9)85Dbo mouse and wildtype controls. At 6 months of age, when Aβ plaques begin to form in AD mice, there is no significant difference detected between AD mice and wildtype controls performance in spatial, contextual, or cued memory tasks. Our functional connectivity data indicates patterns of both hypo and hyper connectivity throughout the brain of

AD mice when compared to WT. This data indicates a potential compensatory mechanism that allows AD mice to have near WT levels of learning and memory performance at the age point when plaque deposition begins. Using a Bayesian network machine learning model, we can begin to predict certain brain regions that may explain the behavioral task readouts at this age point. Areas including the retrohippocampus, insula, and ectorhinal cortex exhibit hyper connectivity, while the parietal lobe and retrosplenial area exhibit hypo connectivity. Using nanoString spatial proteomic technology, we also assessed protein expression profiles in 9 brain regions traditionally studied in models of AD where we also found functional connectivity differences. This revealed differences in proteomic profiles between the AD and WT in both the AD pathology panel and immune response panel. For the first time, the relationship between behavior, functional connectivity, and proteomic profiles are being investigated in a mouse model of AD that will provide new insight into disease progression. This may lead to breakthroughs in early detection and clinical diagnostics for patients with AD.

Disclosures: L. Fadel: None. **E. Hipskind:** None. **N. Ruggiero:** None. **C. Ortiz:** None. **J. Romero:** None. **A. Samee:** None. **R. Pautler:** None.

Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.01

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: Neuronal Prion Reduction with an Engineered Zinc Finger Protein Transcription Factor Achieves Effective Total PrP Reduction in Mouse Brain and CSF

Authors: *S.-W. CHOU[1], K. MARLEN[1], J. HU[1], G. CISBANI[2], F. PETERS[2], F. HARTSTEIN[2], C. MELIS[2], M. A. MORTBERG[3], M. MEHRABIAN[1], A. PHILLIPS[1], A. GOODWIN[1], A. FALCON[1], E. V. MINIKEL[3], S. M. VALLABH[3], A. M. POOLER[1], B. ZEITLER[1];
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Abstract: Prion disease is a fatal, rapidly progressing neurodegenerative disorder caused by aggregation of misfolded prion protein, PrP, encoded by the *PRNP* gene. Lowering endogenous PrP levels by 50% either genetically or with antisense oligonucleotides can approximately double the lifespan of prion-infected mice. We are developing a single-administration AAV approach using zinc finger protein transcription factors (ZF-TFs) to achieve sustained and widespread reduction of PrP in the brain. We are also investigating the potential utility of cerebrospinal fluid (CSF) PrP levels as a surrogate biomarker for brain PrP reduction. While *PRNP* mRNA is expressed in all cell types in the brain, neuronal *Prnp* expression is likely to be necessary and sufficient for neurotoxicity and disease progression in prion-infected mouse models. However, it is unclear what fraction of brain and CSF PrP levels is neuronally-

derived.To evaluate the contribution of different cell types to *Prnp* expression at the bulk and single-cell level in the brain, we generated AAV vectors expressing a highly potent and specific prion-targeted ZF-TF under the control of one of three different promoters: hSYN1, GfaABC1D, or CMV. In cultured mouse primary neurons and astrocytes, ZF-TF treatment reduced *Prnp* mRNA in a dose-dependent manner; while hSYN1-driven expression was restricted to neurons, CMV- and GfaABC1D-driven expression were found in both cell types. In wildtype mice treated with these constructs, all tested promoters showed at least 50% bulk prion mRNA reduction depending on the brain region (hSYN1 ≥ CMV > GfaABC1D) when compared to the control group. Brain hemispheres from the same experiment were analyzed by multiplexed single-cell RNAscope and immunohistochemistry. Neuron-specific expression was observed for the hSYN1 promoter in all brain regions examined. For the CMV group, heterogenous expression was observed, primarily in neurons and astrocytes. Surprisingly, the GfaABC1D groups showed predominantly neuronal expression that was weaker and less homogenous than hSYN1. In all cases, a strong negative correlation between ZF-TF and *Prnp* expression was observed at the single-cell level throughout the brain. Visualization with a PrP-protein-directed antibody (POM2) confirmed prion protein knockdown in transduced brain regions. Finally, CSF PrP levels were 66% and 72% lower than controls in experimental groups with the hSYN1 and CMV promoter, respectively, and 42% lower with the GfaABC1D promoter. Our data demonstrates a *Prnp* targeted ZF-TF can potently reduce prion expression in the brain, and suggests neurons may be the major contributing cell type to total CSF PrP levels in mice.

Disclosures: S. Chou: A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **K. Marlen:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **J. Hu:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **G. Cisbani:** A. Employment/Salary (full or part-time):; Evotec. **F. Peters:** A. Employment/Salary (full or part-time):; Evotec. **F. Hartstein:** A. Employment/Salary (full or part-time):; Evotec. **C. Melis:** A. Employment/Salary (full or part-time):; Evotec. **M.A. Mortberg:** None. **M. Mehrabian:** None. **A. Phillips:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **A. Goodwin:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **A. Falcon:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **E.V. Minikel:** None. **S.M. Vallabh:** None. **A.M. Pooler:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **B. Zeitler:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics.

Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.02

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH grant NS076896

Title: Prion infection leads to an early-to-mid disease specific decrease in hippocampal mGluR5

Authors: D. OJEDA-JUÁREZ[1], K. SOLDAU[1], A. MALIK[1], J. LAWRENCE[1], D. P. PIZZO[2], H. SANCHEZ[3], M. D. GESCHWIND[3], C. SIGURDSON[1,4];
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Abstract: Prion diseases are rapidly progressive neurodegenerative disorders caused by the accumulation of prion protein aggregates (PrP^Sc) in the central nervous system. Synapse loss has been extensively reported as an early neuropathological finding, and the clinical onset correlates with a marked reduction in synaptic proteins. The cellular prion protein (PrP^C) binds prion, amyloid-β, tau, and α-synuclein oligomers, which results in the activation of macromolecular complexes and signaling at the post-synapse, yet the alterations in synaptic proteins are incompletely understood. To determine how synaptic receptor levels change in prion disease, we measured the levels of AMPA, metabotropic, and NMDA receptors (GluA1, mGluR5, and GluN1) in the occipital cortex of sporadic Creutzfeldt-Jakob post-mortem samples by immunoblotting. Surprisingly, there was a marked reduction in mGluR5 dimers, but no change in GluA1 or GluN1. The loss of mGluR5 dimers was not associated with a widespread loss of pre- or post-synaptic proteins, as synaptophysin, PSD95, and VAMP2 were unaffected. Furthermore, higher levels of PrP^Sc in occipital cortex correlated to shorter disease duration, but not to mGluR5 monomer or dimer level. To determine the kinetics of the mGluR5 reduction during prion infection, we conducted a time course study in the hippocampus of prion-infected mice. Interestingly, there was a pronounced decrease in mGluR5 dimer levels over time beginning at early-to-mid (pre-clinical) disease, although the loss of mGluR5 was not due to a decrease in mRNA levels. Additionally, the levels of phosphorylated AMPA receptors (pGluA1-S845) were increased by mid-disease (pre-clinical), and GluN1 decreased in late disease (clinical and near terminal). Together, these findings suggest that mGluR5 receptors are post-transcriptionally reduced in early-to-mid prion disease. Understanding the signaling pathways at the synapse may lead to the discovery of novel therapeutic targets and/or biomarkers to track disease progression for prion disease and other neurodegenerative disorders.

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Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.03

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH Grant R01NS064131
VA Space and Resources

Title: The CUL3 adaptor protein SPOP impacts tauopathy mechanisms

Authors: R. ECK[1], R. L. KOW[2], N. LIACHKO[3], ***B. KRAEMER**[4,1];
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Abstract: Pathological accumulation of the microtubule binding protein tau drives age-related neurodegeneration in a variety of disorders, collectively called tauopathies. In the most common tauopathy, Alzheimer's disease (AD), the accumulation of pathological tau strongly correlates with cognitive decline. The underlying molecular mechanisms that drive neurodegeneration in tauopathies remain unknown. We employed classical forward genetic approaches and identified multiple loss of function alleles in the *C. elegans spop-1 g*ene that ameliorate tauopathy, suggesting SPOP is required for tau mediated neurodegeneration. CRISPR based genome editing methodology enabled the generation of customized *SPOP-1* loss of function and null alleles. Molecular genetics, behavioral, neuronal reporter assays, and biochemical analyses were also employed to characterize the consequences of *spop-1* loss of function on tauopathy related phenotypes in model systems. Knockout of SPOP-1 rescues tau mediated behavioral deficits caused by neuronal dysfunction in tau transgenic *C. elegans.* Biochemical analysis revealed that SPOP-1 loss of function promotes clearance of phosphorylated and total tau species from *C. elegans* neurons, but no change in tau transgene mRNA levels. Tau transgenic animals exhibit obvious neurodegeneration of GABAergic neurons, but loss of *spop-1* rescues neurodegeneration. While SPOP functions as an CUL3 E3 ligase adaptor protein, CUL3 function is not required for SPOP loss of function rescue of tauopathy. Genetic epistasis analysis suggests the nuclear speckle resident poly(A) RNA binding protein *sut-2* and *spop-1* function in a parallel molecular pathway. SPOP is a novel modifier of tauopathy phenotypes. Combined with previous findings investigating ALYREF, PARN/TOE1, SUT-1 and SUT-2/MSUT2, this work suggests phase-separated nuclear speckles are an important cellular site controlling susceptibility to pathological tau. Recent work showing SPOP modification of PR dipeptides derived from C9orf72 expansion suggests common pathways may be at work in the neurodegenerative molecular mechanisms of tauopathy, repeat dipeptides, and perhaps other proteinopathy disorders.

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Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.04

**Topic:** C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: X-linked ubiquitin-specific peptidase 11 increases tauopathy vulnerability in women

Authors: Y. YAN[1], X. WANG[1], D. CHAPUT[3], M.-K. SHIN[2], Y. KOH[1], L. GAN[4], A. A. PIEPER[5], J. A. WOO[1], ***D. E. KANG**[1];
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Abstract: Although women experience significantly higher tau burden and increased risk for Alzheimer's disease (AD) than men, the underlying mechanism for this vulnerability has not been explained. Here, we demonstrate through both *in vitro* and *in vivo* models, as well as human AD brain tissue, that X-linked ubiquitin specific peptidase 11 (USP11) augments pathological tau aggregation via tau deubiquitination initiated at lysine-281. This enhances tau acetylation at lysines 281 and 274. Tau acetylation induced by CBP/p300 or by inhibition of deacetylases SIRT1 or HDAC6 is suppressed by the loss of USP11. USP11 escapes complete X-inactivation, and female mice and people both exhibit higher USP11 levels than males. Genetic elimination of *usp11* in a tauopathy mouse model preferentially protects females from acetylated tau accumulation, tau pathology, and cognitive impairment. USP11 levels also associate positively with tau pathology with a strong female bias in human tauopathies. Thus, inhibiting USP11-mediated tau deubiquitination may provide a new therapeutic approach to protect women from increased vulnerability to tau aggregation in tauopathies, including AD.
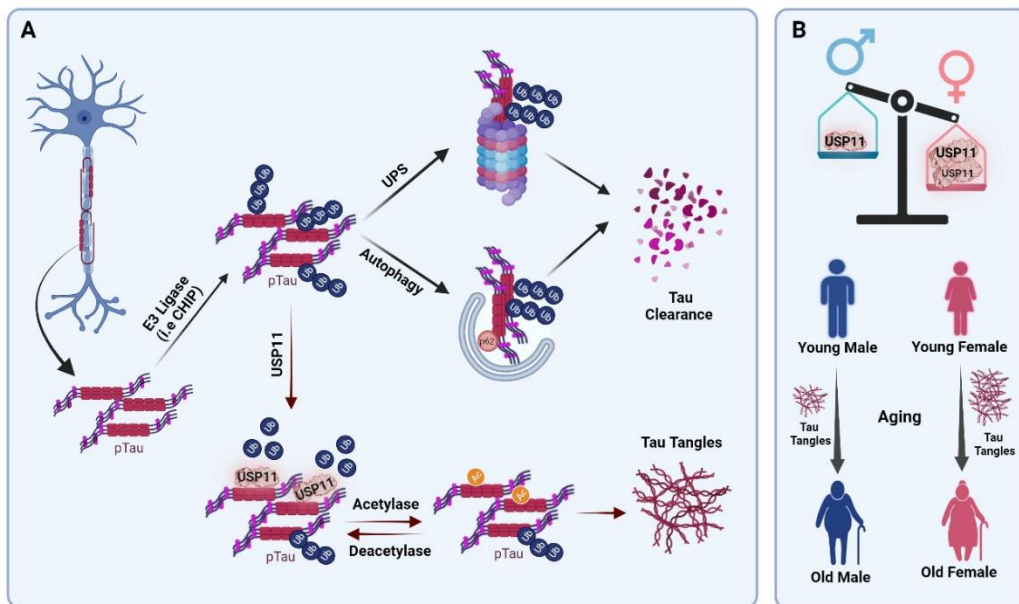
Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.05

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: β-arrestins promote tauopathy by disrupting microtubules and impairing autophagy

Authors: *J. WOO[1], T. LIU[1], Y. YAN[1], T. KEE[2], S. G. CAZZARO[2], C. FANG[3], X. ZHAO[4], X. WANG[1], K. MCGILL-PERCY[1], M. CASTANO[5], J. MATLACK[5], K. YRIGOIN[5], S. LIGGETT[5], D. KANG[1];
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Abstract: Multiple G protein-coupled receptors (GPCRs) are targets in the treatment of dementia, and the arrestins are common to their signaling. β-arrestin1 and β-arrestin2 were significantly increased in brains of patients with frontotemporal lobar degeneration (FTLD-tau), a disease second to Alzheimer's as a cause of dementia. Genetic loss and overexpression experiments using genetically encoded reporters and defined mutant constructs in vitro, and in cell lines, primary neurons, and tau P301S mice crossed with β-arrestin1-/- mice or β-arrestin2-/-, show that β-arrestin1 or β-arrestin2 stabilizes pathogenic tau and promotes tau aggregation. Cell and mouse models of FTLD showed this to be maladaptive, fueling a positive feedback cycle of enhanced neuronal tau via non-GPCR mechanisms. Genetic ablation of β-arrestin1 or β-arrestin2 markedly ablates tau pathology and rescues synaptic plasticity defects in tau P301S transgenic mice. Biochemical and cellular studies show that β-arrestin1 and β-arrestin2 drive tauopathy by destabilizing microtubules and impeding p62/SQSTM1 autophagy flux. Interestingly, atomic force microscopy and cellular studies revealed that oligomerized, but not monomeric, β-arrestin2 increases tau by inhibiting self-interaction of the autophagy cargo receptor p62/SQSTM1, impeding p62 autophagy flux. Hence, reduction of oligomerized β-arrestin2 with virus encoding β-arrestin2 mutants acting as dominant-negatives markedly reduces tau-laden neurofibrillary tangles in FTLD mice in vivo. Reducing β-arrestin1 or β-arrestin2, and its oligomeric status represent a new strategy to alleviate tau pathology in FTLD and related tauopathies.

Disclosures: J. Woo: None. **T. Liu:** None. **Y. Yan:** None. **T. Kee:** None. **S.G. Cazzaro:** None. **C. Fang:** None. **X. Zhao:** None. **X. Wang:** None. **K. McGill-Percy:** None. **M. Castano:** None. **J. Matlack:** None. **K. Yrigoin:** None. **S. Liggett:** None. **D. Kang:** None.

Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.06

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH grant NS076896

Title: Rab7 contributes to glial activation during prion-infection

Authors: *G. FUNK[1], D. OJEDA-JUÁREZ[1], D. P. PIZZO[1], J. WANG[1], C. SIGURDSON[1,2];
[1]UCSD, La Jolla, CA; [2]Univ. of California, Davis, Davis, CA

Abstract: Prion diseases are rapidly progressive and fatal neurodegenerative disorders, caused by the aggregation of a misfolded isoform of the PrP^C protein, known as PrP^{Sc}. Prion conversion has been show to occur in the plasma membrane, recycling endosomes, and multivesicular bodies. *In vitro* studies suggest that PrP^{Sc} reduces endocytic trafficking as well as lysosomal maturation and degradative function by decreasing the membrane association of Rab7. Rab7, a small GTPase, plays a pivotal role in the endosomal maturation, transport of cargo including lipoproteins and signaling receptors from late endosomes to lysosomes, and fusion of phagosomes and autophagosomes with late endosomes and lysosomes. However, how Rab7 function impacts prion disease progression *in vivo* has yet to be defined. To investigate the contribution of Rab7 in prion disease progression, we challenged mice having a conditional knock-out of Rab7 in neurons (*Rab7$^{f/f}$SynCre*), astrocytes (*Rab7$^{f/f}$GFAPCre)*, or microglia (*Rab7$^{f/f}$LysCre)* with the subfibrillar prion strain (ME7) and evaluated mice for survival time and glial activation. Iba1 and GFAP immunohistochemical staining were used as biomarkers for microglia and astrocytes in the dentate gyrus, hippocampal cornu ammonis (CA1), and cerebral cortex. We observed that depletion of Rab7 in astrocytes, microglia, and neurons had no effect on the survival time after prion infection. Surprisingly, we observed that prion-infected *Rab7$^{f/f}$GFAPCre+* mice showed a decrease in astrocytic and microglial activation, as measured by area stained, as compared to the Cre- littermate controls. In contrast, prion-infected *Rab7$^{f/f}$SynCre+* and *Rab7$^{f/f}$LysCre+* showed no differences in astrocyte or microglial activation compared to *Cre-* controls. These results suggest that 1) Rab7 depletion from microglia, astrocytes, or neurons in prion-infected mice does not affect survival time, and 2) Rab7 expression in astrocytes may be necessary for astrocyte and microglial activation during prion infection with a subfibrillar strain. Elucidating the cellular and molecular pathways contributing to prion disease progression are essential for the future development of therapeutics.

Disclosures: G. Funk: None. **D. Ojeda-Juárez:** None. **D.P. Pizzo:** None. **J. Wang:** None. **C. Sigurdson:** None.

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.07

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: Diminished ESCRT-0 function exacerbates AMPA receptor dysregulation and accelerates prion-induced

Authors: *M. POURHAMZEH;
Dept. of Pathology, UCSD, San Diego, CA

Abstract: Diminished ESCRT-0 function exacerbates AMPA receptor dysregulation and accelerates prion-induced neurodegeneration
Jessica A. Lawrence[1], Mahsa Pourhamzeh[1], Patricia Aguilar-Calvo[1], Helen Khuu[1], Katrin Soldau[1], Donald P. Pizzo[1], Jin Wang[1], Daniel Ojeda-Juarez[1], Timothy F. Miles, Brent Aulston, Seung Min Song[1], Julia Callender[1], JoAnn Trejo[2], Nobuyuki Tanaka[3,4], Viviana Gradinaru, Chengbiao Wu[5], Xu Chen[5], Gentry Patrick[6], Christina J. Sigurdson[1,7,*]
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Neuronal endolysosomal defects are central to the pathogenesis of prion and other neurodegenerative diseases. In prion disease, prion oligomers traffic through the multivesicular body and are routed for degradation in lysosomes or release in exosomes, yet how prions impact proteostatic pathways is unclear. Hrs and STAM1 (ESCRT-0), which transport ubiquitinated membrane proteins from early endosomes to MVBs, were shown to be significantly reduced in the brain tissue of prion-affected mice and humans, as well as the in the tau- P301S mouse tauopathy model. To determine how the reduction in ESCRT-0 impacts prion conversion and cellular toxicity *in vivo*, we prion-challenged conditional knockout mice having *Hrs* deleted from neurons. The neuronal Hrs-depleted mice showed a shortened survival and an acceleration in synaptic derangements, including an accumulation of ubiquitinated proteins, deregulation of phosphorylated AMPA and profoundly altered synaptic structure, all of which occurred later in the infected littermate controls. We also found that neuronal Hrs depletion increases surface PrP^C expression, which may contribute to the rapidly advancing disease. Finally, depletion of neuronal Hrs in uninfected mice was linked to a reduction in pGluA1-S845 and -S831, suggesting that Hrs may be important for GluA1 trafficking and synaptic membrane insertion. Taken together, the reduced Hrs in the prion-affected brain hampers ubiquitinated protein clearance at the synapse, exacerbates post-synaptic glutamate receptor deregulation, and accelerates neurodegeneration.
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Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.08

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: Small Molecule Activators of Neuroprotective microRNAs Identified by High-throughput Screening in Human iPSC-derived Neurons

Authors: *L. D. NGUYEN[1], Z. WEI[1], S. BARBERÁN-SOLER[2], C. SILVA[3], R. RABINOVSKY[1], C. R. MURATORE[1], J. M. S. STRICKER[1], T. L. YOUNG-PEARSE[1], S. J. HAGGARTY[3], A. M. KRICHEVSKY[1];
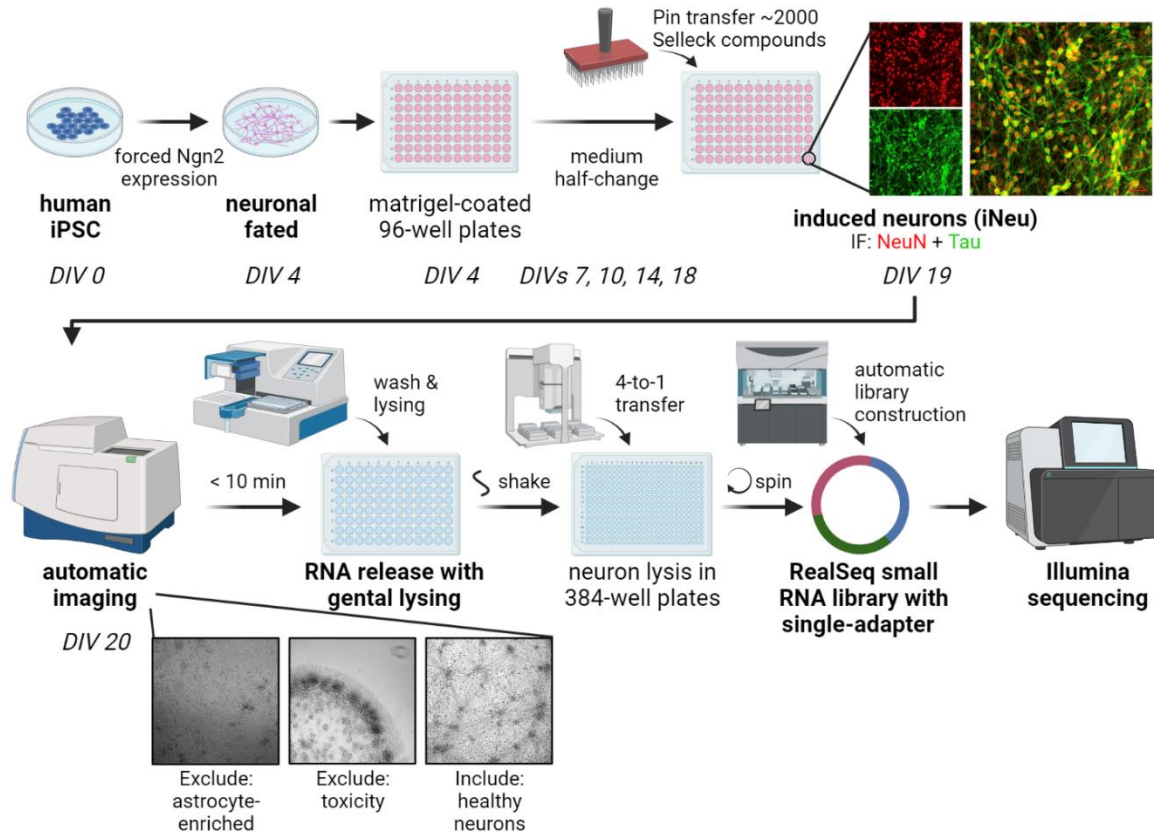[1]Neurol., Brigham and Women's Hosp. and Harvard Med. Sch., Boston, MA; [2]RealSeq Biosci., Santa Cruz, CA; [3]Neurol. and Psychiatry, Massachusetts Gen. Hosp. and Harvard Med. Sch., Boston, MA

Abstract: Background. MicroRNAs (miRNAs) are short, single-stranded RNAs that regulate fundamental biological processes by silencing their mRNA targets. miR-132 is a key miRNA consistently downregulated in Alzheimer's disease (AD) and various tauopathies. We and others have shown that miR-132 overexpression in the brain rescued neurodegenerative phenotypes in several animal models of AD. However, current strategies to upregulate miRNAs through oligonucleotide miRNA mimics or virus-mediated gene delivery are limited by poor distribution, low uptake, and high immunogenicity. Therefore, we aimed to discover and validate small molecule drugs that upregulate miR-132 as an alternative approach.
Methods. To identify small molecule activators of miR-132, we performed a pilot high-throughput miRNA-seq screen of 2000 drugs in human induced pluripotent stem cell (iPSC)-derived neurons. We obtained the expression profiles of 500 miRNAs for 1800 drugs. Focusing specifically on miR-132, we selected 44 compounds for further study and investigated whether these lead compounds were also neuroprotective in cell and animal models.
Results. We successfully validated that several members of the cardiac glycoside family, which are canonical sodium-potassium ATPase inhibitors, consistently upregulated miR-132 at the sub-μM range. The mechanism was through increased transcription of the miR-132/212 locus and was mimicked by knocking down ATP1A1 and ATP1A3 - the major ATPase isoforms in neurons. Treating neurons with sub-μM cardiac glycosides potently downregulated total and phosphorylated tau by more than 70% in human neurons and protected against cell death by N-methyl-D-aspartate (NMDA), rotenone, and Aβ oligomers.
Conclusion. We identified and validated small molecule drugs that upregulated the neuroprotective miR-132 in neurons. Our dataset also represents a comprehensive resource for discovering small molecules drugs that regulate specific miRNAs for therapeutic purposes.

Pin transfer ~2000 Selleck compounds

human iPSC
DIV 0

forced Ngn2 expression

neuronal fated
DIV 4

matrigel-coated 96-well plates
DIV 4

medium half-change

DIVs 7, 10, 14, 18

induced neurons (iNeu)
IF: NeuN + Tau
DIV 19

automatic imaging
DIV 20

< 10 min

wash & lysing

RNA release with gental lysing

shake

4-to-1 transfer

neuron lysis in 384-well plates

spin

automatic library construction

RealSeq small RNA library with single-adapter

Illumina sequencing

Exclude: astrocyte-enriched

Exclude: toxicity

Include: healthy neurons

Disclosures: L.D. Nguyen: None. **Z. Wei:** None. **S. Barberán-Soler:** A. Employment/Salary (full or part-time):; RealSeq Biosciences. **C. Silva:** None. **R. Rabinovsky:** None. **C.R. Muratore:** None. **J.M.S. Stricker:** None. **T.L. Young-Pearse:** None. **S.J. Haggarty:** None. **A.M. Krichevsky:** None.

Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.09

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH
F Prime
DOD
Answer ALS
ALSA

Title: Evidence for Inherited CHMP2bFTD/ALS Resulting from Nuclear Pore Complex and CHMP7 Pathophysiology.

Authors: V. BASKERVILLE[1], *J. D. ROTHSTEIN[2], **A. N. COYNE**[2];
[1]Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Johns Hopkins Univ., Johns Hopkins Univ., Baltimore, MD

Abstract: Altered nucleocytoplasmic transport is emerging as a prominent pathomechanism of multiple neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Frontotemporal Dementia (FTD) and Huntington's disease (HD). The nuclear pore complex (NPC) and interactions between its individual nucleoporin components and nuclear transport receptors regulate nucleocytoplasmic transport, as well as genome organization and gene expression. Specific nucleoporin abnormalities have been identified in sporadic and familial forms of neurodegenerative disease including sporadic ALS and familial C9orf72 ALS/FTD and these alterations are thought to contribute to disrupted nucleocytoplasmic transport. Recently using multimodal imaging of induced pluripotent stem cell-derived neurons (iPSNs) derived from a large number of patients with familial or sporadic ALS, we have demonstrated that nuclear accumulation of the ESCRT-III protein CHMP7 is sufficient to initiate NPC injury thereby linking CHMP7 and the ESCRT-III nuclear surveillance pathway to an early event in ALS pathogenesis. Importantly, these studies demonstrated that the characteristic loss of nuclear TDP-43 function and localization occurs downstream of this CHMP7-mediated NPC injury cascade. Given that mutations in the closely related ESCRT-III protein CHMP2 are causative of familial FTD, we sought to determine whether CHMP2B mutations also disrupt the NPC and downstream TDP-43 function. Here, using isogenic mutant CHMP2B iPSNs and multiple imaging paradigms, we demonstrate the emergence of a significant defect in the nuclear membrane and nuclear pore complex as well as aberrant TDP-43 function as evaluated by qRT-PCR for TDP-43 targets. Additional studies are now underway to comprehensively characterize this pathogenic cascade in the context of CHMP2B mutations and determine is antisense oligonucleotides (ASOs) targeting CHMP7 can repair this injury as we have previously demonstrated for familial and sporadic ALS.

Disclosures: V. Baskerville: None. **J.D. Rothstein:** None. **A.N. Coyne:** None.

Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.10

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: Probing mechanisms for axonal degeneration in Primary Tauopathy using patient derived cerebral organoids

Authors: *X. SUN[1], S. MAHALI[2], C. KARCH[2], P. BAAS[1], L. QIANG[1];
[1]Neurol. and anatomy, Drexel Univ. Col. of Med., Philadelphia, PA; [2]Dept. of Psychiatry, Washington Univ. in St. Louis Sch. of Med., St. Louis, MO

Abstract: In primary tauopathies, such as Frontotemporal Dementia (FTD), tau loses association with microtubules (MTs) in the axon and forms neurofibrillary tangles. Tau is one of the most abundant MT-associated proteins in the axon, but how pathological tau impacts the properties of MTs in tauopathies remains controversial and confusing. Because pathological tau binds less avidly to MTs, the conventional view has been that the levels and stability of axonal MTs are diminished as a result of the pathology. However, this idea is based on the dogma of tau as a MT stabilizer, which our recent studies on rodent neurons have called into question. The situation is further confounded in the case of human disease by the fact that neurons express a very different profile of tau isoforms as they mature, with different isoforms having different MT-binding affinities and propensities for aggregation. To investigate this matter in a more disease-relevant scenario, we used human dorsal forebrain organoids derived from isogenic human induced pluripotent stem cells, including those with tauP301S mutation that occurs in FTD. Significantly elevated levels of immature and mature tau (3R-tau and 4R-tau) were identified in the tauP301S organoids at both mRNA and protein levels. A consistent tau elevation was observed in the postmortem brain of patients with tauopathy. Moreover, this elevation was accompanied by aberrant modifications of tau, which might be a harbinger of subsequent pathological changes. Strikingly, MAP6, a bonafide MT stabilizer that competes with tau's effects on MTs, presented two opposite changes in its levels in the tau mutant organoids at the early and late-developing stages, which were accompanied by the predicted corresponding changes in MT dynamics. Some of these changes coincided with neurodegenerative phenotypes revealed by immunohistochemical and electrophysiological studies. Lastly, tau reduction via antisense-oligonucleotide approaches mitigated some pathological early-stage changes in tauP301S neurons. In all, our results demonstrate distinct bi-phasic changes in MAP6 and corresponding aberrant MT behaviors arising from different aspects of tau pathology at early and late stages of the disease. We conclude that therapies to correct MT abnormalities in tauopathy must be targeted for the specific stage of the disease.

Disclosures: X. Sun: None. **S. Mahali:** None. **C. Karch:** None. **P. Baas:** None. **L. Qiang:** None.

Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.11

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: Lipid droplets facilitate Tau fibrillization

Authors: *X. TIAN[1], Y. BAI[2], A. LONGHINI[2], A. DUBOSE[1], M. VIGERS[1], C. CAMARGO[2], L. FORD[2], S. HAN[1], K. S. KOSIK[2];
[1]Dept. of Chem. and Biochem., [2]Neurosci. Res. Inst., Univ. of California Santa Barbara, Santa Barbara, CA

Abstract: Lipid droplets (LDs) are energy-storing organelles, maintaining cellular lipid homeostasis. Their accumulation has been linked to many neurodegenerative diseases, including Tauopathies. Fibrillization of Tau protein is a hallmark of Tauopathies, yet no studies have shown a direct interaction between Tau and lipid droplets. How LDs contribute to the etiology of Tauopathies is still unknown. Here, we show LDs and Tau interact *in vitro* by fluorescence confocal microscopy (n=3 independent experiments) and such interaction enables Tau fibrillization confirmed by ThT assays and Transmission Electron Microscopy (TEM). We observed two spatial relationships for LDs and Tau: 1. LDs fully overlapping with Tau, suggesting Tau might be concentrated within LDs. 2. Tau surrounding LDs, possibly indicating either that Tau is *en route* to the core of LDs or that Tau is being organized around LDs. We further confirmed different Tau conformers, including monomers, oligomers, and fibrils, can interact with LDs (n=2 independent experiments). Similar spatial relationships were observed for LDs and different Tau conformers. Interestingly, we found Tau oligomers form fibrillar nets along the surface of LDs by TEM (n=2 independent experiments) and such fibrillar nets do not exist without LDs. In cells, we found oligomeric Tau seeded more cells when LD production is induced in those cells (2 biological replicates, 5 fields of view per replicate, p<0.05 by two-way ANOVA test). Taken together, we demonstrate lipid droplets can facilitate Tau fibrillization *in vitro* and our study provides a new direction to explain Tau pathologies.

Summary of results for SfN 2022
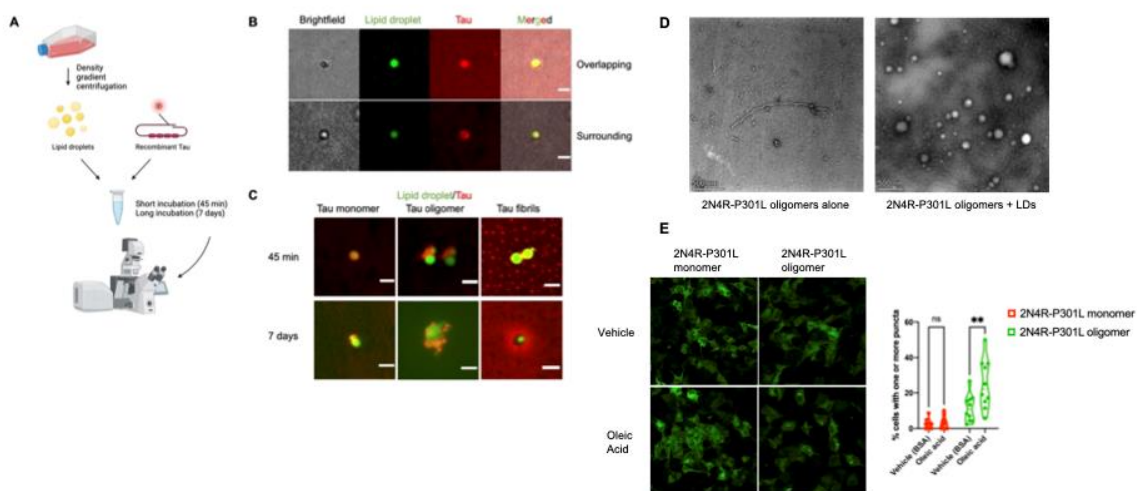
Nanosymposium

427. Cellular and Molecular Mechanisms of Prions and Tauopathies

Location: SDCC 6CF

Time: Tuesday, November 15, 2022, 8:00 AM - 11:00 AM

Presentation Number: 427.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Development and application of a human tissue-based in-lysate assay to demonstrate targeted protein degradation in Alzheimer's disease

Authors: V. BIEBER[1], B. BAJRAMI[1], M. O'SHEA[2], N. STRATMAN[1], J. AHN[2], L. INGANO[1], J. CHOI[1], H. HERING[1], C. ANDERSON[2], D. WALSH[1], ***D. PATERSON**[1];
[1]Biogen, Inc., Cambridge, MA; [2]C4 Therapeut., Watertown, MA

Abstract: Objective: Accumulation of tau fibrils in Alzheimer's disease (AD) correlates with neurodegeneration and with the severity of clinical symptoms. Bifunctional molecules that simultaneously bind tau fibrils and E3 ligases have the potential to facilitate degradation by the ubiquitination-proteasome system (UPS), and as such are an attractive therapeutic approach for AD. Models of tau aggregation often employ mutations associated with frontotemporal dementia and the assemblies formed are not recognized by AD-specific tau tracers. Here we describe the development of an *in-lysate* assay in which test compounds are added directly to AD brain lysate and assessed for their ability to degrade *bona fide* tau aggregates.
Methods: Cortex from AD and non-demented controls were homogenized in lysis buffer (0.8% NP40 in 100 mM Tris-HCl, pH 7.4, 1.5 mM MgCl2, 150 mM NaCl,100X HALT protease inhibitor cocktail), centrifuged at 20,000xg for 20 mins and the supernatant collected and used. Abcam kit (ab107921) was employed to measure proteasome activity, and the presence of competent UPS machinery was determined using a validated degrader of Bruton's tyrosine Kinase (BTK). Putative tau degrader compounds (0.1-10uM) were incubated in lysate up to 48 hrs and tau aggregation measured using a HTRF assay. Separately, a radioligand competition assay was used to measure binding of degraders to [^{3}H] MK6240-positive tau aggregates.
Results: Degrader-mediated reduction of BTK demonstrates the feasibility of screening degrader compounds in brain lysates. However, proteasome activity in lysates from AD brain were on average ~50% lower level than in lysates from control brains, and the lowest activity was observed in AD samples with greatest tau burden. Despite degraders being able to bind to tau, none of the tested compounds reduced the levels of tau aggregates.
Conclusions: The inverse relationship between proteasome activity and tau burden suggests tau aggregation could contribute to impairment of the UPS. Reduced proteasome activity in AD brain and the insoluble nature of tau fibrils may explain why the tested degraders were unable to lower tau aggregate levels. Studies to identify mechanisms where proteasome function may be enhanced to allow degradation of tau fibrils are on-going.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.01

Topic: D.06. Vision

Title: How does the cortical hand representation change following amputation? A pre- and post-amputation fMRI study

Authors: *H. SCHONE[1,2], M. KOLLAMKULAM[1], C. GERRAND[3], R. O. MAIMON MOR[1], C. I. BAKER[2], T. R. MAKIN[1];
[1]Inst. of Cognitive Neurosci., Univ. Col. London, London, United Kingdom; [2]Lab. of Brain and Cognition, NIH, Bethesda, MD; [3]Sarcoma Unit, The Royal Natl. Orthopaedic Hosp. NHS Trust, Stanmore, United Kingdom

Abstract: Sensorimotor experiences throughout our lifespan are thought to shape the neural representation of the body. What happens to the adult brain when it loses a key source of input, for example, following the amputation of an arm? Recent research has demonstrated that despite decades of input loss, and presumed mechanisms for cortical plasticity, the sensorimotor system of amputees preserves the representation of a missing hand. Does this persistent hand representation reflect a canonical representational structure, e.g. due to local network homeostatic constraints, which is stable independently of experience (e.g. innate)? Or does the preserved representational structure reflect a lifetime of sensorimotor experiences with the missing hand? Prior cross-sectional designs addressing this question conflate within- and between-subject variability with respect to the missing hand representation. Here, we longitudinally investigated the stability of the hand representation, before and after hand amputation. Using functional MRI, we interrogated the representational structure underlying activity elicited by real hand movements (pre-amputation) and phantom hand movements (post-amputation). Over a 7-year period and across 10 UK clinic sites, we recruited 8 patients preparing to undergo hand amputations. Due to a multitude of factors (e.g., complications during surgery, MRI safety contraindications, no hand motor control, poor physical mobility etc.), we successfully managed to complete testing on 1 patient with a planned unilateral hand amputation to remove a soft-tissue sarcoma on the right forearm. The patient was scanned twice pre-

amputation surgery and at two separate time-points post-amputation: 3 months and 6 months. Additionally, we scanned 15 age-matched able-bodied control participants across the same timescale (60 scans in total). Using both mapping of digit topography and representational similarity analysis, we show a remarkably consistent inter-digit representational structure for the pre-amputation hand and the post-amputation phantom (missing) hand. Overall, this work provides the first pre- and post-amputation longitudinal evidence for preserved representation of the phantom (missing) hand following amputation.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.02

Topic: D.06. Vision

Support: NS095251

Title: Tactile sensations evoked by brain-machine interfaces are stable over time

Authors: *S. BENSMAIA[1], M. ORTIZ-CATALAN[2], E. MASTINU[2], C. M. GREENSPON[3];
[1]Univ. of Chicago, Chicago, IL; [2]Chalmers Univ. of Technol., Gothenburg, Sweden; [3]Dept. of Organismal Biol. & Anat., Chicago Univ., Chicago, IL

Abstract: Electrical stimulation of tactile nerves in amputated limbs or of somatosensory cortex in subjects with spinal cord injuries both evoke vivid natural sensations reminiscent of those produced by a sensate hand - a phenomenon that can be leveraged to convey tactile feedback through bionic hands. Importantly, stimulation of a given nerve or location produces a distinct and repeatable sensation that subjects can localize to specific parts of the hand, consistent with well established somatotopy. Given that sensory input to cortex is absent or substantially reduced, the extent to which the cortical somatotopy remains stable over time is unclear. One hypothesis is that the lack of input can result in a change in the somatotopic map of the somatosensory cortex. In a case where a bionic limb in sensitized but the locations of the sensors do not match the initial percepts caused by stimulation, one might expect cortex to remap the percepts to the location of the sensors on the limb. An alternative hypotheses is that the somatotopic map is stable and that remapping will not occur nor will the somatotopy degrade over time. To address this question, we examined both amputees and patients with spinal cord injuries and characterized their sensory experiences in response to repeated electrical stimulation over years. In the case of the amputees, we found that after long-term use of a neuromusculoskeletal prosthesis that featured a mismatch between the sensor location and the resulting tactile experience, the perceived location of the touch did not change. Furthermore, we

found that in the patients with spinal cord injuries that the locations of the evoked percepts were stable over time and that the somatotopy within cortex was robust after injury.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.03

Topic: D.06. Vision

Title: Learning alters neural activity to simultaneously support memory and action

Authors: *D. LOSEY[1,2,3], J. HENNIG[1,2,3], E. R. OBY[7,8], M. D. GOLUB[12,2,4], P. T. SADTLER[7,8], K. M. QUICK[8,7], S. RYU[13,14], E. C. TYLER-KABARA[15,9,10,7], A. P. BATISTA[11,7], B. M. YU[1,2,5,6], S. M. CHASE[1,2,6];
[1]Neurosci. Inst., [2]Ctr. for the Neural Basis of Cognition, [3]Machine Learning Dept., [4]Dept. of Electrical and Computer Engin., [5]Dept. of Electrical Engin., [6]Dept. of Biomed. Engin., Carnegie Mellon Univ., Pittsburgh, PA; [7]Ctr. for the Neural Basis of Cognition, [8]Dept. of Bioengineering, [9]Dept. of Neurolog. Surgery, [10]Dept. of Physical Med. and Rehabilitation,, [11]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [12]Dept. of Electrical Engin., Stanford, Palo Alto, CA; [13]Dept. of Neurosurg., Palo Alto Med. Fndn., Palo Alto, CA; [14]Dept. of Electrical Engin., Stanford Univ., Stanford, CA; [15]Neurosurg., Dell Children's Med. Ctr., Austin, TX

Abstract: Suppose an experienced skier learns to snowboard. Skiing and snowboarding require different sets of muscle activations, driven by different neural population activity patterns, to achieve the same goal of getting down the mountain without falling. How does the brain

incorporate the knowledge about how to snowboard without disrupting the knowledge about how to ski? More generally, how are we able to learn new motor behaviors without forgetting previously learned ones?

We found that the neural activity to perform the same behavior is different before versus after the learning experience. Specifically, the neural activity when performing the familiar behavior remained appropriate for the newly learned behaviors.

It is typically difficult to ascertain how appropriate any given neural activity pattern is for different behavioral tasks. To overcome this challenge, we used a brain-computer interface (BCI) learning paradigm, where the relationship (i.e., BCI map) between neural activity and behavior is known. We trained three monkeys to perform a cursor control task using a BCI. We used two different BCI maps in each experimental session. Much like the example of an experienced skier learning to snowboard, a monkey first controlled a computer cursor using a familiar Map A, and then learned how to use a new Map B. Following learning, we reinstated Map A. This allowed us to evaluate whether monkeys used different population activity patterns to control Map A before versus after learning Map B.

We found that the neural activity post-learning was consistent with better performance through Map B than was the pre-learning activity, even though the monkey was controlling the cursor with the Map A. That is, learning left a "memory trace." This memory trace coexisted with proficient performance under the Map A. This was achieved by primarily altering dimensions of neural activity that did not impact behavior under the Map A. This allowed neural activity to simultaneously support a memory of Map B and the required actions through Map A.

Overall, our results reveal that learning can leave a memory trace in neural population activity that need not interfere with the subsequent behavior. The formation of a memory trace may thus provide a mechanism to facilitate the learning of multiple motor skills without interference, instantaneous switching between tasks, and rapid relearning of motor behaviors ("savings").

Disclosures: D. Losey: None. **J. Hennig:** None. **E.R. Oby:** None. **M.D. Golub:** None. **P.T. Sadtler:** None. **K.M. Quick:** None. **S. Ryu:** None. **E.C. Tyler-Kabara:** None. **A.P. Batista:** None. **B.M. Yu:** None. **S.M. Chase:** None.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.04

Topic: D.06. Vision

Title: Experience-dependent metamodal coupling of vibrotactile and auditory speech processing systems through matched stimulus representations

Authors: S. R. DAMERA[1], P. S. MALONE[1], B. W. STEVENS[1], R. L. KLEIN[1], S. P. EBERHARDT[2], E. AUER, Jr.[2], L. E. BERNSTEIN[2], ***M. RIESENHUBER**[1];
[1]Dept. of Neurosci., Georgetown Univ. Med. Ctr., Washington, DC; [2]Dept. of Speech, Language, and Hearing Sci., George Washington Univ., Washington, DC

Abstract: It has been postulated that the brain is organized by "metamodal", sensory-independent cortical modules implementing particular computations, leading to the intriguing hypothesis that brain areas can learn to perform tasks (such as word recognition) not just in "standard" sensory modalities but also in novel sensory modalities. Yet, evidence for this theory, especially in neurotypical subjects, has been variable. We hypothesized that effective metamodal engagement of a brain area requires congruence between the novel and standard sensory modalities not only at the task level (e.g., "word recognition") but critically also a match at the algorithmic level (in Marr's terminology), i.e., at the level of neural representation of the information of interest. To test this hypothesis, we trained participants (N=20) to recognize vibrotactile versions of auditory words using two encoding schemes. The vocoded approach preserved the dynamics and representational similarities of auditory speech while the token-based approach used an abstract phoneme-based code. Although both groups learned the vibrotactile word recognition task, only in the vocoded group did trained-vibrotactile stimuli recruit the auditory speech network and lead to increased coupling between somatosensory and auditory speech areas. In contrast, the token-based encoding appeared to rely on paired-associate learning. Thus, matching neural input representations is a critical factor for assessing and leveraging the metamodal potential of cortical modules. Our study not only critically advances our understanding of metamodal engagement and general principles of brain organization, but also opens the door to designing more efficient sensory substitution algorithms that better interface with existing cortical processing pathways. The ability to "piggyback" onto an existing processing hierarchy (e.g., auditory speech recognition) may facilitate the rapid learning of novel stimuli presented through a spared sensory modality (e.g., vibrotactile processing). Here we demonstrate that an algorithm (vocoding) that improves this interfacing is able to more efficiently convey the same information than an algorithm (token) that does not. Future work should explore whether this observed integration into existing processing streams leads to improved generalization and transfer of learning.

Disclosures: S.R. Damera: None. **P.S. Malone:** None. **B.W. Stevens:** None. **R.L. Klein:** None. **S.P. Eberhardt:** None. **E. Auer:** None. **L.E. Bernstein:** None. **M. Riesenhuber:** None.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.05

Topic: D.06. Vision

Title: A case for an effector-independent, action-type organization for action execution

Authors: *F. MARTINEZ ADDIEGO[1], Y. LIU[1,2], C. O'BRIEN[1], S. SEN[1], N. KHALSA[1], M. RIESENHUBER[1], J. CULHAM[3,4], E. STRIEM-AMIT[1];
[1]Dept. of Neurosci., Georgetown Univ., Washington, DC; [2]Inst. of Neurosci., CAS Ctr. for Excellence in Brain Sci. and Intelligence Technol., Shanghai, China; [4]Brain and Mind Inst., [3]Univ. of Western Ontario, London, ON, Canada

Abstract: The sensorimotor cortex has been shown to be organized somatotopically: specific body parts correspond to distinct areas. However, it is not clear to what extent the sensorimotor system is also organized by functional principles, such as action types. One way to study action-type organization is to determine whether the same action will have the same representation even when performed by different body parts (effectors). We leveraged functional neuroimaging in a group of typically developed controls and several individuals born without hands to map action representation in the brain. fMRI data were collected while participants completed actions such as writing letters, drawing shapes, and spatula-use with their dominant right hand or foot. Preliminary analyses of control subjects (n=12), reveal a similar preference for the execution of different tool-use actions across the hand and foot in the premotor cortex (PMd) and supplementary motor area (SMA). This suggests that some areas of the sensorimotor cortex may have an effector-independent organization for specific actions and that their organization is not only topographical.

Disclosures: F. Martinez Addiego: None. **Y. Liu:** None. **C. O'Brien:** None. **S. Sen:** None. **N. Khalsa:** None. **M. Riesenhuber:** None. **J. Culham:** None. **E. Striem-Amit:** None.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.06

Topic: D.06. Vision

Support: ERC 773121 NovelExperieSense
          Horizon GuestXR 101017884

Title: Can we reopen critical periods in the adult human brain retinotopic areas?

Authors: *A. AMEDI, A. MAIMON, K. CIESLA, E. AGGIUS-VELLA;
Reichman Univ., Herzliya, Israel

Abstract: This talk will discuss principles driving specializations in the human brain and their dependence on specific experiences during development (i.e. critical/sensitive periods) versus learning in the adult brain. Specifically, I will focus on studying Nature vs. Nurture factors in shaping category selectivity in the ventral stream, dorsal stream and retinotopic and topographic specializations in the human brain. A wealth of research (ours and others) has amassed in the past decade showing that visual areas can develop their distinct specializations in the absence of visual experience. While this was shown repeatedly with respect to ventral visual stream areas,

our research now indicates that area V6, part of the dorsal visual stream, can be specialized for navigation even in the absence of visual experience. Furthermore it can develop specialization for non-visual input in just a few days in the adult brain. These findings together indicate that the functioning and development of the brain is task specific and sensory independent (and not sensory specific as commonly accepted).

Disclosures: A. Amedi: None. **A. Maimon:** None. **K. Ciesla:** None. **E. Aggius-Vella:** None.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.07

Topic: D.06. Vision

Title: Kinematic adaptations to early loss of vision in the short-tailed opossum (Monodelphis domestica) in a skilled reaching and grasping task

Authors: *C. R. PINEDA, L. A. KRUBITZER;
Psychology, Univ. of California Davis, Davis, CA

Abstract: Early sensory input dramatically impacts neocortical organization and behavior. In humans, congenitally blind adults adopt new strategies to navigate and acquire new means for written communication such as Braille reading. These behaviors are supported by the spared senses, which blind adults use in new ways to generate adaptive behavior. Despite the importance of these compensatory behaviors, such sensory-mediated compensatory strategies are not systematically studied. Studies in our laboratory in short-tailed opossums (*Monodelphis domestica)* that are bilaterally enucleated at post-natal day 4 (EB), before the formation of retinogeniculate and thalamocortical pathways, have allowed us to quantify the performance of several behaviors mediated by the spared sensory systems. For example, EB opossums have lower texture discrimination thresholds, and lower error rates in complex navigation tasks, which are accompanied by postural differences when compared to sighted opossums (SC) (Englund et al., 2020; Rammamurthy et al., 2021). To examine how EB opossums compensate for blindness in an ethological relevant context, we trained EB and SC opossums in a skilled reaching task. Animals were recorded with 3 video cameras positioned in stereo which allowed us to extract pose kinematics in three dimensions using DeepLabCut, a deep learning algorithm (Mathis et al., 2018). Results from tests under light and dark conditions show that EB opossums reach more accurately compared to SC animals irrespective of the lighting condition. Higher performance accuracy is accompanied by several displacement and kinematic differences. The average maximum vertical speed of the forepaw during the late reach extension epoch is significantly higher in EB opossums than in SC animals. In contrast, the horizontal speed during the early extension epoch of EB opossums is significantly lower than of SC opossums. The difference in

kinematic measures at early and late epochs of the reach extension underlines strategic differences that allow EB opossums to target their reach more accurately. Interestingly, the peak snout elevation of EB opossums is higher than that of SC animals during early extension, potentially indicating that early blind opossums have expanded their exploration of their peripersonal space. We are currently removing input from the remaining senses by cutting the whiskers and inducing anosmia to investigate their relative contribution to compensatory behaviors of EB opossums. Determining how EB opossums use the spared senses to compensate for the lack of vision allows us to see the extent to which the body and the brain adjust to sensory deficits over a lifetime.

Disclosures: C.R. Pineda: None. **L.A. Krubitzer:** None.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.08

Topic: D.06. Vision

Support: DFG Ro 2625/10-1
SFB 936-B1
Hector Fellow Academy

Title: Persistent impairment of Excitatory/Inhibitory balance in the human visual cortex after recovery from congenital patterned visual deprivation

Authors: *R. PANT[1], K. PITCHAIMUTHU[1], J. P. OSSANDON[1], I. SHAREEF[2], S. LINGAREDDY[3], J. FINSTERBUSCH[4], R. KEKUNNAYA[5], B. ROEDER[1];
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Abstract: Excitatory/Inhibitory (E/I) balance in the visual cortex has been shown to be altered after a period of visual deprivation in a wide range of non-human animal models (monocular, binocular deprivation), using different methods (TTX, dark rearing, lid suture) and species. In such studies, altered E/I balance after a period of visual deprivation has been interpreted as evidence of the role of E/I balance in critical period plasticity. However, persistently impaired E/I balance corresponding to disrupted visual experience has not yet been demonstrated in humans. In the present study, we obtained a non-invasive measure of visual cortex E/I balance in 10 individuals who recovered from dense bilateral congenital cataracts (CC), and compared them to 10 age-matched sighted control individuals (SC). CC individuals were deprived of vision for an average of 11.8 years (range = 0.2 to 31.36 years) and tested at least 1 year after cataract removal surgery. We used 3T Magnetic Resonance Spectroscopy to obtain visual cortex Gamma-Aminobutyric Acid (GABA) concentration, Glutamate/Glutamine (Glx) concentration,

and the concentration ratio of Glx/GABA as measures of inhibitory activity, excitatory activity, and E/I balance respectively. Participants were scanned while at rest with their eyes opened (EO) or closed (EC) using the MEGA-PRESS sequence with a 40 x 30 x 25 mm voxel in the visual cortex, and a control voxel in the frontal cortex. Overall, water-normalized Glx and GABA concentrations did not differ between CC and SC individuals' visual cortices. However, CC individuals demonstrated a significantly lower visual cortex Glx/GABA ratio than SC individuals, across EO and EC conditions. In the frontal cortex, GABA, Glx and Glx/GABA concentration measures did not differ between the two groups, suggesting that lowered Glx/GABA concentration in the CC group was specific to visual cortex. Further, N-Acetyl Aspartate concentrations remained stable across all groups and conditions in both visual and frontal cortex, demonstrating that neurochemical changes due to delayed patterned visual experience were specific to Glx/GABA. These results suggest that a period of congenital visual deprivation impairs E/I balance in the human visual cortex, even up to 30 years after sight restoration.

Disclosures: R. Pant: None. **K. Pitchaimuthu:** None. **J.P. Ossandon:** None. **I. Shareef:** None. **S. Lingareddy:** Other; LUCID Diagnostics. **J. Finsterbusch:** None. **R. Kekunnaya:** None. **B. Roeder:** None.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.09

Topic: D.06. Vision

Title: The retinotopic tuning and related structural development of early visual areas depend on early visual experience

Authors: *C. BROCKHAUS[1], M. ZHAN[2], M. LINKE[1], C. HÖLIG[1], R. KEKUNNAYA[3], R. VAN HOOF[4], R. GOEBEL[4], B. RÖDER[1];
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Abstract: The mapping of the visual field onto the early visual cortex follows well-known principles which have been demonstrated both in invasive electrophysiological studies in non-human animals as well as in brain imaging studies in humans. On a fine-scale level, increases in population receptive field (pRF) size, the area in visual space eliciting a response in a population of neurons, are observed with eccentricity and from lower- to higher-level visual areas.

Furthermore, cortical magnification factor (CMF), the cortical space devoted to a location in the visual field, is highest at foveal locations and decreases with eccentricity. Both measures have shown systematic associations with visual acuity and V1 surface area, suggesting a tight link of function and structure. While large-scale retinotopic organization principles have been shown to evolve independently of visual experience, it has been unclear whether this holds true for the fine-scale organization within and across visual areas. In the present study, we acquired 7T fMRI data and performed pRF mapping (Dumoulin & Wandell, 2008) to assess the long-lasting effects of a transient phase of congenital blindness on retinotopic organization. We recruited eight individuals who had been born with dense bilateral cataract, which was removed on average with 21 months (range: 6 - 48 months), and eight age-and gender-matched normally sighted controls. Early visual regions (i.e., V1, V2, V3) were defined by a visual functional atlas. Results in sighted control participants confirmed known retinotopic organization principles: pRF size increased with increasing eccentricity as well as from lower- to higher-level visual regions and CMF decreased with eccentricity. Notably, neither the effect of eccentricity nor of visual region were observed on pRF size in the cataract reversal individuals. Furthermore, while a decrease in CMF with eccentricity was observed in cataract reversal individuals, their CMF was overall smaller and the decrease towards parafoveal regions was less steep than in sighted control individuals. Finally, V1 surface area was smaller in cataract reversal individuals and was strongly positively associated with the average cortical magnification factor across all participants. In addition, better visual acuity was associated with smaller pRF sizes and larger CMF. The present results extend previous research demonstrating that a prototypical retinotopic organization possibly existing at birth needs early visual input to become structurally and functionally refined in order to allow for the integration of visual information across early visual areas for adult visual capabilities.

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Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.10

Topic: D.06. Vision

Title: Plasticity of higher-order visual cortex following large cortical resections

Authors: *T. T. LIU[1], M. C. GRANOVETTER[2], A. S. MAALLO[2], J. Z. FU[1], C. PATTERSON[3], M. BEHRMANN[2];

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Abstract: The visual word form area (VWFA), typically located in the left ventral occipitotemporal cortex (VOTC), emerges during reading acquisition and has privileged connectivity with language regions such as the inferior frontal gyrus (IFG, Broca) and the posterior superior temporal gyrus (pSTG, Wernicke). Small lesions in the VWFA in adults result in an impairment, pure alexia, indicating that this area is necessary for word reading. Paradoxically, in children, large cortical resections that include the left VOTC for the treatment for pharmaco-resistant epilepsy, do not necessarily lead to reading impairments. To understand the neural and behavioral consequences, and the ensuing plasticity, of resections encompassing the left or right, anterior or posterior, VOTC, we mapped category-selective activations (face, scene, object, and word) in four right-handed pediatric patients and 26 age-matched controls using fMRI, and tested their intermediate and high-level vision. We report plasticity of word processing in two patients (SN: M, 12y; TC: F, 13-15y) with cortical resections encompassing the left posterior VOTC: word activations were identified in the right VWFA, right IFG and right pSTG in both patients. In addition, we found an unusual coupling of visual word processing in the right hemisphere and spoken language in the left hemisphere of TC as her presurgical clinical fMRI showed left hemisphere language dominance (left IFG and left pSTG). These findings of atypical hemispheric lateralization for words are in sharp contrast with the topography of a left-lateralized word-processing network in two other patients with resections in the left anterior VOTC (OT: M, 14y-18y) or in the right posterior VOTC (UD: M, 7-10y) and age-matched controls. Parallel to their functional reorganization of the word-processing network, we uncovered atypical underlying representational structure of the category-selective organization in patients SN and TC. Furthermore, in longitudinal comparisons, enhanced competition between face and word representations was observed in the intact left VOTC in patient UD and in the intact right VOTC in patient TC, possibly due to a strong foveal bias for face and word processing, which requires high-resolution vision to discriminate between many visually confusable and homogeneous exemplars. Finally, normal intermediate and higher-order perception was evident in all four patients, attesting to functional plasticity in visual cortex. Together, these findings reveal the sufficiency and reorganization of preserved cortex for word processing and provide insights into dynamic functional changes in extrastriate cortical architecture.

Disclosures: T.T. Liu: None. **M.C. Granovetter:** None. **A.S. Maallo:** None. **J.Z. Fu:** None. **C. Patterson:** None. **M. Behrmann:** None.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

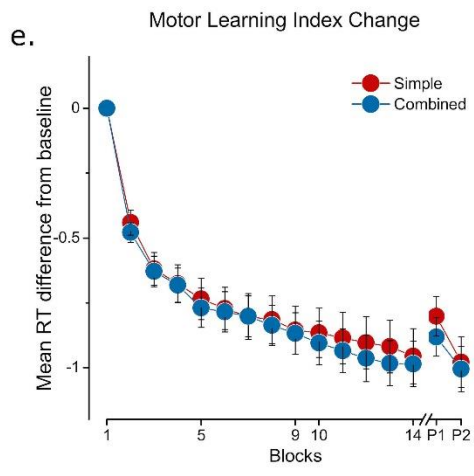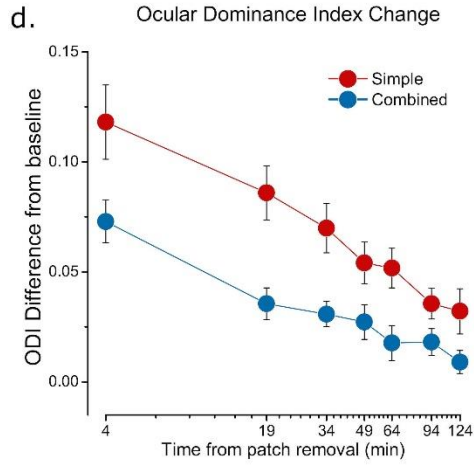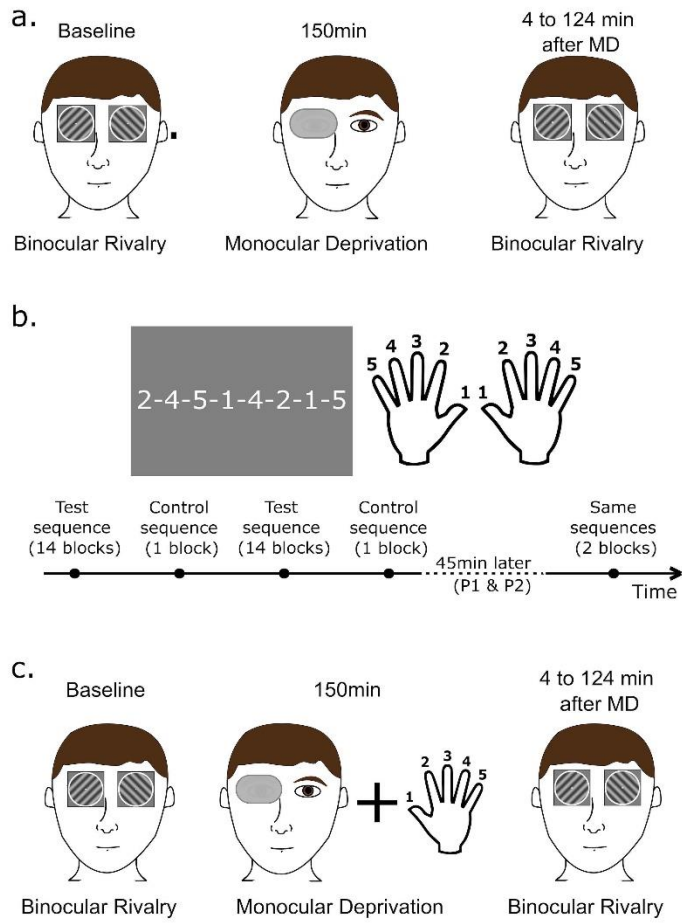**Time:** Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.11

Topic: D.06. Vision

Title: Interaction of visual and motor plasticity in adult humans

Authors: *I. SARI, C. LUNGHI;
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Abstract: In adults, sensory cortices become more or less hardwired in contrast to the motor cortex which remains highly plastic throughout the lifespan. However, recent studies have shown that the adult visual cortex retains a higher degree of homeostatic plasticity than formerly thought, as a short period (2-2.5 hours) of monocular deprivation (MD) shifts ocular dominance in favor of the deprived eye in adult humans (Lunghi et al. 2011, 2013; Zhou et al. 2013; Binda et al. 2018). There is also evidence that both visual and motor plasticity rely on the regulation of GABAergic inhibition (Lunghi, et al. 2015; Stagg et al. 2011). Yet, the direct interaction of visual and motor plasticity has not been tested in adult humans. Here we tackle this issue by measuring visual and motor plasticity either in isolation (simple) or at the same time (combined) in a group of adult volunteers (N= 31). We assessed visual plasticity as the ocular dominance change measured by binocular rivalry after short-term MD (150 min, Fig 1a). Motor plasticity was measured by the change in reaction times after a motor sequence learning task (Fig 1b). In the combined condition the visual and motor tasks were performed simultaneously (Fig 1c). We found that inducing visual and motor plasticity at the same time impairs visual plasticity ($p < 0.01$, $\eta^2_p = 0.32$, Fig 1d), while motor plasticity is spared ($p > 0.05$, $\eta^2_p < 0.1$, Fig 1e). With two control experiments, each on a group of 10 participants, we eliminated the amount of visual stimulation ($p > 0.05$, $\eta^2_p = 0.19$) and the working memory or attentional load ($p > 0.05$, $\eta^2_p = 0.08$) during MD to be the main contributors to this effect. Altogether, our results indicate that there is a unilateral interaction between visual and motor plasticity in adults, in line with previous evidence that plasticity induced by a stroke in the motor cortex impairs ocular dominance plasticity in rodents (Greifzu et al. 2011, Pielecka-Fortuna et al. 2015). This influence of motor on visual plasticity might reflect a global homeostatic mechanism regulating the Excitation/Inhibition balance changes in the entire cortex.

Title: Highly efficient modulation of the innate behavior via function specific corticofugal
projection

Authors: *J. LIU[1], Y. HE[1], G. BOUVIER[2], B. LIU[1];
[1]Cell and Systems Biol. ; Biol., Univ. of Toronto, Mississauga, Mississauga, ON, Canada; [2]Dept. of Physiol., Univ. of California, San Francisco, San Francisco, CA

Abstract: The visual cortex sends massive corticofugal projection to the brainstem, by which this cortical area can modulate the brainstem-mediated functions including innate visual behaviors. The roles of corticofugal projection have been actively investigated in past years. We recently reported that the corticofugal projection is essential in the plasticity of the optokinetic reflex (OKR), an involuntary eye movement to stabilize retinal images. This plasticity allows the OKR to be adaptively modified in response to visual experience or relative to other oculo-motor reflexes in order to maintain image stability. Fascinatingly, the cortico-fugal projection from the visual cortex can enhance its innervation to the brainstem OKR circuit, as a result potentiating the OKR behavior. Despite the importance of the cortico-fugal projection in OKR plasticity, the underlying mechanisms remain unclear. In this study, we used an interdisciplinary approach to examine the response properties and connectivity of the cortico-fugal neurons in the visual cortex which project to the brainstem OKR circuit. First, with two-photon calcium imaging we found that those cortico-fugal neurons shared a unique temporo-nasal direction bias with their postsynaptic targets in the brainstem. Next, we compared the calcium responses of the same cortico-fugal neurons before and after the induction of OKR potentiation. Interestingly, following OKR potentiation the activity of the neurons that bias temporo-nasal visual motion was boosted much more strongly than neurons that bias other directions. These functional specific cortico-fugal neurons with temporo-nasal bias enable the visual cortex to efficiently enhance its input to the NOT-DTN in support of OKR potentiation. Last, with circuit tracing and activity perturbation we found that the corticofugal projection to the brainstem OKR circuit primarily originated from anterior V1 and posterior higher visual areas, and it synapsed on a single brainstem population. Altogether, our results provide strong evidence that this corticofugal projection and its downstream target in the brainstem form a functionally distinct pathway which specializes in adaptively modulating the OKR.

Disclosures: J. Liu: None. **Y. He:** None. **G. Bouvier:** None. **B. Liu:** None.

Nanosymposium

428. Experience-Dependent Plasticity: Gaining Insights Into the Neural Capacity to Adapt

Location: SDCC 5

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 428.13

Topic: D.06. Vision

Support: Wellcome grant HMR01890

Title: Exercise induced structural brain plasticity is mostly transient

Authors: *C. BRATLEY[1], C. LIGNEUL[1], M. KALLER[1], M. TACHROUNT[1], H. JOHANSEN-BERG[2], J. LERCH[1];
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Abstract: Introduction: Multiple studies have shown that the brain is plastic even at the mesoscopic resolution seen by MRI (Draganski et al. 2004; Scholz et al. 2009)). We have been able to replicate these results in the mouse using high-field MRI thereby enabling tighter experimental control and mechanistic studies (Scholz et al. 2015; Lerch et al. 2011; Cahill et al. 2015). Yet unresolved is the persistence of this type of plasticity. We set out to answer this question by giving mice access to a running wheel for 2 weeks while taking high-resolution MRI scans. Methods: 8 C57BL6 male and female adult mice were singly housed. Four received the running wheel for 2 weeks before returning to standard caging and 4 were kept in standard caging throughout. Mice were scanned before, during and after exercise. Mice were injected with a contrast agent (manganese chloride 0.4mmol/kg) 24 hours prior to scanning. We used a high-resolution MRI at 7T (MGE, 3 echoes, 60μm isotropic). After acquisition the 3 echoes from the MGE were averaged and denoised (Manjón et al. 2010). Images were further processed using the pydpiper framework (Friedel et al. 2014) and segmented with MAGeT (Chakravarty et al. 2013). Volumes were analyzed after normalizing to baseline volume. Results: As expected based on prior studies (Cahill et al. 2015) the hippocampus had the most robust response in local volume to exercise, increasing by 4% in CA1-3 and 8% in the dentate gyrus. These changes were transient; after 1 week without exercise the volume increase compared to controls was just 2%. These differences were no longer statistically significant. Discussion: Exercise induces increases in hippocampal volume yet these increases quickly reduce to baseline. The study is ongoing and as mouse numbers increase we will be able to determine whether there is a residual volume change even weeks after exercise, yet it is evident from the data to date that dominant signal in brain volume is transient. This largely matches what has been shown at the cellular level, with dendritic spines, for example, only increasing transiently (Xu et al. 2009)
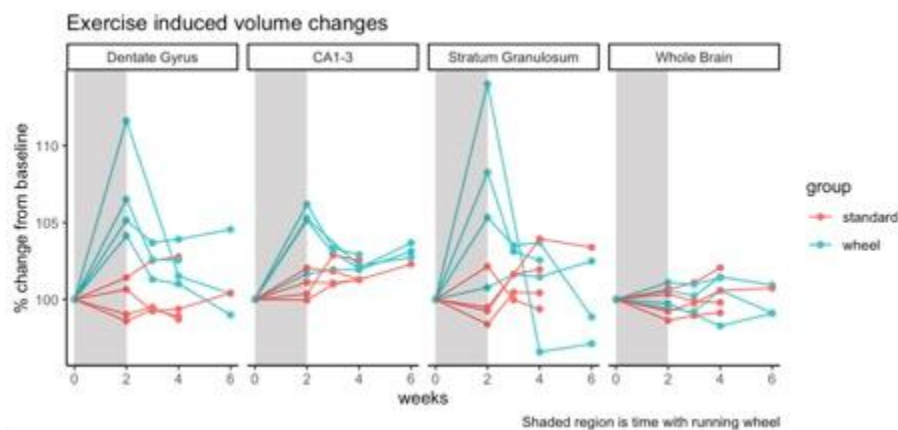
Disclosures: C. Bratley: None. **C. Ligneul:** None. **M. Kaller:** None. **M. Tachrount:** None. **H. Johansen-Berg:** None. **J. Lerch:** None.

Nanosymposium

429. Olfactory Higher Order Processing and Perception

Location: SDCC 23

Time: Tuesday, November 15, 2022, 8:00 AM - 10:15 AM

Presentation Number: 429.01

Topic: D.04. The Chemical Senses

Support: U19NS112953
U19NS107464

Title: The role of the olfactory bulb network for defining a temporal window of concentration invariant odor identity

Authors: *M. KARADAS[1], J. V. GILL[1], S. CEBALLO[1], S. SHOHAM[1,2,3], D. RINBERG[1,4,5];
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Abstract: Odors at different concentrations evoke distinct patterns of neural activity both at the level of olfactory sensory neurons (OSN) and glomeruli, as well as the next level, mitral/tufted (MT) cells. What features of these patterns carry information about odor identity and what are the neural mechanisms defining these features? To approach this problem we designed an all-optical system to monitor the activity of a large number of glomeruli and MT cells with high temporal resolution, and developed a method for establishing functional connectivity between glomeruli and MT cells. We combined two photon Ca2+ imaging with one photon patterned optogenetics in mice expressing a fast calcium indicator (GCaMP6f) in glomeruli and M/T cells, and a light-sensitive opsin (ChR2) in OSNs. We found that the earliest activity of glomeruli and MT cells most reliably represented odor identity across concentrations. To establish the network mechanisms shaping MT cell odor responses, we identified MT cells connected to specific glomeruli using optogenetic probing. We found that MT cells connected to early activated glomeruli exhibited stereotypic excitatory responses following their parent glomeruli. At the same time, the odor responses of MT cells connected to later activated glomeruli were strongly affected by the inhibitory network evoked by earlier activated glomeruli. We probed the responsiveness of MT cells to glomerulus activation using a short optogenetic pulse in the presence of odor stimuli, and found that MT cells connected to later activated glomeruli could effectively transmit a glomerulus signal to the cortex only in a short temporal window at the beginning of the sniff cycle. Beyond this window, MT cell responses were strongly suppressed by inhibition evoked by the presented odor. These findings provide evidence for the previously proposed primacy coding model of temporally-dependent concentration invariant odor coding, and reveal potential mechanisms supporting this code.

Disclosures: M. Karadas: None. **J.V. Gill:** None. **S. Ceballo:** None. **S. Shoham:** None. **D. Rinberg:** None.

Nanosymposium

429. Olfactory Higher Order Processing and Perception

Location: SDCC 23

Time: Tuesday, November 15, 2022, 8:00 AM - 10:15 AM

Presentation Number: 429.02

Topic: D.04. The Chemical Senses

Title: What determines selective vulnerability and circuit integration of olfactory bulb dopaminergic neurons?

Authors: *T. KUNKHYEN, A. A. LAUER, T. R. BRECHBILL, A. N. RANGEL, C. E. J. CHEETHAM;
Univ. of Pittsburgh, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Selective loss of specific sub-populations of neurons is a hallmark of neurodegenerative disease. Around 40% of dopaminergic (DA) neurons in the mouse olfactory bulb (OB) undergo cell death after a month of sensory input blockade but the remaining 60% are resilient. Furthermore, OB DA neurons are continuously generated throughout life, enabling them to fully repopulate after reversal of naris occlusion. OB DA neurons therefore provide an ideal model system in which to understand selective vulnerability and how newborn neurons can functionally integrate to replace previously lost neurons. Systemically injecting the olfactotoxin methimazole enables us to see the impact of rapid elimination followed by gradual restoration of sensory input to the OB. We used chronic *in vivo* 2-photon imaging in DAT-cre;Ai9;Ai162 mice that express both a red fluorescent protein and a green genetically encoded calcium indicator to track the survival and integration, as well as the odor response properties, of individual DA neurons over weeks. We found that loss of DA neurons was significantly elevated during the first week after methimazole treatment but then returned to baseline. We are analyzing the vulnerability of the previously described large embryonically generated vs. small postnatally generated DA neurons and whether there are differences in the odor response characteristics between neurons that are vulnerable or resilient to loss of sensory input. We are also quantifying the rate of newborn DA neuron integration before and after sensory disruption. Determining what distinguishes vulnerable neurons from their resilient neighbors and whether stem cell-derived neurons can restore circuit function may inform novel targeted therapeutic strategies for the treatment of neurodegenerative diseases.

Disclosures: T. Kunkhyen: None. **A.A. Lauer:** None. **T.R. Brechbill:** None. **A.N. Rangel:** None. **C.E.J. Cheetham:** None.

Nanosymposium

429. Olfactory Higher Order Processing and Perception

Location: SDCC 23

Time: Tuesday, November 15, 2022, 8:00 AM - 10:15 AM

Presentation Number: 429.03

Topic: D.04. The Chemical Senses

Title: Object representation in the piriform cortex

Authors: *P. HERRERO-VIDAL[1,2], E. CHONG[3], C. SAVIN[1], D. RINBERG[2,1];
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Abstract: The piriform cortex (PCx) is believed to play a key role in creating meaningful perceptual representations of odors, but how it encodes them remains unclear. We approached this question by studying PCx responses to "synthetic odors", which we generated by optogenetically activating spatiotemporal patterns of activity in the olfactory bulb (OB). This enables independent and precise control of the neural activity patterns that drive PCx, something unattainable with natural odorants. We record PCx responses in naive mice and measure how they change in response to parametric perturbations of OB activity. We find that PCx neurons are sensitive to spatial and temporal aspects of OB activity, with PCx responses affected more strongly when perturbing earlier elements in the OB sequence. We use decoding to assess the behavioral relevance of PCx responses by comparing neural predictions to behavioral outputs in trained animals. For behavior, we trained animals to discriminate a target (T) from many non-target (nT) patterns (with the same number of activated spots in the same temporal window). For neural decoding, using the same T vs nT task, we found that a linear discriminant analysis of full spatiotemporal patterns of PCx responses in naïve animals performs very similar to behavior. Furthermore, we compared the generalization to various perturbations of the T pattern both in the behavioral task and the neural recordings. Remarkably, for all perturbations, the neural predictions closely match the perceptual changes seen in behavior. We proposed that PCx neural representation of synthetic odors lies on a smooth convex manifold. On this manifold, identification of any one object can be performed as linear discrimination of one pattern versus many other patterns. Importantly, the local geometry of this representation explains the behavioral generalization performance. The fact that PCx responses to perturbations in naive animals can reproduce behavioral reports in trained animals suggests that we are tapping into the intrinsic computations that make PCx a key circuit for the perception of odors.

Disclosures: P. Herrero-Vidal: None. **E. Chong:** None. **C. Savin:** None. **D. Rinberg:** None.

Nanosymposium

429. Olfactory Higher Order Processing and Perception

Location: SDCC 23

Time: Tuesday, November 15, 2022, 8:00 AM - 10:15 AM

Presentation Number: 429.04

Topic: D.04. The Chemical Senses

Title: Tuning, sequences and geometry of olfactory representations

Authors: *J. V. GILL[1], M. KARADAS[1], S. SHOHAM[2,1,3], D. RINBERG[1,4,5];
[1]Neurosci. Inst., [2]Tech4Health Inst., [3]Dept. of Ophthalmology, New York Univ. Langone Hlth., New York, NY; [4]Ctr. for Neural Sci., [5]Dept. of Physics, New York Univ., New York, NY

Abstract: Animals depend on their senses for survival. Mice, who rely on olfaction to navigate the world, can rapidly identify odors within a single sniff across a wide range of concentrations. What circuit computations support this ability? In the mouse olfactory bulb, odor information is conveyed to the cortex through ~50,000 mitral and tufted cells (MTCs). MTCs differentially respond to odors by changing both the rate and timing of spikes relative to inhalation, resulting in reliable, odor specific sequences that evolve over a single sniff. Despite the consistency and ubiquity of these MTC sequences, how rate and timing-based odor encoding are organized and related has yet to be explored. To address this, we presented a large battery of odors at multiple concentrations to awake mice while recording odor evoked MTC responses using 2-photon calcium imaging. Importantly, we substantially improved the temporal resolution of MTC imaging by expressing the recently developed fast calcium indicator jGCaMP8f, allowing us to monitor the sub-sniff timing of MTC sequential responses. We found that single-sniff odor responses had a low-dimensional embedding which well-preserved the distances in odor tuning between neurons, suggesting that neural activity was constrained to a low-dimensional encoding subspace. Further, we found that the geometrical structure of these subspaces were preserved across mice, meaning that the relationships between odors and MTC tuning were consistent across animals and independent MTC populations. Next, we examined the relationship between the response magnitude, or 'tuning', of MTCs across odors, and the timing of their activation sequence for a given odor. We found that sequences propagated across the odor encoding subspace according to the distance in tuning between MTCs, with sequences originating in a set of similarly tuned neurons, then propagating to more distantly tuned neurons as a linear function of latency from inhalation. Lastly, we explored the temporal organization of responses to different concentrations of a given odor, finding that while the later MTC responses varied substantially across concentrations, the earliest responses in the sequences were consistent, defining a temporal window of concentration invariant odor coding. Our results shed new light on spatiotemporal neural encoding of olfactory information. Moreover, constructing and studying a computational model for sequence-based unsupervised training of synapses from MTCs to the piriform cortex reveals that sequential activity permits perceptual generalization for novel odors and the transmission of concentration invariant odor representations.

Disclosures: J.V. Gill: None. **M. Karadas:** None. **S. Shoham:** None. **D. Rinberg:** None.

Nanosymposium

429. Olfactory Higher Order Processing and Perception

Location: SDCC 23

Time: Tuesday, November 15, 2022, 8:00 AM - 10:15 AM

Presentation Number: 429.05

Topic: D.04. The Chemical Senses

Title: Investigating cortical readout of temporal codes for olfaction

Authors: *R. BLAZING[1], K. M. FRANKS[2];
[1]Duke Univ. Neurobio. Grad. Program, Durham, NC; [2]Neurobio., Duke Univ., Durham, NC

Abstract: Stereotyped temporal sequences of neural activity that correlate with features of the external world have been identified across a variety of neural circuits. These sequences, which generally last on the order of tens to hundreds of milliseconds, are thought to mediate essential cognitive processes including navigation, memory encoding and retrieval, and sensory discrimination. However, the extent to which the fine temporal structure of neural sequences impacts the activity of downstream reader circuits remains relatively unexplored. The rodent olfactory system presents an ideal model circuit in which to investigate the sensitivity of cortical circuits to precisely timed input sequences. In this system, odors activate stereotyped spatiotemporal sequences of olfactory bulb glomeruli that project to downstream piriform cortex (PCx). Whether and how the PCx reads out this finely-structured temporal input is unknown. Using targeted patterned optogenetic stimulation of glomeruli in mice while recording from large populations of PCx neurons, we have revealed that neural population responses in PCx are highly sensitive to the specific timing and order of sequences of glomerular inputs stimulated over the course of a single sniff cycle (~150 ms). We hypothesized that this temporal sensitivity may be conferred by intra-cortical inhibitory circuits, which have been shown to restrict the time window for summation of excitatory inputs onto PCx cells in-vitro. To test this hypothesis, we stimulated sequences of glomeruli while optogenetically suppressing PCx inhibitory interneurons. Surprisingly, our preliminary data suggest that decreasing cortical inhibition improves the network's temporal selectivity by increasing the gain of cortical excitatory responses. This suggests that sequence selectivity may instead be primarily computed in the OB, then relayed to downstream cortical networks. Together, our findings will provide novel insights into the computational principles and mechanisms that govern the encoding and decoding of precise neural sequences in cortical circuits.

Disclosures: R. Blazing: None. **K.M. Franks:** None.

Nanosymposium

429. Olfactory Higher Order Processing and Perception

Location: SDCC 23

Time: Tuesday, November 15, 2022, 8:00 AM - 10:15 AM

Presentation Number: 429.06

Topic: D.04. The Chemical Senses

Title: Binge eating suppresses flavor representations in the mouse olfactory cortex

**Authors: *H. LO**[1,3], A. SCHOENHERR[1], M. L. TANTIRIGAMA[2,4], M. E. LARKUM[4], B. JUDKEWITZ[1,2,3], K. STUMPENHORST[5], M. RIVALAN[6], Y. WINTER[5], D. SCHMITZ[1,3,2,7], F. JOHENNING[1,3];

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Abstract: Appropriate feeding behavior is the foundation of maintaining homeostasis. Elevated feeding rate (binge eating) is a common trait of eating disorders, and it is associated with obesity. It is also known that flavor perception has an active role in regulating feeding. However, the effects of feeding rate on flavor sensory feedback remain unknown. We developed a liquid food delivery system that mice can consume flavored milk with different feeding rates, e.g., slow eating mode (4-second interval) and binge eating mode (0.4-second interval). Using miniscope in mice, we show that binge eating suppresses neuronal activity in the anterior olfactory (piriform) cortex (aPC), while slow eating does not. The strength of binge-induced suppression in the aPC predicts animals' consumption and duration of feeding. This suppression is unlikely due to the activation of local GABAergic interneurons (PV[+] & SOM[+]) in the aPC. Odor inputs from olfactory bulb mitral cells remain stable upon binge eating, suggesting the suppression is not due to degraded odor inputs. We further excluded the inhibitory effect from serotonergic modulation in the aPC by using *in vivo* serotonin imaging. Taken together, our results provide clear circuit mechanisms of binge-induced flavor modulation, which may contribute to binge-induced overeating due to reduced sensory feedback of food items.

Binge eating suppresses flavor representations in the mouse olfactory cortex (piriform cortex) — Lo et al., 2022
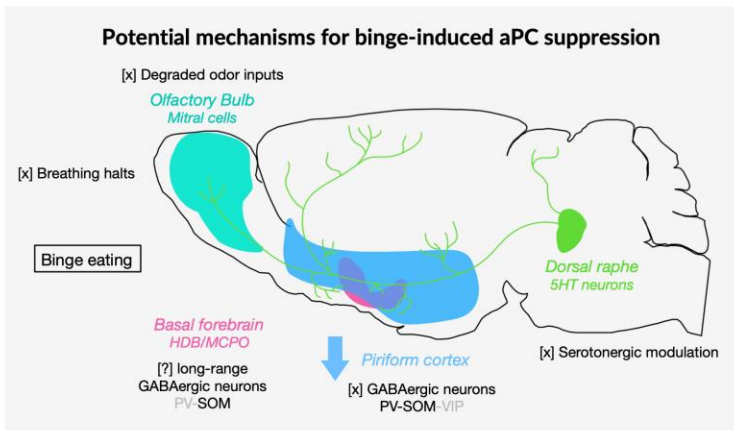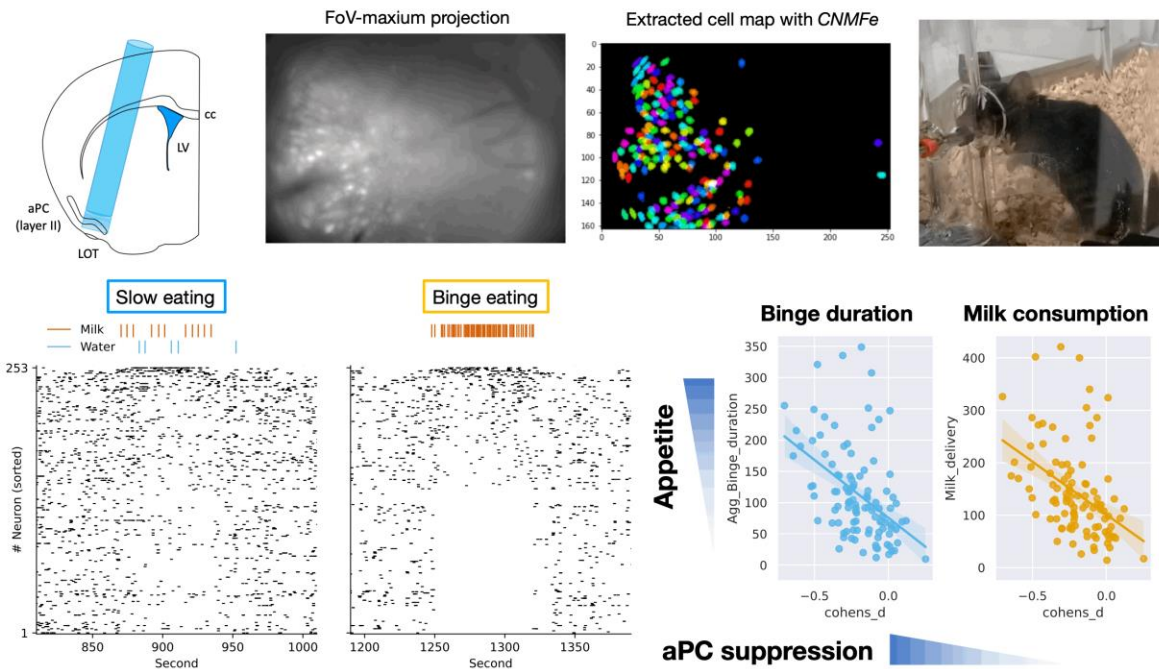
Disclosures: **H. Lo:** None. **A. Schoenherr:** None. **M.L. Tantirigama:** None. **M.E. Larkum:** None. **B. Judkewitz:** None. **K. Stumpenhorst:** None. **M. Rivalan:** None. **Y. Winter:** None. **D. Schmitz:** None. **F. Johenning:** None.

Nanosymposium

429. Olfactory Higher Order Processing and Perception

Location: SDCC 23

Time: Tuesday, November 15, 2022, 8:00 AM - 10:15 AM

Presentation Number: 429.07

Topic: D.04. The Chemical Senses

Support: WT

Title: Olfactory processing in thalamocortical circuits in monkeys

Authors: B. A. L. PERRY[1], E. COURTIOL[2], *A. S. MITCHELL[1,3];
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Abstract: The olfactory system, as opposed to the other sensory systems, does not have a primary thalamic relay. However, the mediodorsal thalamus receives inputs from different primary olfactory areas including the piriform cortex and has strong reciprocal connection with the orbitofrontal cortex, a secondary olfactory area. The mediodorsal thalamus has been shown to be involved in some olfactory processing in mammals however only few studies detailed the encoding of olfactory information in this structure, especially in monkeys. In addition, given the strong relationship between the mediodorsal thalamus and the orbitofrontal cortex, it is *essential* to compare the odor responses in both structures and determine how they interact during odor presentation. To do so, we recorded neural signals (single units as well as local field potentials) from both structures simultaneously in four male rhesus macaque monkeys during repeated presentations of various olfactory stimuli including monomolecular odorants as well as biological odorants (i.e. female urine). Here, we present the results of single unit responses in these structures. Both mediodorsal thalamus and orbitofrontal cortex contained units that responded to one or more of the four odors presented in each session.

Disclosures: B.A.L. Perry: None. **E. Courtiol:** None. **A.S. Mitchell:** None.

Nanosymposium

429. Olfactory Higher Order Processing and Perception

Location: SDCC 23

Time: Tuesday, November 15, 2022, 8:00 AM - 10:15 AM

Presentation Number: 429.08

Topic: D.04. The Chemical Senses

Support: RIKEN Brain Science Institute
Howard Hughes Medical Institute
JPB Foundation

Title: Knowledge is abstracted across overlapping memory engrams

Authors: *A. J. AQRABAWI, Q. R. V. FERRY, M. PIGNATELLI, A. HAMALIAN, S. TONEGAWA;
Picower Inst. For Learning and Memory, MIT, Cambridge, MA

Abstract: Knowledge affords an organism the ability to appropriate past experience for ongoing survival. Gradually and persistently, our memories build an associative network of abstractions derived from the statistical regularities existing between episodes. This culminates into a practical model of the world that guides prediction and future action. The hippocampus and prefrontal cortex have been implicated in knowledge formation and instantiation, yet the cellular basis of organization remains enigmatic. We hypothesized that the repeated strengthening of neurons encoding similar features across events, serves as an efficient, passive mechanism for generalizing the commonalities. The engram is the fundamental unit of memory. Here we show that abstract knowledge representations emerge via the iterative process of overlapping engrams. We devised a genetic strategy to exclusively label and manipulate the population of neurons holding overlapping engrams and demonstrated their functional role in transitive inference, a knowledge-dependent form of deductive reasoning. Furthermore, we found that overlapping engrams are positioned at a superior hierarchical standing in terms of informational capacity, occupying the tail-end of a log-normal rate distribution. Their coordinated activity was met with near-simultaneous inhibition of a parallel non-overlapping population and appeared to exhibit the dynamical properties of attractors, firing independently of bottom-sensory input. We further illustrated how overlapping engrams form sequentially within nested hippocampal-cortical feedback loops. Lastly, we reveal the fractal geometry of memory, whereby overlapping engrams self-organize into a cauliflower-like manifold possessing a measured fractal dimension of ~2.79. Collectively, these data uncover an intuitively understandable biological mechanism for knowledge representation in the brain.

Disclosures: A.J. Aqrabawi: None. **Q.R.V. Ferry:** None. **M. Pignatelli:** None. **A. Hamalian:** None. **S. Tonegawa:** None.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.01

Topic: H.08. Learning and Memory

Support: NSF CAREER IOS-2145814
NIH DP2 MH129958-01

Title: Understanding the role of medial entorhinal cortex in interval timing

Authors: *J. G. HEYS, E. R. BIGUS, A. A. CHAGOVETZ;
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Abstract: The ability of the nervous system to keep track of time on the scale of seconds to minutes (i.e. interval timing) is critical to cognition and behavior. In particular, episodic memory (i.e. memories of specific personal events) requires rapid and flexible learning of the duration and temporal sequence of events, suggesting brain circuits required for encoding of episodic

memory could play a critical role in interval timing. Therefore, we hypothesize that medial entorhinal cortex (MEC), a circuit well established for spatial and episodic memory, might also function as a neural clock to provide timing information specifically during behavior that requires rapid, flexible learning of timing information. To address this question we have developed a novel temporal delay non-match to sample (tDNMS) paradigm in which mice learn to report whether sequentially presented pairs of odor stimuli are the same or different in duration. By chemogenetically inactivating MEC, we observe that tDNMS learning is disrupted, whereby mice expressing the inhibitory chemogenetic receptor hM4Di behave at chance throughout consecutive inactivation training sessions. In a separate series of experiments, using high-throughput in vivo cellular resolution Ca2+ imaging, we find that a large fraction of neurons in MEC display time-locked activity during this task. Interestingly, we find that many time cells display context-dependent, trial specific timing activity, with neural dynamics that differentiate trial type precisely at moments during each trial when there is sufficient information to discriminate match or mis-match trial type. These results demonstrate that MEC shows a robust representation of elapsed time through sequential neural activation. Importantly, we find that distinct sequences of MEC neural activity emerge as animals perceive tDNMS trial type, suggesting that MEC encodes context specific timing information. We speculate that similar circuit architecture and related neural dynamics in MEC used to drive path integration in populations of grid cells, may also be used to produce clock-like activity seen in context-dependent time cell sequences.

Disclosures: J.G. Heys: None. **E.R. Bigus:** None. **A.A. Chagovetz:** None.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.02

Topic: H.08. Learning and Memory

Title: Linking the representation of space and time in the human brain

Authors: *D. BUETI[1], V. CENTANINO[2], G. FORTUNATO[3];
[1]Intl. Sch. For Advaced Studies, [2]Cognitive Neurosci., Intl. Sch. For Advanced Studies (SISSA), Trieste, Italy; [3]Cognitive Neurosci., SISSA, Trieste, Italy

Abstract: Being able to combine spatial and temporal information in a unique percept is a fundamental aspect of our sensory experience of the world and of our capacity to interact with it. However, in the human brain whether space and time are processed jointly or independently

remain elusive. Using high-spatial resolution fMRI (7-Tesla) and a single-interval duration discrimination task of visual stimuli varying in both duration (from 0.4 to 0.8 s) and spatial location (foveal and parafoveal), we tested different neural response models to investigate how different brain areas (from occipital to frontal cortex) encode and represent stimulus durations and spatial locations. Results showed that early visual areas (V1-V3) process stimulus duration monotonically i.e., the longer the duration the greater the hemodynamic response, while tuned-like responses became increasingly prevalent in parietal and frontal regions. Moreover, in these brain regions temporal maps unfold over spatial maps revealing a tight link between spatial and temporal representations.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.03

Topic: H.08. Learning and Memory

Title: The neural basis of an internally generated, temporally organized action sequence

Authors: B. WU[1], M. ZHOU[1], H. JEONG[1], J. FLOEDER[1], V. LEE[2], A. TAYLOR[1], D. BURKE[1], A. MORSI[2], A. SANDERS[1], *V. NAMBOODIRI[1];
[1]Univ. of California San Francisco, San Francisco, CA; [2]Univ. of California Berkeley, Berkeley, CA

Abstract: Actions span a continuum along multiple dimensions. They may be either 1) entirely triggered by external sensory stimuli or internally generated; 2) performed at random moments or occur in sequences with exquisite temporal organization; and 3) frequently or sparsely reinforced. Actions in the real world are often internally generated, occur in sequences with a temporal structure and are sparsely reinforced (e.g., playing music, getting dressed, or playing sports). Though the neural basis of action generation has been a subject of key interest in neuroscience over many decades, internally generated, temporally organized action sequences that are only sparsely reinforced have not been studied in the laboratory. Here, we develop a new behavioral task in which head-fixed mice learn to develop an action strategy in which they lick metronomically at regular intervals (i.e., temporally organized) in the absence of any external sensory cue (i.e., internally generated) with each timed action having a <2% probability of reinforcement (i.e., sparsely reinforced). Mice (n=30, both sexes) show evidence of clear behavioral learning in this task within ~3 days and reliably produce periodic metronomic licks (with a period ranging from 1.5-3 s between individuals) for an entire behavioral session (except for consummatory bouts) lasting ~40 minutes. Overall, mice produce >1,000 timed licks within a

single session, even though they obtain only ~20 sucrose reward drops (5 uL each) over this period. Hence, this task is highly valuable to study the basis of internally generated, temporally organized action sequences. Using this task, we studied the encoding of such actions by mesolimbic dopamine release in the nucleus accumbens. Surprisingly, we found that even though the metronomic licking is necessary for obtaining rewards (i.e., it is an operant action), mesolimbic dopamine dipped around these actions (n=3 animals, n=5 sessions each after stable behavior, p<0.05 in each animal). Interestingly, the magnitude of the dip in dopamine levels predicted the moment of the next lick, i.e., the next internally-generated timed action (mean r = -0.20, p = 6.6E-6, t(14)=-7.0; n=15 sessions from n=3 mice). Such a fast timescale correlation between dopamine and ongoing movement is rarely observed and hence, we hypothesize that the study of internally generated temporally organized action sequences may be critical to study the role of dopamine in movement. Overall, we developed a highly reliable task in mice to study internally generated, temporally organized actions with sparse reinforcement, and show that mesolimbic dopamine level predicts the upcoming movement time.

Disclosures: B. Wu: None. **M. Zhou:** None. **H. Jeong:** None. **J. Floeder:** None. **V. Lee:** None. **A. Taylor:** None. **D. Burke:** None. **A. Morsi:** None. **A. Sanders:** None. **V. Namboodiri:** None.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.04

Topic: H.08. Learning and Memory

Title: Dopaminergic control of self-timed movement.

Authors: *A. E. HAMILOS[1], G. SPEDICATO[1], Y. HONG[1], F. SUN[2], Y. LI[2], J. A. ASSAD[1,3];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Peking Univ., Beijing, China; [3]Inst. Italiano di Tecnologia, Genoa, Italy

Abstract: Insights from movement disorders and pharmacological studies have long suggested dopamine tunes the pace of the internal clock, but how does the endogenous dopaminergic system influence the timing of movement? We examined the relationship between dopaminergic signaling and movement timing in mice. Animals were trained to lick after a self-timed interval following a start cue; reward was delivered if the animal's first lick fell within a rewarded window (3.3-7 s). The distributions of first-lick timing displayed the scalar property of timing,

and we leveraged the considerable variability in these distributions to determine how the activity of the dopaminergic system related to the animals' timing. We recorded dopaminergic signals with fiber photometry in gender-balanced cohorts of mice as they prepared to initiate self-timed movements (GcaMP6f in genetically defined dopamine neurons (n=12); the dopamine indicator dLight1.1 (n=5) or DA2m (n=4) in striatal neurons). We found that these dopaminergic signals were highly predictive of single-trial movement timing via a slow "ramp-up" that unfolded over the course of seconds between the start-timing cue and the self-timed movement, reminiscent of a ramp-to-threshold process. This effect was robust even when accounting for spurious movements, task variables, and trial history. Rather than directly triggering movement initiation, optogenetic activation of dopamine neurons during self-timing caused systematic early-shifting of the timing distribution (n=12 mice). Likewise, rather than prevent movement, inhibition caused late-shifting (n=4 mice), and no-opsin stimulation caused no consistent behavioral effect (n=5 mice). Optogenetic stimuli were also subthreshold for generating/preventing movement outside the task. Altogether, these results suggest that rather than directly driving movement initiation (e.g., like a motor neuron), dopaminergic activity instead modulates movement onset by adjusting the moment-to-moment probability of unleashing a planned movement, possibly via dopamine's push-pull influence over the ongoing activity of the downstream direct and indirect pathways. Consistent with this view, the dynamics of the endogenous dopaminergic signals quantitatively predicted the moment-to-moment probability of movement initiation during self-timing and recapitulated the hazard function of the movement timing distribution. We conclude that ramping dopaminergic signals, possibly reflecting changes in reward expectation leading up to the timed movement, probabilistically modulate the moment-to-moment decision of when to move.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.05

Topic: H.08. Learning and Memory

Title: A Discrete Motor-Independent Signature of Urgency During Human Perceptual Decision Making

Authors: *R. G. O'CONNELL[1], **H. MCCONE**[2], C. DEVINE[3], J. DULLY[2], E. MCNICKLE[2], S. KELLY[4];
[1]Trinity Col. Dublin, [2]Trinity Col. Dublin, Dublin, Ireland; [3]Dublin Business Sch., Dublin, Ireland; [4]Univ. Col. Dublin, Dublin, Ireland

Abstract: When faced with a strict deadline, how does the brain adjust its decision processes to take account of the passage of time? Computational modeling and electrophysiological investigations have pointed to a key role for dynamic 'urgency' processes that serve to progressively reduce the quantity of evidence required to reach commitment as time elapses. To date, such urgency dynamics have been observed exclusively in neural signals that accumulate evidence for a specific motor plan, taking the form of an additional time-dependent build-up component. Here, using data from two complementary experiments manipulating time and accuracy demands, we demonstrate that a long-studied component of the human event-related potential, the contingent negative variation (CNV), traces urgency in a movement-independent fashion. In correspondence with dynamic urgency effects identified through accumulation-to-bound model fits, the CNV builds over time until choice commitment with an onset and rate that is modulated by speed pressure. The CNV exhibits two additional properties not observed in previously reported urgency signals: First, it provides a discrete representation of urgency, growing only as a function of time and not evidence strength. Second, when choice reports must be withheld until stimulus offset, the CNV peaks and decays long before response execution on trials with low difficulty, mirroring the dynamics of motor-independent evidence accumulation signals. These properties suggest that the brain uses urgency signals not only to expedite motor planning but also to hasten cognitive deliberation.

Disclosures: R.G. O'Connell: None. **H. McCone:** None. **C. Devine:** None. **J. Dully:** None. **E. McNickle:** None. **S. Kelly:** None.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.06

Topic: H.08. Learning and Memory

Support: HRB ILP-PHR-2017-014

Title: Sex-specific association between allostatic load and multisensory integration in middle-aged and older adults

Authors: Z. AZIZI[1], R. J. HIRST[1], F. N. NEWELL[1], R. KENNY[1], *A. SETTI[2];
[1]Trinity Col. Dublin, Dublin, Ireland; [2]Univ. Col. Cork, Cork, Ireland

Abstract: Multisensory integration, the ability of the brain to integrate information from different sensory modalities, is critical for responding to environmental stimuli. While older adults show changes in multisensory integration with age, the impact of allostatic load (AL) (i.e.

the effect of exposure to chronic stress, which can accelerate ageing) on multisensory perception remains understudied. AL is inversely associated with general physical and cognitive functioning in older adults. Gamma-aminobutyric acid (GABA) level shapes individual differences in audio-visual integration and perception. In turn, GABA neurotransmission modulates AL. We explored the relationship between multisensory integration and AL in 1307 adults aged 50+ from The Irish Longitudinal Study on Ageing. The Sound Induced Flash Illusion (SIFI) was used to test multisensory integration at multiple audio-visual temporal asynchronies. The AL score was created using a battery of 12 biomarkers representing the activity of four major physiological systems: immunological, cardiovascular, metabolic, and renal. The number of factors for which a participant was 473bstract473ed in the highest risk quartile using sex-specific cutoffs was used to produce an overall AL score. We also included medication use when calculating our AL score. We analysed the accuracy to illusion trials in SIFI with a 473bstract473ed logistic mixed effects regression model, adjusted for age, education, self-reported vision and audition, visual acuity score, number of cardiac diseases, MoCA score, physical activity, accuracy on multisensory congruent and unimodal trials, and pre-post condition. Considering known differences between males and females in both AL and multisensory literature, our analyses were conducted separately for each sex. Results revealed that lower accuracy in integration (i.e., higher SIFI susceptibility with larger temporal asynchronies) was associated with higher AL in women but not men. These findings suggest that AL is associated with distinct patterns of multisensory integration in ageing, particularly in women. The modulation of neurotransmitter GABA – associated with Gamma Band activity, which is registered during SIFI perception – by AL is a potential mechanism at play deserving further investigation.

Disclosures: Z. Azizi: None. **R.J. Hirst:** None. **F.N. Newell:** None. **R. Kenny:** None. **A. Setti:** None.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.07

Topic: H.08. Learning and Memory

Title: Evaluation of integrations of magnitude information across modalities (visual and auditory) and domains (time and numerosity), using Bayesian hierarchical modeling and Maximum Likelihood Estimation

Authors: *Y. YOTSUMOTO, T. OTSUKA, H. YOSHIMATSU;
Dept. of Life Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: While time is an entity above and beyond each sensory modality, its inputs are constrained to sensory modalities. When we perceive the duration of an external stimulus, it is first encoded in the modality-specific cortex and then becomes available for cognitive processing. In such hierarchical processing, the duration information encoded in multiple modalities must be integrated. Moreover, it has been proposed that our neural system represents an474bstractt magnitude that is common to the perception of time, number, and space, thus indicating that time is further integrated with other domains (number and space).

The present study examined how information is integrated between modalities (visual and auditory) and domains (time and numerosity) and proposed models that could explain the integrations.

First, we examined the duration integration of visual and auditory modalities. We conducted an experiment where we presented visual flickers and auditory flutters in synchrony and instructed the participants to attend to the visual modality, the auditory modality, or both. After confirming that the attended modality modulated the point of subjective equivalence, we applied Bayesian hierarchical modeling to evaluate how attention could change the weights placed on each modality. The model showed that attention can increase the weight placed on the attended modality and that the auditory stimulus received more weighs even after controlling for reliabilities.

Second, we examined the integration of time and numerosity information. In the experiment, we presented time and numerosity information simultaneously and instructed the participants to judge the magnitude of time, numerosity, or both domains. We systematically manipulated the stimuli reliability and calculated just noticeable differences. The results were partially consistent with the Maximum Likelihood Estimation account; time and numerosity information was integrated in a statistically optimal fashion in the sense that the integrated estimate was found to be most reliable.

Our results suggest that integrations of magnitude information across modalities and across domains may differ in terms of the roles of the reliabilities. We will further discuss how integrations may be achieved by employing different strategies in hierarchical cortical processing.

Disclosures: Y. Yotsumoto: None. **T. Otsuka:** None. **H. Yoshimatsu:** None.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.08

Topic: H.08. Learning and Memory

Title: Neural signatures of evidence accumulation in temporal decisions

Authors: *A. N. LANDAU, N. OFIR;

The Hebrew Univ. of Jerusalem, The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Cognitive models of interval timing can be formulated as an accumulation-to-bound processes. However, the physiological manifestation of such an accumulation process has not yet been identified. We measured electroencephalography (EEG) of participants while they performed a temporal-bisection task in which they were requested to categorize the duration of visual stimuli as short or long. We found that the stimulus-offset and response-locked activity depends both on stimulus duration and the participants' decision. To relate this activity to the underlying cognitive processes, we used a drift-diffusion model. The model includes a noisy accumulator starting with the stimulus onset, and a decision threshold. According to the model, a stimulus duration will be categorized as "long" if the accumulator reaches the threshold during stimulus presentation. Otherwise, it will be categorized as "short." We found that, at the offset of stimulus presentation, an EEG response marks the distance of the accumulator from the threshold. Therefore, this model offers an accurate description of our behavioral data as well as the EEG response using the same two model parameters. We then replicated this finding in an identical experiment conducted in the tactile domain. We also extended this finding to two different temporal ranges (sub- and supra-second). Taken together, the work provides a new way to study the cognitive processes underlying temporal decisions, using a combination of behavior, EEG and modeling.

Disclosures: A.N. Landau: None. **N. Ofir:** None.

Nanosymposium

430. Neurobiological Basis of Timing

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Presentation Number: 430.09

Topic: H.08. Learning and Memory

Support: Hessian initiative for the development of scientific and economic excellence (LOEWE) Neuronal Coordination Research Focus Frankfurt (NeFF)

Title: Predictive coding explains increased reliance on perceptual priors in older adults and ASD – a magnetoencephalography study

Authors: *J. CHAN[1], M. WIBRAL[2], P. WOLLSTADT[3], M. SINIATCHKIN[4], M. J. NAUMER[5], C. M. FREITAG[6], J. KAISER[7];

[1]Univ. Col. Cork, Cork, Ireland; [2]Göttingen Grad. Ctr. For Neurosciences, Biophysics, and Mol. Biosci., Georg-August University, Göttingen, Göttingen, Germany; [3]MEG Unit, Brain Imaging Ctr., Frankfurt, Germany; [4]Universitätsklinik für Kinder- und Jugendpsychiatrie und Psychotherapie, Univ. Clin. Owl, Bielefeld, Germany; [5]Univ. Koblenz · Landau, Koblenz, Germany; [6]Goethe Univ. Frankfurt, Frankfurt/Main, Germany; [7]Inst. Of Med. Psychology, Goethe-University Frankfurt, Frankfurt am Main, Germany

Abstract: The multisensory temporal binding window (TBW) is a period when stimuli from different sensory modalities are combined into a single percept. The sound-induced flash illusion (SiFI) exploits this TBW to create an illusory second flash when presented with two rapid beeps along with a single flash. Despite the bottom-up sensory information there is also a top-down expectation that is required to perceive the illusion. Older adults and different patient groups (e.g., ASD and older adults with MCI) with different symptomology exhibit an extended TBW and thus perceive more illusions compared to age-matched controls. The aim was to determine if there are similar different neurological networks under pinning this behavior between groups. Two studies were conducted: Study 1 compared older adults (N = 28) to young adults (N = 25). Study 2 compared ASD (N = 17) to aged-matched typically developed (TD) adults (N = 15). In both studies, participants were presented the SiFI while inside a 275-channel MEG. Anatomical MRIs were also acquired for source analyses. After artifact rejection, neural oscillations were calculated using frequency-dependent Morlet wavelet between 2-60 Hz. Linear beamforming was used to solve the inverse problem and estimate source locations. Furthermore, by using a combination of transfer entropy (TE) and dynamic causal modeling (DCM) the information transfer for each group and condition was estimated. In Study 1, there was increased overall beta band activity (12-25 Hz) in older adults compared to young adults with more top-down information from the middle frontal gyrus to the fusiform areas in older compared to young. In Study 2, we found greater alpha (8-12Hz) and beta-band activity in ASD participants compared to TD for illusions compared to no illusions with similar brain areas being the source of the activity. We interpret this data from a predictive coding perspective. As we age, our unisensory acuity declines, and we increasingly rely multisensory perception. The increased template that a sound and visual stimulus should occur can be driving the increased illusions. People with ASD are more rule-based and therefore have a greater expectation that multisensory stimuli have spatial and temporal congruency.

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Nanosymposium

430. Neurobiological Basis of Timing

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Presentation Number: 430.10

Topic: H.08. Learning and Memory

Support: Institute for Biohealth Innovation Grant

Title: Error Monitoring for Time – How Humans learn and adapt to Temporal Intervals

Authors: *M. WIENER[1], A. MOTALA[2], F. BADER[1];
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Abstract: Error monitoring is an essential human ability underlying learning and metacognition. In the time domain, humans possess a remarkable ability to learn and adapt to temporal intervals, yet the neural mechanisms underlying this are not well understood. Here, we describe a series of experiments designed to elucidate error monitoring for time intervals in humans. We employed a modified version of a visual time reproduction task with random suprasecond intervals (1-5s) in which, crucially, subjects were allowed to "re-do" each trial after receiving adaptive feedback on their performance. Further, feedback was nondirectional, and so provided no details on whether subjects had over or under-estimated the target interval. Remarkably, subjects (n=20) improved performance on re-do trials by increasing both accuracy and precision, suggesting a metacognitive awareness of time errors. Removing feedback in a second group (n=20) still led to improvements in accuracy, but no increase in precision. In a further experiment (n=50), changing the frequency of re-do trials had no impact on improvement. Finally, a fourth group of subjects (n=24) performed this task while recording fMRI responses. Here, we observed that estimating duration on initial trials led to activation in timing network regions such as the supplementary motor area (SMA), basal ganglia, and inferior parietal lobe (IPL), whereas during re-do trials, activation of the default mode network (DMN), including posterior cingulate, precuneus, and superior frontal gyrus (SFG). Additionally, when reproducing on re-do trials, only the SMA and SFG became active. These findings suggest that humans maintain an awareness of their own timing that relies on interactions between the default-mode and timing networks, such that errors can be flexibly adapted to update time representations.

Disclosures: M. Wiener: None. **A. Motala:** None. **F. Bader:** None.

Nanosymposium

430. Neurobiological Basis of Timing

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Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.11

Topic: H.08. Learning and Memory

Title: Dissociable roles of theta and alpha oscillations in sub-second and supra-second time reproduction: An investigation of their links to depression and anxiety

Authors: M. LIANG, S. LOMAYESVA, **E. ISHAM**;
Univ. of Arizona, Tucson, AZ

Abstract: The Striatal Beat Frequency model (Matell & Meck, 2004) suggests that multiple cortical oscillators contribute to human temporal cognition. Based on the theory, the current study examined the interactions between interval timing and neural oscillations, as predicted by timing deficits found in neuropsychiatric conditions. The interaction is predicted to manifest in frontal-midline theta and occipital alpha oscillations, which are two important cortical oscillatory sources engaged in working memory and time perception. Therefore, the main goal of the study is to examine how depression and anxiety modulate frontal-midline theta and occipital data during the encoding of sub- and supra-second intervals. In the current study, participants

reproduced sub- (400, 600, 800ms) and supra-second (1600, 1800, 2000ms) intervals while they underwent scalp EEG recordings. Anxiety and depression levels were measured via self-report psychometrics. We found that higher levels of self-reported anxiety and depression were associated with shorter reproduction of lengths of durations. Further, time-frequency analysis of scalp EEG revealed a dissociation where state anxiety altered the links between frontal-midline theta power and sub-second interval reproduction, while depression and trait anxiety altered the association between occipital alpha power and supra-second interval reproduction. Our results suggest that anxiety and depression alter time perception, and this differentially relates to frontal-midline theta and occipital alpha as substrates for sub- and supra-second interval timing.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.12

Topic: H.08. Learning and Memory

Title: Killing or counting time: causally test a sensorimotor theory of action timing

Authors: P. S. STRASSMANN[1,2], ***X. CAI**[1,2], C. D. HOWARD[1,3], J. R. COOK[1,2,4], X. JIN[1,5,6];
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Abstract: It has been recently reported that a sensorimotor mechanism is employed by the brain to track time in mice, in which the secondary auditory cortex transduces self-generated audiomotor feedback to control action timing (Cook et al. 2022). Animals have been often observed to develop a stereotyped pattern of behavior (generally referred as 'waiting-period actions' here) during timekeeping. Traditionally, these waiting-period behaviors have been viewed as superstitious or compulsive, with such actions being merely collateral to or reflective of timekeeping. However, the recently discovered sensorimotor mechanism underlying action timing suggests these behaviors may serve as a pacemaker for the internal clock, used to count but not kill time (Cook et al. 2022). Here, we took an optogenetic approach to precisely manipulate actions antecedent to timing decision and causally test this sensorimotor theory of timing. It was found that optogenetic stimulations of different cell types in various brain regions including dorsal striatum, thalamic nuclei and midbrain during the waiting period result in

significant changes in both animal's action and timing. Importantly, bidirectional regulation of actions during the waiting period leads to corresponding temporal shifts of timing, with a strong negative correlation between changes in the amount of waiting-period actions and the timing duration. Notably, manipulation of nigrostriatal dopamine affects timing if, and only if, when waiting-period actions were also regulated. These effects can be quantitatively reproduced in a computational model of action timing based on the recently discovered sensorimotor mechanism. Together, these results further support the sensorimotor theory of action timing, in which the waiting-period actions serve as the pacemaker for the internal clock, and suggest that certain brain regions are involved in interval timing via control of action, rather than due to a non-motor, dedicated role in time perception.

Disclosures: P.S. Strassmann: None. **X. Cai:** None. **C.D. Howard:** None. **J.R. Cook:** None. **X. Jin:** None.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.13

Topic: H.08. Learning and Memory

Title: The influence of semantic context on sequence memory and event segmentation

Authors: *O. RACCAH[1], K. DOELLING[3], L. DAVACHI[4], D. POEPPEL[2];
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Abstract: How the brain integrates and represents muti-item sequences, and retrieves this information from memory, is one of the most foundational problems in psychology and neuroscience (Lashley, 1950). A key aspect of sequence processing is the ability to encode and subsequently recall the relative order of items or past events, independent of their timing (Lewandowsky & Murdock, 1989). This capacity, referred to as *temporal order memory (ToM)*, is a hallmark of episodic memory, allowing us to anticipate impending events and to guide our future actions (Davachi & Dubrow, 2015). It has long been recognized that our existing semantic knowledge has a direct impact on our ability to retain new information (Craik & Lockhart, 1972). However, the influence of semantic knowledge on our ability to remember ordered sequences is largely unexplored. To address this question, we administered a sequence memory paradigm, in which participants listened to lists of spoken words, where each set of six consecutive words belong to a distinct semantic category before transitioning to a new semantic category. Following each list, participants were given a temporal order memory test within and across semantic categories. We parameterized semantic similarity across our word stimuli using an autoencoder model (Google Word2Vec). Using this approach, we show that semantic

similarity within-categories strongly drives temporal order memory performance (n = 28; R^2 = .43; P < 0.001). Next, we evaluated how prior semantic knowledge may interact with our encoding of temporal order *over time*. To this end, we computed semantic similarity across word pairs based on their serial position during encoding. This analysis revealed that only certain word pairs – i.e., those occupying the middle serial positions of a categorical event – reliably predict subsequent temporal order recall. Furthermore, we found that item pairs at the boundary between categories predict ToM as a function of semantic *dissimilarity*, indicating a semantically defined event boundary. Together, these findings suggest the possibility that the accumulation of semantic information serves to bind items into composite events in memory and additionally serves to critically inform neural models of sequence memory.

Nanosymposium

430. Neurobiological Basis of Timing

Location: SDCC 33

Time: Tuesday, November 15, 2022, 8:00 AM – 11:30 AM

Presentation Number: 430.14

Topic: H.08. Learning and Memory

Title: A supplementary motor response enables acquisition of multiple prior distributions in human coincidence timing

Authors: *S. NATSUME[1], J. HERON[3], N. W. ROACH[4], M. MIYAZAKI[1,2];
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Abstract: Sensory signals are inherently variable, but the central nervous system minimizes the impact of sensory noise by integrating signals with prior knowledge. Successful Bayesian integration in complex environments relies on the ability to acquire multiple prior distributions. Recently, our research group found that in a coincidence timing task, participants can concurrently learn two prior distributions when they are assigned to two different motor effectors (e.g., left hand vs. right hand) (Matsumura et al., FENS2020). However, in practice, associating a distinct motor effector to each new prior distribution would quickly become infeasible. Inspired by findings from bimanual motor learning studies (Noazaki et al., 2006, Nat Neurosci), we hypothesized that in a timing task, multiple prior acquisition might be supported by the addition of a supplementary motor response. To test this hypothesis, we conducted psychophysical experiments where three sequential visual stimuli (S1, S2, and S3) were presented either on one side (corresponding to the participant's dominant hand) or both sides (left and right) of a fixation

point. Time intervals for S1-S2 and S2-S3 were identical in each trial. Across trials, intervals for one-side and two-side presentation conditions were randomly sampled from either short-time (424-988 ms) or long-time (1129-1694 ms) prior distributions. Participants were asked to anticipate the appearance of S3 based on the S1-S2 interval and press a key to coincide with its onset. When participants used their dominant index finger to respond on all trials, they were unable to concurrently learn both short-time and long-time priors. In contrast, participants who responded to one-side presentations with one finger and both-side presentations with both fingers successfully acquired both priors. These results indicate that the addition of a supplementary motor response can aid the acquisition of multiple prior distributions, even when using the same motor effector in a coincidence timing task.

Nanosymposium

431. Neural Mechanisms of Aging I

Location: SDCC 25

Time: Tuesday, November 15, 2022, 8:00 AM – 9:45 AM

Presentation Number: 431.01

Topic: H.12. Aging and Development

Title: A tale of two systems: Altered linear coupling between blood flow and oxygen metabolism in the aging human brain

Authors: *M. P. TURNER[1], Y. ZHAO[2], D. H. ABDELKARIM[2], P. LIU[3], J. S. SPENCE[1], J. L. HUTCHISON[2], D. SIVAKOLUNDU[4], B. P. THOMAS[5], N. A. HUBBARD[6], H. LU[7], B. P. RYPMA[2];
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Abstract: Neural-vascular coupling (NVC), the delivery of oxygen and nutrients to metabolically active neuronal tissue by vasculature, varies with brain state and is affected in

healthy aging. Calibrated fMRI (cfMRI) allows separate characterization of BOLD signal and its constituents, cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMRO$_2$), providing a more complete picture of changes to the NVC system with age and stimulus intensity. Combining measurement of CBF (using pseudo-continuous arterial spin labeling) with estimation of baseline deoxyhemoglobin (using hypercapnia calibration) permitted us to calculate CMRO$_2$, which in turn permitted estimation of NVC. 33 younger and 27 older participants underwent cfMRI scanning during both an attention-controlled visual stimulation task in which participants periodically viewed checkerboards flickering at either 2Hz, 4Hz, or 8Hz. Our results replicated prior results from our lab, namely: (1) significantly greater task-evoked BOLD signal in younger relative to older adults, (2) no significant age-group difference in task-evoked CBF, and (3) significantly greater task-evoked CMRO$_2$ in older relative to younger adults. Most importantly, we observed a significant Age-Group × Stimulus-Intensity interaction in the NVC ratio. Specifically, with increasing stimulus intensity, the NVC ratio increased monotonically in younger adults while it remained unchanged in older adults. We also explored relationships between individual cognitive performance and the task-evoked neurometabolic and neurovascular parameters. Taken together, these results support the hypothesis that NVC mechanisms are adversely affected in aging.

Disclosures: M.P. Turner: None. **Y. Zhao:** None. **D.H. Abdelkarim:** None. **P. Liu:** None. **J.S. Spence:** None. **J.L. Hutchison:** None. **D. Sivakolundu:** None. **B.P. Thomas:** None. **N.A. Hubbard:** None. **H. Lu:** None. **B.P. Rypma:** None.

Nanosymposium

431. Neural Mechanisms of Aging I

Location: SDCC 25

Time: Tuesday, November 15, 2022, 8:00 AM - 9:45 AM

Presentation Number: 431.02

Topic: H.12. Aging and Development

Title: Liver expressed antimicrobial peptide 2 is elevated during aging and associated with hippocampal pathology

Authors: *J. TIAN[1], H. DU[1], R. H. SWERDLOW[2];
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Abstract: Normal aging populations experience, though subtle, cognitive changes associated with hippocampal atrophy. However, the molecular mechanisms underlying hippocampal

abnormality in normative aging remain unclear. Ghrelin and the receptor growth hormone secretagogue receptor 1a (GHSR1a) plays a pivotal role in regulating hippocampal synaptic function. Liver expressed antimicrobial peptide 2 (LEAP2) is an endogenous GHSR1a antagonist that modulate GHSR1a activity with ghrelin. Here, we report an age-dependent increase of circulating LEAP2 in cognitive norms, resulting in imbalanced ghrelin and LEAP2 and associated hippocampal pathology in the tested subjects. Further examination shows elevated LEAP2 in aging wildtype (wt) mice, which is in accordance with hippocampal lesions resembling GHSR *null* mice. Consistent with the deleterious impact of LEAP2 against ghrelin-induced GHSR1a activity, LEAP2 inhibits ghrelin signaling-mediated hippocampal synaptic activation. Moreover, acute hippocampal slices from aging mice exhibit blunted response to GHSR1a agonist stimulation. These findings suggest that LEAP2 deregulation is an age effect that contributes to GHSR1a dysfunction and associated with hippocampal dysfunction in normative aging. Targeting LEAP2 will hold promise to preserve cognitive function during aging and prevent a transition from normative to pathological aging.

Nanosymposium

431. Neural Mechanisms of Aging I

Location: SDCC 25

Time: Tuesday, November 15, 2022, 8:00 AM - 9:45 AM

Presentation Number: 431.03

Topic: H.12. Aging and Development

Title: Aging-related vulnerbility of synaptic mitochondria in humanized amyloid beta-expressing mice

Authors: *K. JIA[1], J. TIAN[4], T. WANG[2], L. GUO[5], H. DU[3];
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Abstract: Aging is an inevitable stage of lifecycle and closely associated with a spectrum of disorders including Alzheimer's disease (AD). Although the mechanistic links between brain aging and AD remain largely unresolved, increasing evidence suggests that the two conditions are tied to mitochondrial dysfunction. However, current translation of aging-related mitochondrial defects into an AD context is complicated by an interplay between mitochondria and AD-associated pathological molecules including amyloid beta (Aβ) and pathological tau. Previous findings from aging mice with excess Aβ production and/or tau pathology raise a

question of whether the observed mitochondrial deficits are bona fide pathological alterations of degenerative aging or, in fact, a result of superlative toxicity of Aβ and/or tau. In contrast, aging wildtype rodents, due to their lack of AD-related key pathophysiological factors, do not fully represent individuals at AD risk. In this context, mice expressing human form of Aβ at physiological levels (hAβ-KI mice) hold potential to reconcile this dilemma. Here, we report that hAβ-KI mice undergo aging-related deficits in synaptic mitochondrial function including compromised bioenergetics, decreased calcium retention capacity, altered morphological control towards mitochondrial fission, as well as increased oxidative damages as compared with their counterparts from age- and gender-matched nontransgenic (nonTg) controls. Moreover, aging hAβ-KI mice exhibit increased accumulation of Aβ42 in their synaptic mitochondria, which correlates with mitochondrial dysfunction. In contrast, nonsynaptic mitochondria from aging hAβ-KI mice are relatively resistant to human Aβ. Lastly, synaptic mitochondrial defects in aging hAβ-KI mice correlate with decreased synaptic density, increased synapse elimination by microglia, and impaired recognition memory. Our findings indicate synaptic mitochondrial vulnerability in abnormal aging, in which aging factors and Aβ toxicity converge. Furthermore, hAβ-KI mice may constitute a novel mouse model for the study of mitochondrial abnormalities in the elderly at AD risk.

Disclosures: K. Jia: None. **J. Tian:** None. **T. Wang:** None. **L. Guo:** None. **H. Du:** None.

Nanosymposium

431. Neural Mechanisms of Aging I

Location: SDCC 25

Time: Tuesday, November 15, 2022, 8:00 AM - 9:45 AM

Presentation Number: 431.04

Topic: H.12. Aging and Development

Title: Neuroadaptive remodeling of hippocampal circuits supports successful odor recognition memory in aging

Authors: *P. MORENO-CASTILLA[1], E. L. R. MELENDEZ[2], J. M. LONG[3], P. R. RAPP[4];
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Abstract: Recognizing a stimulus as previously encountered is a crucial capacity in everyday life that frequently deteriorates with aging. Nonetheless, cognitive decline is not an inevitable feature of growing old. Rodent models faithfully recapitulate this hallmark of aging, reliably demonstrating increased interindividual variability in memory relative to younger adults. To investigate the differential recruitment of medial temporal lobe circuits in relation to cognitive outcome in aging, we developed an olfactory recognition task for young and aged Long-Evans rats that directly varies memory demand (short- and long-term retention). Animals were

euthanized immediately after the retention test, and quantification of cFos immunolabeled cells was used to map the distribution of memory-induced neuronal activation. We found that aged rats performed on par with young on the short-term memory test, displaying robust odor recognition and significant medial temporal lobe activation. In stark contrast, on the long-term odor recognition test aged rats displayed substantial interindividual variability that strongly correlated with spatial learning capacities measured in the water maze. The pattern of cFos+ induction was also prominently affected, such that the number of activated cells was blunted in the dentate gyrus, entorhinal, perirhinal, and postrhinal cortices, and tightly correlated with poorer recognition performance in aged animals. Most notably, cross-correlation network analyses revealed coherence patterns between brain regions that were vastly different across groups. Despite scoring as well as young, aged rats with intact memory displayed an enhanced network density of increased interregional correlation coefficients, including greater intra-hippocampal correlations, and elevated parahippocampal correlations with the piriform cortex. In contrast, aged animals with impaired memory failed to engage intrahippocampal connections and had reduced coherence with the piriform area. These data signal that, alongside brain maintenance and other potential accounts, a process of dynamic neuroadaptive network reorganization may be among the mechanisms that supports positive cognitive outcomes in aging.

Disclosures: P. Moreno-Castilla: None. **E.L.R. Melendez:** None. **J.M. Long:** None. **P.R. Rapp:** None.

Nanosymposium

431. Neural Mechanisms of Aging I

Location: SDCC 25

Time: Tuesday, November 15, 2022, 8:00 AM - 9:45 AM

Presentation Number: 431.05

Topic: H.12. Aging and Development

Support: NIH Grant R37 AG025667
Abbott Nutrition (C4712) through the Center for Nutrition, Learning, and
Memory at the University of Illinois

Title: Sustained moderate-to-vigorous physical activity is related to enhanced resting state functional connectivity in cognitively salient brain networks in seniors

Authors: *D. M. PINDUS[1], M. AI[4], L. CHADDOCK-HEYMAN[2], A. Z. BURZYNSKA[5], M. VOSS[6], N. GOTHE[1], E. A. SALERNO[7], J. FANNING[8], S. A. ANTERAPER[3], A. N. CASTANON[9], S. WHITFIELD-GABRIELI[4], C. H. HILLMAN[4], E. MCAULEY[1], A. F. KRAMER[4];
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Exercise Sci., Wake Forest Univ., Winston-Salem, NC; [9]Dept. of Speech, Language, and Hearing Sci., Boston Univ., Boston, MA

Abstract: Functional connectivity (FC) in brain networks important for cognitive control decreases during adult aging. Physical activity (PA) may help optimize FC in these brain networks. However, the relationship between daily PA and resting state FC of the aging brain remains poorly understood. We evaluated the associations between total and bouted moderate-to-vigorous PA (m-vPA) and FC in 118 seniors (M_{age} = 64.8 ± 1.71 yrs, 84 females) using baseline data from the Fit & Active Seniors Trial (NCT01472744). Total daily m-vPA (all 60 s epochs recording > 1040 accelerometer counts per minute; min/d) and bouted m-vPA (time in m-vPA bouts lasting ≥ 10 consecutive minutes; min/d) were measured with an ActiGraph GT3X accelerometer. We hypothesized that m-vPA variables would be associated with voxel seeds in cognitively salient brain networks: dorsal attention (DAN), ventral attention (VAN), frontoparietal (FPN), and the default mode network (DMN). We expected enhanced FC between total and bouted m-vPA-related seeds and voxel clusters in the DAN, VAN, and FPN. DMN analyses were considered exploratory. Multivariate pattern analysis, implemented in CONN toolbox, was used to identify multivariate patterns of voxel clusters associated with m-vPA variables. To determine which FC patterns explained the main results, clusters with > 50 voxels related to m-vPA variables were used as seeds in the post hoc seed-to-voxel analyses. Controlling for accelerometer wear time, age, sex, education, and aerobic fitness, bouted m-vPA was associated with one cluster with a peak region in the left posterior middle temporal gyrus, a part of the DMN (voxel threshold $p < 0.001$, FWE-corrected cluster threshold, $p < 0.05$). In the post hoc analyses, bouted m-vPA was associated with correlations between its seed cluster in the DMN and two clusters primarily comprising regions within cognitively salient brain networks: DAN, VAN, DMN, and FPN, and a cluster in the somatosensory network. Bouted m-vPA was associated with *negative correlations* between its seed cluster in the DMN and voxels in the DAN and FPN. Conversely, bouted m-vPA was related to *positive correlations* between its seed, and voxels in the DMN, and somatosensory network. Correlations between the seed cluster and VAN were inconsistent (positive and negative). Our preliminary findings suggest that bouted m-vPA may help (i) attenuate age-related decline in anticorrelations between the DMN and DAN, an indicator of age-related network dedifferentiation linked to poorer cognitive performance; and (ii) enhance within-network FC in the DMN, suggestive of greater network segregation previously linked to better cognitive performance.

Disclosures: D.M. Pindus: None. **M. Ai:** None. **L. Chaddock-Heyman:** None. **A.Z. Burzynska:** None. **M. Voss:** None. **N. Gothe:** None. **E.A. Salerno:** None. **J. Fanning:** None. **S.A. Anteraper:** None. **A.N. Castanon:** None. **S. Whitfield-Gabrieli:** None. **C.H. Hillman:** None. **E. McAuley:** None. **A.F. Kramer:** None.

Nanosymposium

431. Neural Mechanisms of Aging I

Location: SDCC 25

Time: Tuesday, November 15, 2022, 8:00 AM - 9:45 AM

Presentation Number: 431.06

Topic: H.12. Aging and Development

Title: Functional and Structural Neuro-markers Predict Healthy Lifestyle Engagement in Older Adults

Authors: *M. AI[1], **T. P. MORRIS**[2], **A. NORIEGA DE LA COLINA**[4], **J. TREMBLAY-MERCIER**[5], **S. VILLENEUVE**[6,5], **A. F. KRAMER**[3], **M. R. GEDDES**[7];
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Abstract: Objective: Prior research has demonstrated the importance of a healthy lifestyle to enhance cognition and diminish dementia risk in later life. Therefore, understanding the mechanisms underlying lifestyle engagement, particularly in older adults at risk for Alzheimer's Disease (AD) can give insights in providing efficient behavioral change interventions for those in need. Cognition and personality are key for facilitating health behaviors. Additionally, multimodal measures with behavior and functional and structural brain have previously been shown to predict exercise adherence. We hypothesize that multimodal measures will predict multidomain lifestyle behaviors in older adults. Methods: 175 cognitively normal older adults with a family history of AD from the Prevent-AD longitudinal aging cohort were included (age=66.3±5, 130 female, education years=15.5±3). Self-reported physical activity engagement, cognitive activity engagement, diet adherence, and social support were entered into k-means clustering, in an attempt to phenotype participants based on their combined lifestyle behaviors. Two groups were identified for overall healthier and riskier lifestyle. Three classification prediction models were applied to distinguish people within the two lifestyle risk groups (i.e., healthier and riskier groups), within a final sample of 139 participants: Functional connectivity matrices and grey matter brain features (model 1), behavioral measure assessing cognitive function and personality (model 2), and a combination of behavioral measures and neuroimaging features (model 3). Support Vector Machine (SVM) learning algorithms were used for the classification in a nested cross-validation manner. Averaged coefficients for features were extracted and ranked to indicate the importance of each feature in the final model. Results:The SVM classifier was 81% accurate in separating groups in model 1, 60 % accurate in model 2, and 83 % accurate in model 3. In model 3, the first 10 features with the highest absolute coefficients included a delayed memory task score, and functional connectivity among default mode network, dorsal attentional network, somatomotor network, and ventral attention network. Conclusion:Neuroimaging features, especially functional connectivity, are able to provide more precise prediction of older adults' healthy behavior, compared to behavioral measures alone. Our results provide preliminary evidence of neural mechanisms that distinguish those who engage in healthy lifestyle habits to those who do not.

Disclosures: M. Ai: None. **T.P. Morris:** None. **A. Noriega de la Colina:** None. **J. Tremblay-Mercier:** None. **S. Villeneuve:** None. **A.F. Kramer:** None. **M.R. Geddes:** None.

Nanosymposium

431. Neural Mechanisms of Aging I

Location: SDCC 25

Time: Tuesday, November 15, 2022, 8:00 AM - 9:45 AM

Presentation Number: 431.07

**Topic:** H.12. Aging and Development

Support: F32AG07462

Title: Dynamic brain states are disrupted by aging and Alzheimer's disease

Authors: *J. N. ADAMS, S. M. KARK, L. STITH, M. G. CHAPPEL-FARLEY, M. A. YASSA;
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Abstract: Cortical networks that dynamically reconfigure are essential to memory and may be vulnerable to the effects of aging and Alzheimer's disease (AD). While functional connectivity (FC) has traditionally been measured by averaging across the entire time series in resting state fMRI (rsfMRI), recent analytic advances now enable characterization of dynamic brain states that fluctuate over the duration of the rsfMRI scan. We tested if brain state dynamics change with age and severity of clinical status across the AD continuum. Using rsfMRI data from the Alzheimer's Disease Neuroimaging Initiative, we performed dynamic FC analyses on 86 older adults (76 ± 7 yrs, 41 F) ranging from cognitively unimpaired (CU; n = 46), low cognitive impairment (CI-; n = 21; subjective memory complaints or early mild cognitive impairment/MCI) or high cognitive impairment (CI+; n = 21; MCI or AD). Data were preprocessed with the CONN Toolbox. Mean time series were extracted from 246 Brainnetome Atlas ROIs. K-means clustering identified six reliable brain states that explained the most variance. We characterized fractional occupancy (% of scan frames assigned to a state), appearance rate (# of times a state occurs/minute), and dwell time (mean duration of a continuous state) for each brain state. Older age was associated with lower fractional occupancy and appearance rate of a brain state corresponding to a positive limbic network (Fig 1A). Fractional occupancy, appearance rate, and dwell time of this positive limbic network were reduced in CI+ compared to CU participants (Fig 1B), independent of age. There was also a trend towards a reduction in fractional occupancy and dwell time in CI+ compared to CI-. These results suggest that brain states reflecting connectivity within the limbic network, which includes temporal lobe regions critical to memory and vulnerable to AD pathology, are particularly disrupted with age and impaired clinical status. Future work will assess the role of AD pathology and structural integrity on brain state characteristics and how dysfunctional brain states may contribute to memory decline.
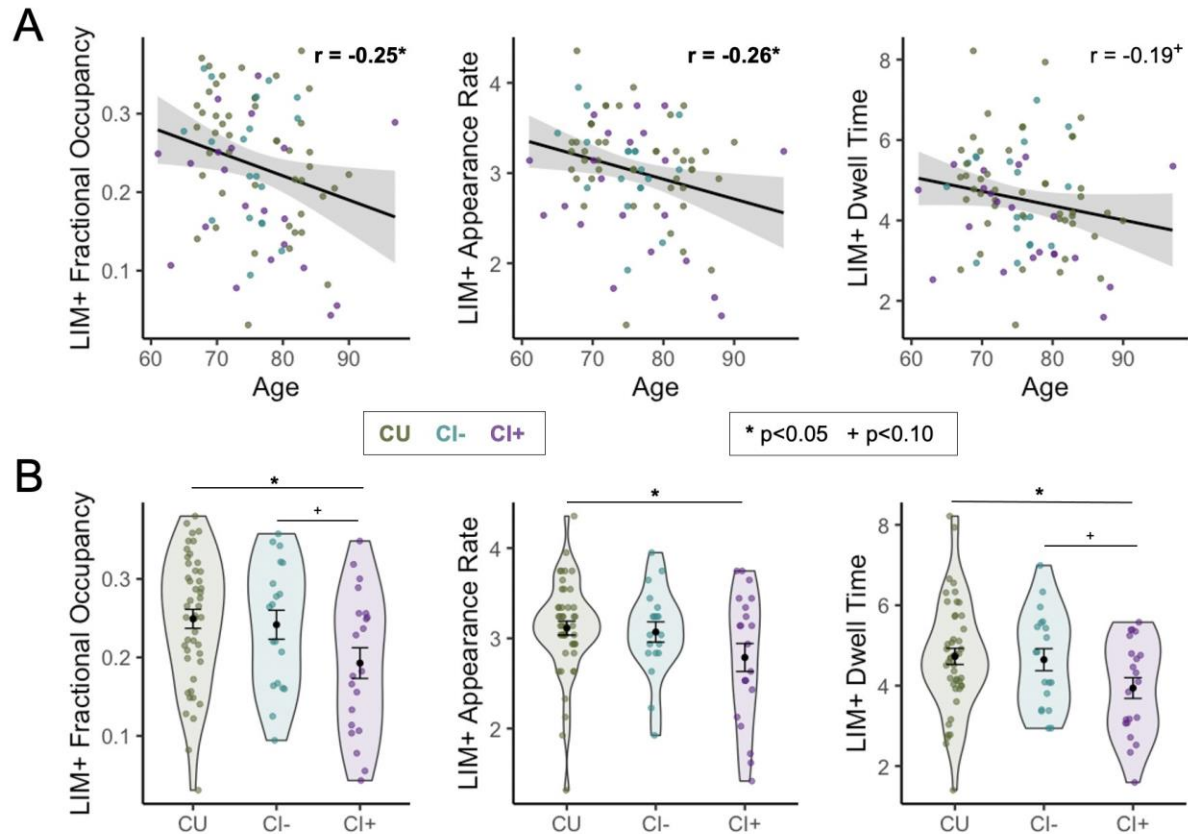
Nanosymposium

432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.01

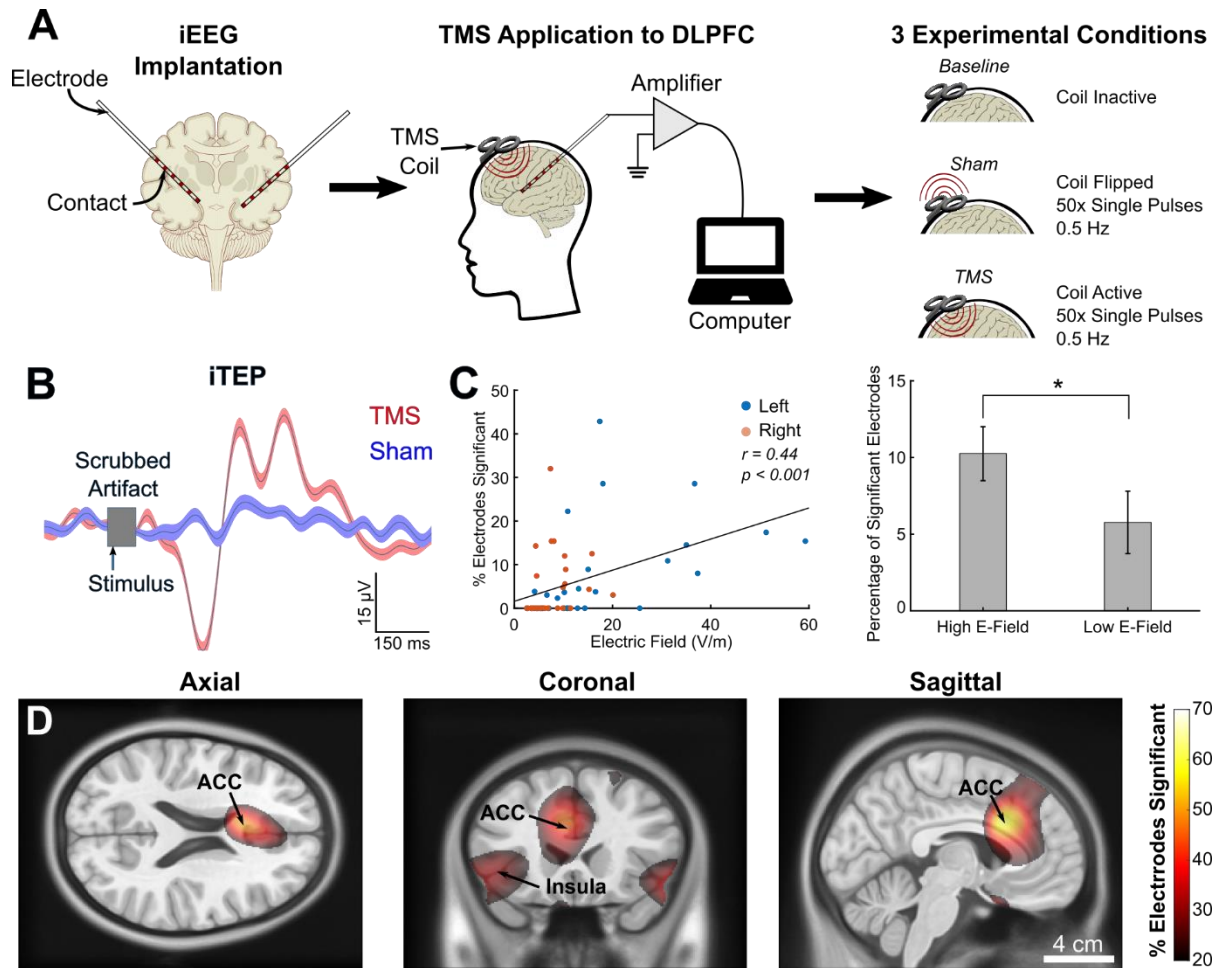**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Effects of transcranial magnetic stimulation on the human brain recorded with intracranial electrocorticography: First-in-human study

Authors: *J. B. WANG[1], J. E. BRUSS[3], H. OYA[4], B. D. UITERMARKT[3], N. T. TRAPP[5], P. E. GANDER[4], M. A. HOWARD, III[4], C. KELLER[2], A. D. BOES[6];
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Abstract: Transcranial magnetic stimulation (TMS) is increasingly used as a noninvasive technique for neuromodulation in both research models and humans. Clinically, it is FDA cleared for depression, smoking cessation, migraines, and obsessive-compulsive disorder, with clinical trials underway for many other neuropsychiatric disorders. It is also increasingly used as an experimental tool. The neurophysiological effects of TMS in animal models have been investigated extensively. However, efforts to understand the physiological effects of TMS in humans have been hampered by methodological limitations, specifically the lack of either spatial or temporal resolution, such as with surface electroencephalography (EEG) and functional magnetic resonance imaging (fMRI), respectively.
Here, we present the first in-human study evaluating the effects of TMS using intracranial electrocorticography (iEEG) in neurosurgical patients with high spatiotemporal resolution (Fig. A). We evaluate brain-wide intracranial responses to single pulses of TMS to the dorsolateral prefrontal cortex (dlPFC), the therapeutic target for depression and other neuropsychiatric disorders (N=10, 1414 electrodes). First, we demonstrate that TMS induces evoked potentials that are specific to TMS compared to sham stimulation (Fig. B). These iTEPs are induced locally within the dlPFC at sites with higher electric field strength (r = 0.44, p &lt 0.001) (Fig. C). We also show that downstream targets functionally connected to the dlPFC are also activated by single pulses of TMS, including regions central to depression such as the anterior cingulate and anterior insular cortex (Fig. D). We find that areas that are functionally connected to the dlPFC per fMRI are more likely to be activated by TMS to the dlPFC (2-Sample Student's T-test, p &lt 0.001). Together, these findings show that 1) TMS-iEEG is a safe and viable tool for studying the electrophysiological effects of TMS on the human brain and that 2) TMS induces responses both locally and in functionally connected downstream neuronal populations in humans.

A iEEG Implantation · TMS Application to DLPFC · 3 Experimental Conditions

B iTEP

C

D Axial · Coronal · Sagittal

Nanosymposium

432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.02

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Role of prefrontal cortex and lateral temporal lobe in healthy and anxious-depressed mental states during the performance of a cognitive control task

Authors: A. SHEKARA[1], A. C. PAULK[3], S. S. CASH[4], A. S. WIDGE[5], J. SHEEHY[1], *I. BASU[2];
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Abstract: Neuropsychiatric disorders are the number one cause of disability and health-related economic burden in the United States. Established therapies primarily focus on diagnostic labels, however a more robust approach would be to use objective constructs such as brain circuitry underlying functional deficits. Cognitive control is often compromised across mood and anxiety disorders and can be estimated with an interference task where subjects must suppress a natural response to overcome response conflict. Conflict evokes robust electrophysiologic signatures, such as theta (4-8 Hz) oscillations in the prefrontal cortex (PFC). However, we do not know much about how depressed/anxious (A/D) mental states modulate such circuits/rhythms. Furthermore, there is little indication about the role of lateral temporal lobe (LTL) in higher executive function. The objective of this work is to determine the neural signatures of PFC and LTL modulating cognitive control in healthy and A/D states. We recorded intracranial EEG (iEEG) from PFC and LTL of 16 human subjects with intractable epilepsy undergoing invasive monitoring while they performed a multi-source interference task (MSIT). Based on neuropsychological evaluation, we labeled the subjects' mental state as healthy or A/D. We estimated power in theta, alpha (8-15 Hz), beta (13-30 Hz), gamma (30-55 Hz) and high gamma (65-110 Hz) frequency bands. For each frequency band and brain region of interest, we fit a generalized linear mixed effects model (GLME): *Response ~ Conflict + State + (1|Subject)* where Conflict and State are binary variables coding the trial conflict and mental state of a participant respectively. We observed temporal differences in PFC and LTL spectrograms of healthy and A/D states, and fit subsequent GLMEs: *Response ~ Conflict + State +Time + State*Time + (1|Subject)* to examine interactions between mental state and time following stimulus presentation. In our first GLMEs, Conflict type was a significant predictor of theta power in dorsolateral PFC (dlPFC) and LTL, theta, alpha, and beta power in the dorsomedial PFC (dmPFC), and alpha power in dorsal anterior cingulate cortex (dACC). Mental state was a significant predictor of beta power in dACC. Our subsequent GLMEs found significant State*Time interactions on theta power in the dlPFC and LTL, and theta and alpha power in dmPFC and dACC. Our results demonstrate roles of both PFC and LTL in conflict encoding independent of mental state. A/D state influenced temporal features of PFC and LTL response, encouraging future exploration of time-dependent effects of mental state on conflict evoked oscillations.

Disclosures: A. Shekara: None. **A.C. Paulk:** None. **S.S. Cash:** None. **A.S. Widge:** None. **J. Sheehy:** None. **I. Basu:** None.

Nanosymposium

432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.03

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Discovering and Tracking Targets for Closed Loop Responsive Neurostimulation in Treatment-Resistant Post Traumatic Stress Disorder

Authors: *J. GILL[1], J. SCHNEIDERS[2], M. STANGL[2], Z. AGHAJAN[2], S. HILLER[2], U. TOPALOVIC[3], M. VALLEJO[4], C. S. INMAN[8], D. VILLAROMAN[2], A. BARI[2], A. ADHIKARI[5], V. R. RAO[9], M. S. FANSELOW[10], M. CRASKE[2], S. E. KRAHL[11], J. W. CHEN[6], M. VICK[12], N. R. HASULAK[13], J. C. KAO[2], R. KOEK[11], N. A. SUTHANA[7], J.-P. LANGEVIN[2];
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Abstract: [**Objective**] Though threat detection systems are critical for survival, they may lead to maladaptive behaviors if overactive following trauma. Identifying the neurophysiological correlates of these mechanisms in individuals with post-traumatic stress disorder (PTSD) is critical for the development of neural therapies, such as closed-loop responsive neurostimulation (RNS). In a first-of-its-kind study, we characterize the neurophysiological correlates of aversive stimulus processing and how it is modified using RNS therapy delivered to the amygdala.[**Methods**] 2 participants were implanted with the NeuroPace RNS System targeting the amygdala bilaterally for the purposes of treatment-resistant PTSD (TR-PTSD). Intracranial electroencephalography (iEEG) was collected during 1) presentation of unpleasant visual and audio laboratory stimuli (emotional image task and fear conditioning) and 2) at-home periods of symptom exacerbation. Additionally, iEEG activity of the amygdala was recorded in 6 human epilepsy participants without TR-PTSD who had the RNS device implanted during identical laboratory tasks.[**Results**] Analysis revealed that theta power was elevated during experimental and clinical aversive events in TR-PTSD. These changes were less pronounced following chronic RNS therapy. We found that negative stimulus-induced theta increases were reduced in epilepsy subjects without TR-PTSD. [**Implications**] We present the first identification of amygdala theta power as an ecologically valid signal that relates to self-reported, at-home symptom exacerbations and aversive laboratory stimuli in TR-PTSD. The TR-PTSD specific theta increase found in the present study may reflect disease-related alterations in amygdala circuitry. Lastly, we demonstrate that negative stimulus induced theta activity may be reduced

following chronic RNS therapy, identifying closed-loop stimulation of the amygdala as a potentially effective treatment strategy for PTSD.

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432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.04

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH R25 NS090978

Title: Default mode network theta power as a novel biomarker of depressed mood

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Abstract: A third of patients with major depressive disorder (MDD) are refractory to standard therapies. Progress in therapeutic neuromodulation for depression has been challenging because of the widespread, network basis of mood and lack of neurophysiologic biomarkers. Neuroimaging studies of depression demonstrate increased connectivity within the default mode network (DMN), which subserves internal processes such as self-reflection, mind wandering, and memory. Invasive electrophysiology in the resting state has demonstrated that DMN connectivity corresponds to correlations in theta-band power. DMN activity is anti-correlated with the dorsal attention network (DAN), which, in contrast, subserves externally oriented cognition (i.e., top-down attention), and demonstrates alpha-specific resting-state correlations. We investigated the relationship between frequency-specific activity within functional networks and mood fluctuations under the hypothesis that mood-specific electrophysiologic power fluctuations are theta-specific within the DMN and alpha-specific within the DAN. We used hourly, self-reported measures of mood and video facial affect recognition to estimate mood valence in patients undergoing invasive stereotactic electroencephalography for seizure monitoring. We analyzed power spectra and functional connectivity (via band-limited power correlations) in epochs corresponding to mood estimates. Depressed mood was correlated with increased theta power,

most significantly in electrodes within the posterior cingulate cortex, a key hub of the DMN. Conversely, alpha power was increased during elevated mood, most significantly in the intraparietal sulcus (a DAN region). Concordant with power changes, theta connectivity within the DMN was correlated with depressed mood and whereas alpha connectivity (in the DAN among other networks) was correlated with elevated mood. Our results recapitulate the frequency specificity of resting-state functional connectivity. Theta and alpha power within DMN and DAN networks, respectively, represent important biomarkers of mood and may guide neuromodulatory therapies. Future studies of the neural correlates of mood should consider adopting a network-based perspective of depression neurophysiology informed by resting-state physiology.

Disclosures: C. Hacker: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sora Neuroscience. **P. Brunner:** None. **J.T. Willie:** None. **E.C. Leuthardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sora Neuroscience, Inner Cosmos, Neurolutions.

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432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.05

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: 1UH3NS103550
                 2R01MH102238

Title: Deep brain stimulation of the subcallosal cingulate evokes two distinct electrophysiologic responses based on differential activation of distinct white matter targets

Authors: *A. SEAS[1], M. NOOR[1], K. CHOI[2], M. OBATUSIN[2], J. DAHILL-FUCHEL[2], A. VEERAKUMAR[3], C. C. MCINTYRE[1], H. S. MAYBERG[2], A. C. WATERS[2], B. HOWELL[1]; [1]Dept. of Biomed. Engin., Duke Univ., Durham, NC; [2]Ctr. for Advanced Circuit Therapeut., Icahn Sch. of Med. at Mount Sinai, New York City, NY; [3]Dept. of Psychiatry, Schulich Sch. of Med. and Dentistry, Univ. of Western Ontario, London, ON, Canada

Abstract: *Background*. Deep brain stimulation (DBS) of the subcallosal cingulate (SCC) region is an emerging therapy for treatment-resistant depression. Imaging studies have identified four white-matter tracts adjacent to the SCC that are activated during symptom-reducing DBS. Of these four, the forceps minor (FM) and bilateral cingulum bundles (CBs) are activated in the greatest proportions at therapeutic stimulation settings. Activating these bundles produces a reliable 150 ms cortical response. However, it is yet unknown which bundle activations drive this DBS-evoked potential (DBS-EP). The objective of this study is to characterize the SCC DBS-EP

of bundle-specific activations. *Methods.* Connectomic models of bilateral SCC DBS were constructed for seven patients. Stimulation parameters were optimized *in silico* to maximize unilateral stimulation of either the ipsilateral CB, or the adjacent aspect of the FM bundle in isolation. Low frequency (2 Hz) unilateral stimulation was performed at each of these bundle-specific settings. Control stimulation was also performed at 2 Hz at contacts used for therapeutic stimulation. The subsequent DBS-EPs were recorded using a 256-sensor scalp electroencephalography (EEG) system. *Results.* We observed two distinct DBS-EPs: FM-specific and control stimulation produced a similar 150 ms response that was symmetric across hemispheres based on visual inspection and calculation of a scalar metric of inter-hemispheric symmetry. This symmetry held true regardless of the side of stimulation. Unilateral CB stimulation produced a 30 ms asymmetric response in the hemisphere of stimulation that was significantly more asymmetric than the control DBS-EP, again without differences between L and R stimulation (p < 0.001 at 30 and 60 ms). DBS-EP magnitudes and transit times were not well predicted by the degree of pathway activations (all $R^2 < 0.16$). However, CB DBS-EPs were linearly separable from FM and control DBS-EPs through application of a support vector machine (SVM). This SVM utilized the degrees of FM and CB activation, as well as a scalar metric of inter-hemispheric symmetry, to accurately classify 83.3% of DBS-EPs. These distinct EEG responses evoked by bundle-specific stimulation outline two potential circuits modulated by SCC DBS. Further investigation is needed to clarify the role of these circuits in the therapeutic response to SCC DBS. *Impact.* DBS-EPs from track-specific and control stimulation provide insight into underlying circuit architecture of various white matter bundles around the SCC DBS target region. These findings demonstrate that the SCC DBS-EP can be tuned through differential FM and CB activation.

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432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.06

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Suboptimal lead placement in BROADEN SCC DBS trial for depression - Tractography analysis

Authors: *K. CHOI[1], J. KHANG[1], P. RIVA-POSSE[2], L. HIMES[3], B. CHEERAN[3], H. MAYBERG[1];
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Abstract: Background Open-label studies of SCC DBS for TRD have demonstrated sustained response and remission rates that are clinically significant. A patient-specific tractography-based targeting approach has further streamlined lead implantation surgery with improved clinical outcomes. This is in contrast to the halted multi-site, double-blind, randomized sham-controlled clinical trial (BROADEN). Heterogeneity in patient selection, as well as variability in targeting, accuracy of lead placement, and programming are potential contributors for the halted trial. As subjects in BROADEN were implanted using anatomical rather than tractography methods, we hypothesize that response variability may be explained by differences in WM activation pathways (WMAP) impacted by trial-and-error adjustments during the open-label follow-up period. We test this hypothesis here using a retrospective normative tractography analysis. **Methods**Fifty-five patients (of 90 enrolled) completed 2 years of open-label SCC DBS. WMAP was calculated for 47 patients (with adequate imaging data) using Lead DBS (https://www.lead-dbs.org). All 47 patients were assigned to one of four outcome groups: remitter (HDRS-17 &lt7, n=16), responder (HDRS-17 improvement &gt40%, n=8), low-responders (HDRS-17 improvement &lt40%, n=13), and non-responders (no HDRS-17 improvement at any time over 2 years, n=10). Standard imaging processing pipelines were used for generating common WM activation pathways. The anatomical location of active contacts was first compared among four groups. Then, common activation pathways were calculated using the HCP dMRI dataset, producing whole-brain common group activation pathways by Lead Group Analysis. **Results**There were no anatomical location differences in active stimulation contacts among the groups. Group tracts explained differences in outcomes. Remitters show complete WMAP of three bundles: forceps minor (FM), uncinate fasciculus (UF), and cingulum bundle (CB). Responders show similar CB and UF to remitters, but incomplete FM activation. Critically, non-responders showed no activation and low-responders showed incomplete activation of the left CB. **Conclusions**These findings provide evidence that poor outcomes in BROADEN were due to suboptimal lead implantation that could not be appreciated by anatomical location alone. As with independent experimental studies, patient-specific tractography-based targeting may be necessary to standardize and optimize SCC DBS.

Disclosures: **K. Choi:** F. Consulting Fees (e.g., advisory boards); abbott neuromodulation. **J. Khang:** None. **P. Riva-Posse:** None. **L. Himes:** A. Employment/Salary (full or part-time):; Abbott Neuromodulation. **B. Cheeran:** A. Employment/Salary (full or part-time):; Abbott Neuromodulation. **H. Mayberg:** F. Consulting Fees (e.g., advisory boards); Abbott Neuromodulation.

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432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.07

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Early experiences with Percept: LFP changes in two patients receiving VC/VS DBS for OCD.

Authors: *S. OLSEN[1], C. SULLIVAN[1], E. DASTIN-VAN RIJN[1], J. AMAN[1], D. DARROW[1], M. C. PARK[1], D. D. DOUGHERTY[2], A. S. WIDGE[1];
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Abstract: Obsessive compulsive disorder (OCD) has been repeatedly linked to hyper-connectivity of cortico-striatal-thalamo-cortical (CSTC) loops, which may be reflected in hyper-synchrony (or coherence) of neural firing between loop structures. Deep brain stimulation (DBS) of the ventral capsule/ventral striatum (VC/VS) has shown promise for treating OCD, but could be improved through targeted network disruption of multiple sites on the CSTC circuit. In our Early Feasibility study (https://clinicaltrials.gov/ct2/show/NCT03184454), we showed changes in coherence with combined stimulation of VC/VS and the supplementary motor area (SMA), which were linked to subjective feelings of symptom improvement in one patient with OCD. In preparation for our next patient, we have collected local field potential (LFP) recordings from Medtronic Percept in two patients receiving VC/VS DBS for OCD throughout their treatment. Early results show broadband increases in power with stimulation, which are related to improvement in OCD symptoms for the patient who responded to the treatment. We hope to leverage the knowledge gained from these single-site recordings for our second patient in the combined stimulation study.

Disclosures: S. Olsen: None. **C. Sullivan:** None. **E. Dastin-Van Rijn:** None. **J. Aman:** None. **D. Darrow:** None. **M.C. Park:** None. **D.D. Dougherty:** None. **A.S. Widge:** None.

Nanosymposium

432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.08

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Longitudinal study of the effects of subcallosal cingulate deep brain stimulation for treatment-resistant depression on the resting electroencephalogram

Authors: *T. NAUVEL[1], S. ALAGAPAN[2], M. OBATUSIN[1], S. HEISIG[1], J. DAHILL-FUCHEL[1], P. RIVA-POSSE[3], C. ROZELL[2], A. WATERS[1], H. S. MAYBERG[1];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Georgia Inst. of Technol., Atlanta, GA; [3]Psychiatry and Behavioral Sci., Emory Univ., Atlanta, GA

Abstract: Deep brain stimulation (DBS) of the subcallosal cingulate (SCC) white matter is a promising new treatment for treatment resistant depression. The aim of this project is the development of brain-based biomarkers that can track antidepressant response and guide clinical decisions including type and timing of parameter adjustments. The resting state EEG is the only clinical non-invasive recording technique able to directly measure the summation of large populations of neurons firing in the cortex, reflecting underlying cortical networks and circuitry. Thus, the resting EEG can be used to index basal brain activity and will give us insight into the dynamics of whole brain networks changing in these patients. Here, progress towards these goals is reported. We have begun analyses of the completed PC+S cohort (n=10), as well as a new ongoing cohort using the RC+S system (n=7). All patients are followed on a monthly basis at 8 time points: before implantation of the electrodes; after 4 weeks of implantation without stimulation; once a month following active stimulation for six months. Recordings consist of 5-minute eyes open and eyes closed resting EEG data (EGI, 256 channels), collected with bilateral stimulation both ON and OFF. Results suggest that patients show significant changes in their EEG over the course of treatment. The most consistent differences appear in the alpha and beta frequency band, either a shift in frequency, or a significant power change (increase or decrease). We find that enroute to wellness, the brain passes through multiple states which can be tracked using the resting state EEG. These findings demonstrate that the resting EEG can track fundamental changes in the brain demonstrating both short and long-term changes induced by the therapy. These findings invite further consideration of underlying biological mechanisms associated with the alteration of corticothalamic network activities reflected in the EEG.

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432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

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Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH NINDS BRAIN Initiative Grant 1UH3NS103549
                 NIH NINDS F99 Fellowship F99NS124181

Title: Characterization and modulation of neural substrates underlying cognitive control in treatment-resistant depression

Authors: *A. ALLAWALA[1], S. VARTANY[2], R. MATHURA[4], H. RITZ[1], J. ADKINSON[4], D. OSWALT[7], R. GADOT[4], A. SHENHAV[3], W. GOODMAN[5], N. POURATIAN[8], K. R. BIJANKI[6], S. A. SHETH[4], D. A. BORTON[1];
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Abstract: Deficits in cognitive control, defined as the ability to adjust response or attention in the face of competing or changing demands, are thought to be responsible for the inflexible behaviors observed in neuropsychiatric diseases. Here, we investigated frontotemporal networks underlying dysfunctional cognitive function using intracranial stereo-EEG (sEEG) recordings and deep brain stimulation (DBS) in human subjects with treatment-resistant depression (TRD). We employed a forced two-choice cognitive control task that parametrically and independently varied the strength of the target and the distractor interference of a visual stimulus, resulting in granular variation in response congruence. This task was performed in 13 total subjects, 10 of whom underwent sEEG implants for epilepsy monitoring, whereas 3 subjects were participating in a clinical trial of DBS for TRD and were additionally implanted with DBS leads in the ventral capsule/ventral striatum and subcallosal cingulate. As expected, reaction time (RT) and accuracy worsened with increasing levels of conflict across all subjects (Cohen's $d_{accuracy}$=0.7,and Cohen's d_{RT}= 0.2). Single-trial regression analyses of sEEG recordings revealed a parametric effect of task conflict with increasing theta-band (4-7 Hz) power across contacts in the dorsolateral prefrontal cortex (dlPFC), dorsal anterior cingulate cortex (dACC), orbitofrontal cortex (OFC), insula and the posterior cingulate cortex across all subjects. In subjects with TRD, high frequency stimulation (130 Hz) improved behavioral performance demonstrated by a reduction in RT from 1080±10ms SEM to 840±50ms SEM, and improved accuracy from 70±9.8% SEM to 90±5% SEM. While we observed an overall increase in theta-band power across contacts in the dlPFC and dACC in two TRD participants following DBS, the effect of conflict in theta-band power was reversed (i.e. theta-band power decreased with increasing conflict in the dlPFC and OFC). Further, we observed differences in the spatial distribution of contacts that exhibited increased theta band power as a function of conflict from pre- to post-DBS states. Finally, we investigated differential encoding of target vs. distractor dimensions across regions of interest using representational similarity analysis. Leveraging this unique behavioral paradigm with concurrent distributed intracranial recordings and causal manipulation broadens our understanding of neural substrates of controlled decision-making as well as the mechanisms of therapeutic action of DBS for TRD and other psychiatric disorders.

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432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.10

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH UH3 NS103468

Title: At home adaptive deep brain stimulation (DBS) for Parkinson's disease (PD) utilizing proportional plus integral control.

Authors: *S. L. SCHMIDT[1], A. CHOWDHURY[1], Q. GAO[1], K. MITCHELL[2], J. J. PETERS[1], K. GENTY[1], W. M. GRILL[1], M. PAJIC[1], D. A. TURNER[2];
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Abstract: Continuous DBS (cDBS) is an effective treatment for the motor symptoms of PD at fixed stimulation parameters independent of patient state. However adaptive deep brain stimulation (aDBS) may offer both better symptom control for fluctuating patient state and side effects reduction. But aDBS biomarker measurement and control parameter selection add considerable complexity, requiring additional expertise and time to tune. Further, the embedded controller available on present implantable pulse generators may not be optimal for simultaneous control of multiple neural biomarkers. In a trial cohort of six patients with PD with leads implanted in both subthalamic nucleus (STN) and globus pallidus (GP) bilaterally connected to a single Medtronic Summit RC+S implantable pulse generator (IPG) we implemented an external proportional plus integral (PI) controller and developed an experimental pipeline to determine aDBS parameters. All study activities were approved by the FDA and Duke IRB and all participants provided informed consent.
We first established STN beta amplitude as a biomarker for bradykinesia in 5 of the 6 participants. In random amplitude experiments, the range of possible aDBS amplitudes (between 0 and 100% of clinical amplitude) was randomly sampled in short time windows. All participants had at least one brain region with high correlation between beta amplitude and stimulation amplitude. The beta frequencies most responsive to DBS but free of artifacts were selected as the beta band input to the controller. We then selected an initial setpoint of the controller to match the mean beta amplitude in response to 70% of stimulation amplitude, using manual tuning of the proportional and integral gains. These initial aDBS parameters provided a similar level of symptom control as continuous DBS (cDBS) on the Unified Parkinson's Disease Rating Scale in all participants. In 5 participants stimulation power was reduced by >20%. We then applied PI aDBS in the home setting for one tremor-dominant participant, comparing 14 hours of PI aDBS and 47 hours of cDBS. In such a setting, the probability of tremor (>1mm) within a one minute window averaged 13.1% [-2.25 to 28.5%, 95% confidence interval] with PI aDBS control compared to 0.43% [-0.70% to 1.56%] on cDBS. During PI aDBS control the average stimulation power was reduced by 20.5% [3.98% to 37.1%] compared to cDBS. In the future PI aDBS could be embedded within IPGs to provide a more effective strategy for managing

fluctuating symptoms than the highly limited internal controllers now available. This work was supported by UH3 NS103468 and devices were donated by Medtronic PLC.

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432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.11

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Identification of candidate neural biomarkers of obsessive-compulsive symptom intensity and response to deep brain stimulation

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Abstract: Despite the success of deep brain stimulation (DBS) for treatment of refractory obsessive-compulsive disorder (OCD), there are currently no robust neural signatures for obsessive-compulsive (OC) symptoms or initial mood and energy improvements often associated with DBS. This may be due to limited opportunities available for conducting intracranial electrophysiological recordings in natural environments where fluctuations in symptoms take place. Recently available DBS platforms offer a way over this hurdle, allowing for streaming of intracranial neural activity both at home and in the clinic. Here, our goal was to identify neural correlates of both OC symptom intensity and acute changes in mood and energy. We conducted longitudinal intracranial recordings in nine participants with refractory OCD implanted with recording-capable DBS devices targeted to ventral capsule/ventral striatum (VC/VS). Four of the nine participants were implanted with additional sensing electrodes placed over the orbitofrontal cortex. We captured local field potentials at home during naturalistic exposures to OCD triggers, and in the clinic during variations in stimulation amplitude. All five participants who completed

the study were clinical responders to DBS therapy. Using the intracranial data collected during OCD exposures, we computed correlations between spectral power and OCD symptom severity. We identified low delta-band power as a candidate neural biomarker of OC symptom intensity during symptom provocations in one participant (left VC/VS: R=-0.59, p=0.01; right VC/VS: R=-0.56, p=0.04). Electrophysiological analysis of acute response to stimulation revealed a peak in VC/VS alpha band activity that was suppressed with optimal DBS. In OFC, we found a native beta band peak that shifted to alpha band with optimal DBS. We consider these VC/VS and OFC spectral changes in alpha and beta bands as preliminary biomarkers of the initial changes in mood and energy commonly seen during VC/VS DBS. These signals have potential utility for classification of symptom intensity and increased mood and energy in adaptive DBS systems for OCD. Continued opportunities for long-term, naturalistic intracranial electrophysiological recordings will propel biomarker discovery for OCD and other psychiatric disorders.

Nanosymposium

432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.12

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: A novel subcallosal cingulate biomarker of deep brain stimulation mediated stable recovery

Authors: S. ALAGAPAN[1], S. HEISIG[2], K. CHOI[2], A. WATERS[2], A. VEERAKUMAR[3], V. TIRUVADI[4], M. OBATUSIN[2], T. J. NAUVEL[2], J. CHA[2], M. FIGEE[2], A. L. CROWELL[4], P. RIVA-POSSE[4], R. J. BUTERA[1], H. S. MAYBERG[2], C. ROZELL[1];
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Abstract: Subcallosal cingulate deep brain stimulation (SCC DBS) has effectively treated patients with treatment-resistant depression (TRD). While gold standard clinical assessments are effective in determining when patients reach stable recovery, they do not help distinguish impending relapse from transient fluctuations in mood or emotional state, thereby impeding clinical decisions such as dose adjustment. Thus, there is a need for a biomarker that differentiates momentary instabilities from relapse. To address this gap, we aimed to identify features of SCC dynamics underlying stable recovery from local field potentials (LFP). LFPs were acquired weekly during the first 6 months of therapeutic SCC stimulation using Medtronic Activa PC+S. We used a neural network classifier to differentiate the beginning and the end of the 6 months using spectral features extracted from LFPs. We used a novel explainable artificial intelligence (xAI) tool to identify 'spectral discriminative factors (SDCs)' that identified the feature changes that explained the classifier performance. Depression severity was measured using Hamilton Depression Rating Scale (HDRS). Instead of tracking weekly HDRS scores, we binarized the HDRS into 'sick' and 'stable response' states and compared them to the states derived from SDC to verify that the SDC tracked this critical clinical outcome. The neural network classifier LFP was able to differentiate the early and late stages of DBS treatment (Area under ROC curve: 0.87 ± 0.09). The binarized states ('sick' and 'stable response') derived from SDC predicted corresponding states derived from HDRS (Area under ROC curve: 0.94 ± 0.04). In addition, the SDC predicted relapse in a participant whose LFP data was not used for training the classifier or the xAI model, suggesting that SDC generalizes beyond the training dataset. Notably, the SDC was significantly affected by changes in stimulation dose.
The results indicate that the SDC satisfies two essential requirements for a biomarker - tracking relevant changes in disease state and responding to intervention. Thus, the SDC may be a potential biomarker for SCC DBS, enhancing scalability in the DBS approach and reducing variability in outcomes.

Disclosures: S. Alagapan: None. **S. Heisig:** None. **K. Choi:** None. **A. Waters:** None. **A. Veerakumar:** None. **V. Tiruvadi:** None. **M. Obatusin:** None. **T.J. Nauvel:** None. **J. Cha:** None. **M. Figee:** None. **A.L. Crowell:** None. **P. Riva-Posse:** None. **R.J. Butera:** None. **H.S. Mayberg:** None. **C. Rozell:** None.

Nanosymposium

432. Real Time Monitoring of Human Brain Networks During Therapeutic Neuromodulation

Location: SDCC 24

Time: Tuesday, November 15, 2022, 8:00 AM - 11:15 AM

Presentation Number: 432.13

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Comparing cortical and thalamic signals for closing the loop on deep brain stimulation for essential tremor

Authors: *B. PARKS, L. ALMEIDA, M. S. OKUN, J. D. HILLIARD, K. D. FOOTE, A. GUNDUZ;
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Abstract: Essential tremor (ET) is a neurological disorder that is predominantly characterized by action-kinetic tremor of the bilateral upper limbs and is one of the most common neurological disorders. While medication can reduce these tremors, medication may not work for everyone or it may produce severe side effects. Deep brain stimulation (DBS) of the ventral intermediate nucleus (Vim) region of the thalamus in an open loop setting is a potential surgical strategy for tremor control. Continuous stimulation when there is no tremor to reduce may lead to side effects such as speech impairment or gait/balance issues, and may prematurely deplete the battery. Closed-loop DBS (CL-DBS) is a method of recording neurophysiology and triggering stimulation on when a specific biomarker, or surrogate biomarker, is detected. During clinical care, a depth lead is placed within the VIM region for delivering stimulation. Our study also places a cortical strip over the primary motor/somatosensory cortex, specifically targeted at the hand knob region. Using an investigational bidirectional neurostimulator (Summit RC+S, Medtronic PLC), we recorded movement-related changes in spectral content from the cortical strip and the depth lead simultaneously to implement cortical-based closed-loop (CL_C-DBS), and depth-based closed-loop (CL_D-DBS). The frequency band was independently chosen for each channel based on statistical testing and power analysis. During online classification, power values are calculated at selected intervals and compared to a given threshold, and stimulation is dynamically triggered on or off based on the power data. Here we show data collected from four patients with up to 18 months of follow-up post-surgery. CL_C-DBS is shown to be effective at turning on stimulation during continuous movement at all visits, while CL_D-DBS is more sporadic in its feasibility. When the closed-loop paradigm is tested, clinical efficacy for both is similar to conventional open-loop DBS, as determined by the Fahn-Tolosa-Marin tremor rating scale (TRS) score. We have shown that there is potential to use the depth lead alone for recording neurological signals and subsequently triggering stimulation on/off, but more research is required to understand the variability in separability between spectral components in different conditions. Translating these results to a broader population may decrease number of battery placement surgeries while maintaining clinical benefit.

Disclosures: B. Parks: None. **L. Almeida:** None. **M.S. Okun:** None. **J.D. Hilliard:** None. **K.D. Foote:** None. **A. Gunduz:** None.

Nanosymposium

511. Parkinson's Disease Molecular Mechanisms

Location: SDCC 1

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 511.01

Topic: C.03. Parkinson's Disease

Title: Dopamine compartmentalization defects initiate auxilin-linked Parkinson's disease

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Abstract: Background: Auxilin, a co-chaperone, participates in the uncoating of clathrin-coated
vesicles (CCVs) and facilitates synaptic vesicle (SV) regeneration at presynapses. Auxilin
(*DNAJC6/PARK19*) loss-of-function mutations cause early-onset Parkinson's disease (PD).
Here, we utilized auxilin knockout (KO) mice to elucidate the mechanisms through which
auxilin deficiency and clathrin-uncoating deficits lead to PD. **Methods:** We performed
longitudinal behavioral and histopathological analysis of wildtype (WT) and auxilin KO mice
and evaluated development of PD characteristics. To obtain unbiased insights into mechanisms,
we performed proteomic analysis of the whole brain, synaptosomes, and CCVs derived from 3-
month-old auxilin KO and WT mice. Taking cues from the proteomic results, we performed
neurochemical analyses to measure levels of dopamine and its metabolites, and *in vivo* fast-scan
cyclic voltammetry along with a new computational analysis to quantitate striatal dopamine
release and reuptake kinetics. Dopamine transporters (DAT) membrane localization was
evaluated by immunostaining, novel dichloropane-based *ex-vivo* imaging, and conventional and
immuno-electron microscopy (EM). Monoaminergic neurotransmitter transporters and endocytic
proteins were also evaluated by immunostaining. The SVs, CCVs and autophagic vacuoles in the
dorsal striatum were evaluated using EM. **Results:** We show that auxilin KO mice display the
cardinal features of PD, including progressive motor deficits, α-synuclein pathology, nigral
dopaminergic neuron loss, decreased striatal dopamine levels, and gliosis. Through proteomic
and neurochemical analyses, we demonstrate that dopamine homeostasis is disrupted in auxilin
KO brains, including slower dopamine reuptake kinetics *in vivo*, an effect associated with DAT
misrouting into axonal membrane deformities in the dorsal striatum. We also show that elevated
macroautophagy and defective SV protein sorting contribute to ineffective dopamine
sequestration and homeostasis, ultimately leading to neurodegeneration. **Conclusions:** Our
findings indicate that auxilin mutations and clathrin uncoating deficits lead to PD through four
distinct mechanisms: cytoplasmic dopamine accumulation, DAT mis-trafficking, SV sorting
deficits and autophagic overload, which collectively lead to dopamine compartmentalization
defects. This study advances our knowledge of how presynaptic endocytosis deficits lead to
dopaminergic vulnerability and pathogenesis of PD.

Nanosymposium

511. Parkinson's Disease Molecular Mechanisms

Location: SDCC 1

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 511.02

Topic: C.03. Parkinson's Disease

Title: Electrophysiological and histological features of curcumin neuroprotection on rotenone induced neurodegeneration in a rat model of Parkinson's disease

Authors: *N. BEHNAM DEHKORDI, L. V. DARBINYAN, K. V. SIMONYAN, L. E. HAMBARDZUMYAN, L. P. MANUKYAN, N. V. SARGSYAN, V. H. SARKISIAN;
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Abstract: Objectives: Parkinson's disease is an age-related neurodegenerative disease characterized clinically as a movement disorder. Motor symptoms in PD are caused by the selective degeneration of dopaminergic neurons in the ventral midbrain's Substantia nigra (SN), which depletes dopamine levels in the striatum. Rotenone is involved in the degeneration of dopaminergic neurons, and curcumin may prevent or effectively slow the progression of Parkinson's disease . The present study involves investigation of rotenone-induced histological changes in the brain area, hippocampus using Nissl staining after 35 day of subcutaneous injection of rotenone in adult male rats **Methods:**. The link between electrophysiological characteristics and neuronal morphology has not been found yet. To investigate these issues, we recorded and classified the electrical activity of individual SNc neurons in the control, Rotenone and Curcumin-treated PD rat models. Then, we analyzed the spike activity and the morphological changes in the SNc following a rotenone-induced degeneration. **Results:** In this manner, the electrophysiological impairments could be related to the morphological alterations in the SNc in the pathophysiology of PD. We sought to determine whether curcumin could protect against rotenone-induced dopaminergic neurotoxicity in a rat model by in vivo electrical recording from Substantia nigra pars compacta (SNc). **Conclusion:** Results obtained show that Curcumin administered through the i.p. route provides substantial protection to SN DA neurons in a rotenone rat model of PD. Our study provides evidence for the therapy of PD as well as the underlying mechanism of Curcumin's neuroprotective activity. Given the intricacy of molecular and neurological systems, more research is needed to pinpoint the precise process. Curcumin treatment significantly improved electrical activity of neurons in the SNc Keywords Curcumin • Rotenone • Substantia nigra pars compacta • In vivo electrophysiology

Disclosures: N. Behnam Dehkordi: None. **L.V. Darbinyan:** None. **K.V. Simonyan:** None. **L.E. Hambardzumyan:** None. **L.P. Manukyan:** None. **N.V. Sargsyan:** None. **V.H. Sarkisian:** None.

Nanosymposium

511. Parkinson's Disease Molecular Mechanisms

Location: SDCC 1

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 511.03

Topic: C.03. Parkinson's Disease

Title: The NLRP3 inhibitor RRx-001 crosses the blood brain barrier and alleviates neuroinflammation in experimental models of Parkinson's disease

Authors: S. JEWELL[1], M. YULE[1], N. BIRCH[1], R. J. ADAM[2], J. O'SULLIVAN[1], *****B. ORONSKY[3], T. R. REID[3], R. GORDON[4];
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Abstract: There are currently no effective disease modifying treatments for Parkinson's disease (PD) which continues to increase in prevalence globally with an ageing population. Chronic immune and inflammasome activation are pathological hallmarks of PD which are thought to drive progressive dopaminergic neuron loss and synuclein pathology leading to disease progression. RRx-001, a small molecule anticancer and chemoprotective agent in Phase 3 clinical trials, was recently confirmed to be a covalent NLRP3 inflammasome inhibitor (IC_{50} = 117 nM) that, in animal models, is effective in mitigating inflammatory pathology. We hypothesized that RRx-001 crosses the blood brain barrier and could inhibit chronic inflammasome activation, neuroinflammation and neuronal death in the Central Nervous System (CNS). To evaluate the CNS uptake of RRx-001, a quantitative whole-body autoradiography (QWBA) study was performed with 10 mg/kg of ^{14}C-labeled RRx-001 administered to 3 male Hanover Wistar rats over 10 time points from 0.083 hour (h) to 72h. NLRP3 inflammasome activation was evaluated with RRx-001 *in vitro* using primary microglia and RAW-Blue NF-κB reporter cells stimulated with inflammasome activators relevant to Parkinson's disease (PD). We also evaluated the neuroprotective efficacy of RRx-001 in preclinical models of PD. From 24 to 168 hours, significant increases in the concentration of radioactivity were observed in meninges (23-fold) and pituitary gland (8-fold) as well as the meninges and brain choroid plexus, indicating that drug-derived radioactivity crossed the blood-brain barrier. Our results showed that RRx-001 was not cytotoxic in macrophages or microglial cells and significantly reduced inflammasome activation and neuroinflammation markers in experimental models of PD pathology. Additionally, our mechanistic studies in dopaminergic neuronal cultures suggest that RRx-001 can prevent mitochondrial dysfunction and fragmentation induced by the Parkinsonian neurotoxicant MPP^+. Together, our data demonstrates that RRx-001 crosses the blood brain barrier and can mitigate inflammasome activation in microglia and macrophages. This highlights the neuroprotective properties of RRx-001 as a novel disease-modifying agent for PD and other neurodegenerative diseases linked to NLRP3-driven pathology.

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Nanosymposium

511. Parkinson's Disease Molecular Mechanisms

Location: SDCC 1

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 511.04

Topic: C.03. Parkinson's Disease

Support: Parkinson Canada Basic research fellowship

Title: Function and neuroprotective potential of Flcn knockout in Parkinson's disease

Authors: *J. OBERGASTEIGER[1], O. FERGUSON[1], C. BOLDUC[1], A. BILODEAU[1], T. DURCAN[2], F. LAVOIE-CARDINAL[1], E. METZAKOPIAN[3], M. LEVESQUE[1];
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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by various motor and non-motor symptoms in patients. One of the main hallmarks of the disease is the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). The second hallmark is the accumulation of alpha-synuclein (aSyn) in so called Lewy bodies. However, there is still no cure for PD and current treatments only alleviate the symptoms of the disease. Therefore, there is an unmet need to provide new therapeutic targets. We performed a CRISPR-based, genome-wide screen to identify new target genes rescuing the degeneration of dopaminergic neurons. From our screen, we identified several new targets and one of them is the Flcn gene. Flcn is implicated in the mTOR pathway, able to regulate autophagy and to interact with several Rab GTPases involved in endocytic trafficking. Furthermore, Flcn was shown to regulate mitochondrial biogenesis. Importantly, when exposed to oxidative stress, Flcn KO increases the viability of dopaminergic neurons *in vitro*. To understand the physiological role of Flcn in DA neurons and validate its potential neuroprotective effect, we knocked out Flcn in mouse dopamine neurons of the SNc. For modelling PD, we used AAV-mediated expression of human aSyn in the SNc. We performed locomotor assessment and histological analysis of the brain 16 weeks after the injection of the AAVs. Flcn KO in SNc dopamine neurons ameliorates the motor deficits induced by aSyn overexpression. Furthermore, Flcn KO rescues the loss of dopaminergic neurons in the midbrain and their terminals in the striatum. Interestingly, Flcn KO also modulates the levels of phosphorylated aSyn in the SNc. To validate our results, we used iPSC-derived dopaminergic neurons as a model of human PD *in vitro*. To account for biological variability, iPSC lines with PD-related mutations were used with their respective isogenic controls. Preliminary results indicate that the Flcn KO in human dopaminergic neurons rescues mitochondrial function deficits, reduced reactive oxygen species and the modulates the number of autophagosomes/lysosomes. In this study, we used an unbiased screening method to identify new neuroprotective targets for PD. Following target validation, we will try to identify drugs capable of modulating Flcn expression and test their efficacy to modify disease onset and/or progression.

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Nanosymposium

511. Parkinson's Disease Molecular Mechanisms

Location: SDCC 1

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 511.05

Topic: C.03. Parkinson's Disease

Title: Advancements in a HTS drug discovery pipeline targeting intrinsically disordered proteins: Moving toward models of neurodegenerative disease phenotypes

Authors: *A. R. BRAUN, E. E. LIAO, N. NATHAN KOCHEN, K. A. SMITH, J. N. SACHS; Biomed. Engin., Univ. of Minnesota TC, MINNEAPOLIS, MN

Abstract: Our group has been developing a high-throughput screening (HTS) drug discovery pipeline that target the pathological misfolding of intrinsically disordered proteins (IDP) associated with neurodegenerative diseases. Our successful HTS campaigns targeting alpha-synuclein (aSyn), tau, and Huntington protein have identified hit compounds capable of rescuing cellular pathological phenotypes in orthogonal secondary assays, while displaying direct target engagement. We have continued the evolution of our cellular fluorescence lifetime FRET (FLT-FRET) biosensors to improve the signal response, reduce false-positives from interfering compounds, and increase the scalability of our HTS models.With these improvements we have begun to explore heterogenous protein biosensors and organelle specific FLT-FRET systems, thereby targeting specific IDP populations and protein-protein interactions for aSyn and tau. Coupling these efforts with hit compounds identified from small compound libraries with known mechanisms of action (MOA) provides insight into the direct or indirect cellular pathways that are involved in modulating aSyn and tau misfolding. Lastly, we are expanding our drug discovery pipeline to include phenotypic cellular screening (e.g., dysfunctional protein trafficking, proteasomal and autophagic dysfunction) as well as computational docking and biophysical assays to identify novel hit compounds and further elucidate the MOA of current hits.

Disclosures: A.R. Braun: None. **E.E. Liao:** None. **N. Nathan Kochen:** None. **K.A. Smith:** None. **J.N. Sachs:** None.

Nanosymposium

511. Parkinson's Disease Molecular Mechanisms

Location: SDCC 1

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 511.06

Topic: C.03. Parkinson's Disease

Title: Fosgonimeton, a small molecule positive modulator of hepatocyte growth factor (HGF)/MET, protects against neuronal damage and motor deficits in preclinical models of Parkinson's disease

Authors: S. SETTI, A.-A. BERTHIAUME, S. REDA, R. TAYLOR, M. KNEIP, J. JOHNSTON, *K. CHURCH;
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Abstract: Parkinson's disease (PD) is a progressive and debilitating neurodegenerative disorder that is characterized by severe movement dysfunction. The primary pathological mechanism of the disease is the loss of dopaminergic neurons and associated signaling in the substantia nigra. Hepatocyte growth factor (HGF) and its receptor MET form a neurotrophic system with potent neuroprotective properties supporting neurite outgrowth and synaptogenesis. Here we evaluate the efficacy of fosgonimeton, a small molecule positive modulator of HGF/MET, in reducing pathological markers of PD in vitro and in vivo. PD can be modeled preclinically using the dopaminergic neuron-specific toxin 6-hydroxydopamine (6-OHDA). In primary cultures of mesencephalic neurons, exposure to 6-OHDA precipitates a loss of tyrosine hydroxylase-positive (TH+) dopaminergic neurons, diminished TH+ neurite networks, and accumulation of alpha-synuclein protein. In the current work, treatment of primary mesencephalic cultures with concentrations of fosgo-AM (the active metabolite of fosgonimeton) ranging from 1 nM to 1 µM led to a statistically significant prevention of all assessed 6-OHDA-induced effects. In vivo, a unilateral striatal injection of 6-OHDA results in loss of dopaminergic neurons in the substantia nigra, extensive reductions in striatal dopaminergic projections and neurite networks, and subsequent deficits in motor function. Daily fosgonimeton administration at 0.25 mg/kg (s.c.) beginning 2 weeks following 6-OHDA lesioning in rats ameliorated motor function deficits across several behavioral assessments, including the cylinder test, apomorphine induced rotation, grip strength, and rotarod. Immunohistochemical evaluation suggests that these beneficial effects may be mediated by protection of dopaminergic neurons in the striatum, as evidenced by preserved TH+ staining. In summary, at clinically relevant concentrations, fosgonimeton exerts therapeutic effects against PD-related pathology in vitro and in vivo, with benefits ranging from neuroprotection of dopaminergic neurons, to a mitigation of alpha synuclein accumulation, to functional improvements in motor performance. These findings highlight the potential value of fosgonimeton in the treatment of clinical PD. Fosgonimeton is currently being evaluated in SHAPE, a phase 2 clinical study for Parkinson's disease dementia and dementia with Lewy bodies (NCT04831281), and LIFT-AD, a phase 3 study for Alzheimer's disease (NCT04488419), with recently reported topline data in ACT-AD, a phase 2 study for Alzheimer's disease (NCT04491006).

Disclosures: S. Setti: A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder,

Nanosymposium

511. Parkinson's Disease Molecular Mechanisms

Location: SDCC 1

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 511.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Elucidating the role NK cells in preformed fibril alpha synuclein mice and profiling blood NK cell subsets in patients with Parkinson's disease.

Authors: *J.-K. LEE[1], S. WEBER[1], K. B. MENEES[1], J. CHUNG[1], R. N. ALCALAY[2,3];
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Abstract: The hallmark of PD pathology includes the accumulation of misfolded α-syn, a component of Lewy bodies. Alpha-synuclein (α-syn) can self-assemble to form fibrillar aggregates and induces neuroinflammation. Natural killer (NK) cells belong to innate immune cells and are reported increased in numbers and decreased in the expression of the inhibitory receptor (NKG2A) in PD patient blood. Our recent studies demonstrated that NK cells are present in the parenchyma of PD, are capable of clearing α-syn, and the depletion of NK cells resulted in exacerbated motor deficits and increased insoluble α-syn deposits in a preclinical mouse PD model. To assess the role of NK cells in peripheral synuclein pathologies, NK cells were depleted in the preformed fibril α-syn-induced PD mice and we assessed a fecal output assay and water contents. To characterize NK phenotypes in PD. We performed flow analysis on

cryopreserved PBMC from PD patients and measured the NK subsets (CD56 and CD16) and the phenotypic expression of inhibitory, activating, and homing receptors on their surface. Our data suggests that PFF α-syn injection induced progressive impairment of gut motility in M83 Tg mice, however, there was no significant differences on gut motility in NK cell depleted mice. Our data showed that there were no major differences in leukocytes composition between PD and controls and the frequencies of CD56^{bright}, CD56^{dim}, CD56$^-$ NK subsets between PD and controls. Importantly, within the disease group, we found that the frequencies of NKG2D expressing CD56^{bright} and CD56^{dim} NK subsets were significantly increased upon disease severity. The frequencies of NKG2A expressing CD56^{bright} NK subset was significantly increased upon disease severity. Our data implicate that the neuroprotective phenotype of NK cells is presented in the CNS and periphery and highlight the potential capability of NK characterization as a potential biomarker for the early diagnosis of PD.

Nanosymposium

512. Traumatic Injury: Repair and Mechanisms

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 512.01

Topic: C.10. Brain Injury and Trauma

Title: Brain extracellular matrix alters local ion concentrations and responses to injury

Authors: *K. NORMOYLE[1], V. DHZALA[1], K. LILLIS[1], K. EGAWA[2], J. GLYKYS[3], N. RAHMATI[4], K. STALEY[1];
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Abstract: The reversal potential of GABA receptors (E$_{GABA}$) is dependent upon the chloride concentrations on both sides of the neuronal membrane. The chloride on the extracellular aspect of the membrane ([Cl$^-$]$_o$) is canonically considered to equal the chloride in the bulk cerebrospinal fluid. However, neurons are surrounded by an extracellular matrix (ECM) comprised of variably

sulfated glycosaminoglycans that can alter local chloride concentration and can be hydrolyzed by active matrix metalloproteinases released by tissue injury. Having synthesized a single-wavelength, pH-insensitive chloride-sensitive fluorophore and constrained it to the extracellular space by conjugation with 10 kilodalton dextran, we used 2-photon Fluorescence Lifetime IMaging (FLIM) to measure extracellular chloride in acute and organotypic hippocampal slices, and in vivo cortex. We first tested whether the extracellular sulfate moieties change the baseline local chloride concentration. We found that $[Cl^-]_o$ between neurons in the depths of acute hippocampal slices and at all depths of organotypic hippocampal slice cultures was only half of the bulk CSF chloride. We next measured $[Cl^-]_o$ after the sulfate moieties in the matrix were freed by endogenous matrix metalloproteinases (MMPs) after brain injury. We found a strong dependence of $[Cl^-]_o$ vs distance from injury, with Cl concentration increasing to the ACSF levels near the injured surface of acute slices or proximity to photolysed neurons in organotypic slices, respectively. These changes in $[Cl^-]_o$ should also alter the neuronal intracellular chloride ($[Cl^-]_i$) via the activity of the high-velocity equilibrative membrane chloride transporters. We have previously reported such changes in slices and confirmed them in both injury models here, in addition to in vivo mouse and piglet models presented here. If the injury-induced increases in $[Cl^-]_o$ and $[Cl^-]_i$ were due to release of extracellular sulfates and replacement by chloride, these sulfates should be released to the perfusate, and we confirmed this using colorimetric assays of chondroitin sulfate. Finally, the release of sulfates should be inhibited by MMP antagonists, which we confirmed with both broad-spectrum and more specific inhibition (ZX-1 or SB3CT, respectively) each of which reduced $[Cl^-]_o$ and $[Cl^-]_i$ and neuronal volume at the surface of cut slices and in proximity to photolysed neurons. We conclude that $[Cl^-]_o$ is partially displaced by sulfates in the (ECM) and that damage to ECM following brain injury alters the transmembrane chloride distribution. These findings have immediate implications for the treatment of cytotoxic edema and seizures after acute brain injury.

Disclosures: K. Normoyle: None. **V. Dhzala:** None. **K. Lillis:** None. **K. Egawa:** None. **J. Glykys:** None. **N. Rahmati:** None. **K. Staley:** None.

Nanosymposium

512. Traumatic Injury: Repair and Mechanisms

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 512.02

Topic: C.10. Brain Injury and Trauma

Title: Responses to mild blast-induced traumatic brain injury in mice

Authors: *B. A. CITRON[1,3,4], K. E. MURRAY[1,4], V. A. STIRITZ[2,4], T. P. COMINSKI[2], A. R. RAVULA[5], B. J. PFISTER[5], K. D. BECK[2,3,4], V. DELIC[1,3,4];
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Abstract: Approximately 400,000 traumatic brain injuries (TBIs), most of them mild, have been sustained among the 2 million service personnel deployed from 2000 to 2020. In training and combat zones, TBIs are typically caused by exposure to blast waves from a variety of sources, and long-term neurodegenerative deficits can develop without an effective treatment. Additional attention is needed to better understand genetic predispositions for susceptibility vs. resilience and repair. Our injury model utilizes a well-established blast tube system designed to mimic pressure waves experienced during a field explosive detonation. With a variety of genetically distinct mouse strains, batteries of behavioral tests are executed at baseline prior to the injury and into chronic phases after blast exposure to assess the role of genetics in recovery of functional outcomes while we also compare TBI-induced regional changes in markers of neurodegeneration and alterations in gene expression. We found that the acoustic startle response, a three-synapse reflex reflecting brain sensorimotor processing, indicated sex differences. At 30 days after mild blast exposure (with ears protected), the magnitude of the response was reduced 55% compared to sham male mice but only reduced 41% in injured females compared to sham females. This indicates that there are differential deficits in sensorimotor function. There were no differences between groups at lower level intensities of the acoustic stimulus. This research represents a multi-level examination of how certain genes may influence blast-induced TBI and recovery and provides an essential foundation for understanding how these genes play roles in the post-injury outcomes in order to advance the identification of therapeutic targets that could be modulated to improve the health of Veterans and others with histories of blast exposures.

Disclosures: B.A. Citron: None. **K.E. Murray:** None. **V.A. Stiritz:** None. **T.P. Cominski:** None. **A.R. Ravula:** None. **B.J. Pfister:** None. **K.D. Beck:** None. **V. Delic:** None.

Nanosymposium

512. Traumatic Injury: Repair and Mechanisms

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 512.03

Topic: C.10. Brain Injury and Trauma

Title: Understanding Chromatin Regulatory Mechanisms Underlying CNS Injury and Repair

Authors: *Y. CHENG[1], F. TIAN[2], Z. HE[3], D. H. GESCHWIND[1];
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Abstract: How mature CNS neurons can survive acquire a growth-permissive state after being damaged is a central question in the field of neural repair. A specific cell state ultimately represents the readout of specific gene expression programs, whose transcription are driven by chromatin regulatory mechanisms. We hypothesize that chromatin regulations of survival- and growth-associated gene programs play a major role in setting the permissive or non-permissive growth state of CNS-injured neurons. Here, we leveraged state-of-the-art functional genomics approaches to gain insight into chromatin state changes driving gene regulation in response to CNS injury. Joint profiling of ATAC-seq and RNA-seq in RGCs following optic nerve injury revealed widespread but correlative chromatin accessibility and gene expression changes, potentially driven by a set of injury-reactive transcriptional factors (TFs). To further define these TFs' actions, we intersected with our recent genome-scale, *in vivo loss-of-function* CRISPR screens of ~1,800 TFs critical for injury-induced neuro-degeneration or regeneration. This analysis converged on two distinct sets of TF regulators critical for RGC cell death and axon regeneration, which are essentially non-overlapping. The degeneration screens converged on ATF3/4, C/EBPγ and CHOP as repressors of RGC survival, which act as two groups (ATF3/CHOP and ATF4/C/EBPγ) to activate two distinct, but complementary, pro-death gene programs. Co-deletion of one TF from each of the two complementary sub-groups results in higher survival compared to a single deletion, achieving nearly full neuroprotection. In the regeneration screen, we identified two groups of TFs as negative regulators of axon regeneration, predicted to act on complementary growth-associated gene programs. One group of TFs consists of CTCF, SIN3A/REST, TCF3, TGIF1 and EBF3, binding to genes involved in neuronal maturation, synaptic function and neurotransmission. The other group of TFs includes LHX2 and LHX6, targeting genes important for cell migration and motility. The core transcriptional interactions of these regeneration-associated TFs are currently under investigation. Together, these findings reveal key chromatin regulators of neuronal survival and axon regeneration, advancing our understanding of chromatin regulatory mechanisms that underlie CNS injury and repair.

Disclosures: Y. Cheng: None. **F. Tian:** None. **Z. He:** None. **D.H. Geschwind:** None.

Nanosymposium

512. Traumatic Injury: Repair and Mechanisms

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 512.04

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS093073

Title: The Mechanical Microenvironment Shapes Central Axon Ultrastructure Visualized by Cryo-Electron Tomography

Authors: *C. GU;
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Abstract: Axon shafts mediate unidirectional conduction of action potentials and long-distance transport of proteins and organelles from neuronal soma to its synaptic terminals. How these long slender structures are mechanically regulated remains poorly understood. Combining confocal microscopy and cryo-electron tomography (Cryo-ET) with *in vivo* and *in vitro* systems, we report that non-uniform mechanical interactions with microenvironment can lead to more than 10-fold diameter enlargement in an axon of the central nervous system (CNS). In the normal brain of adult transgenic mice with some neurons expressing yellow fluorescent protein (YFP), individual axons in the cortex displayed significantly higher diameter variation than those in the corpus callosum. When being cultured on lacey carbon film coated electron microscopy (EM) grids, CNS axons formed varicosities almost exclusively in holes, with enriched mitochondria, multivesicular bodies (MVBs) and small vesicles, similar to axonal varicosities induced by mild fluid puffing. Microtubules (MTs) remained constant in axonal varicosities induced by non-uniform support, and MT bundles were unevenly split at axon branch points often containing MT free ends. When axons were fasciculated mimicking *in vivo* axonal bundles in white matter, varicosity levels reduced. Taken together, the results reveal these novel features of three dimensional ultrastructures of central axons in response to the mechanical microenvironment.

Disclosures: C. Gu: None.

Nanosymposium

512. Traumatic Injury: Repair and Mechanisms

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 512.05

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 1R01NS113969-01
NIH Grant 1RF1NS125578-01

Title: Inflammasome activation in the heart after traumatic brain injury

Authors: *J. P. DE RIVERO VACCARI[1], B. CYR[3], R. HADAD[2], R. W. KEANE[4];
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Abstract: Traumatic brain injury (TBI) results in systemic complications, including the cardiovascular system, that influence long-term outcomes. Here we characterized the inflammasome-mediated inflammatory response in the brain and heart as well as the composition of extracellular vesicles (EV) in serum after TBI in mice. TBI induced elevations in the levels of inflammasome signaling proteins (AIM2, ASC, Caspases-1, -8 and -11) in the atrium of injured mice at 3 days after TBI, while IL-1β was elevated in the ventricles. In addition, significant elevation in the levels of the inflammasome signaling proteins (IL-1β, ASC, caspases-1, -8 and -11) were measured in the cortices of injured mice at 3 days after injury and compared to sham uninjured animals. EV from serum at 3 days after injury were characterized for size, concentration and protein profile using nanoparticle tracking analysis (NTA) and immunoblotting. Serum-derived EV from injured animals contained an elevation in inflammasome signaling proteins when compared to sham animals. Thus, our results indicate that inflammasome activation occurs primarily in the atrium of the heart following TBI, and that the systemic inflammatory response following TBI is contributed in part by the release of EV containing a cargo of inflammasome signaling proteins into serum.

Disclosures: 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. **B. Cyr:** A. Employment/Salary (full or part-time):; University of Miami. **R. Hadad:** 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.

Nanosymposium

512. Traumatic Injury: Repair and Mechanisms

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 512.06

Topic: C.10. Brain Injury and Trauma

Title: Morphological Changes of Astrocytes and Microglia Following Mild Traumatic Brain Injury

Authors: *G. D. NAH[1], N. PORT[2], A. G. HOHMANN[1], J. D. CRYSTAL[1];
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Abstract: Mild traumatic brain injury (mTBI) is the most common type of traumatic brain injury, and it leads to temporary memory impairment as well as an excitotoxic response in the brain, particularly the hippocampus. Much emphasis has been placed on studying the expression of astrocytes and microglia as neuroinflammatory markers of injury in the hippocampus. However, the extent of the inflammation response in relation to memory impairment has not been thoroughly investigated. Wayne State modified weight drop rat model of mTBI accurately recapitulates the elements of a sport-related injury, as well as the excitotoxic response in the hippocampus. Is this study, we measured the expression of astrocytes and microglia 24hr following mTBI in sprague-dawley rats. The rats underwent either a weight drop or sham treatment using the Wayne State model. We measured the expression of astrocytes and microglia by quantifying five parameters of individual cells using the Leica LAS X microscope software: roundness, perimeter, area, length, and shape factor. Astrocytes in the weight drop group were significantly different than the sham group on all five parameters ($p < 0.01$). Microglia in the weight drop group were also significantly different than the sham group on all five parameters ($p < 0.001$). In future studies, we will conduct a time-course to track the expression of astrocytes and microglia in the dentate gyrus of the hippocampus of rats.

Disclosures: G.D. Nah: None. **N. Port:** None. **A.G. Hohmann:** None. **J.D. Crystal:** None.

Nanosymposium

512. Traumatic Injury: Repair and Mechanisms

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 512.07

Topic: C.10. Brain Injury and Trauma

Title: Diazepam prevents deficits in strategy flexibility following a controlled cortical impact injury in mice

Authors: K. DOAN[1], S. YETURU[2], A. WEINGARTEN[1], *L. VILLASANA[1];
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Abstract: The generation and integration of new neurons (neurogenesis) is important for several aspects of learning and memory and is also thought to facilitate cognitive recovery after traumatic brain injury (TBI). Neurogenesis robustly increases in response to several different

types of TBI models. In the controlled cortical impact (CCI) mouse model however, the neurons generated shortly after injury are not the same as those observed the hippocampus of non-injured mice. Neurons generated after CCI mislocalize and have aberrant dendritic morphologies, questioning their functional contributions within their network. Previously we showed that administration of the GABA-A agonist, diazepam, immediately after CCI, prevents CCI-induced increases in neurogenesis and normalizes the morphology of new neurons. To determine how inhibiting atypical post-CCI neurogenesis affects hippocampal-dependent memories, male and female wild-type mice received a sham or CCI and were immediately implanted with osmotic pumps containing diazepam or vehicle for one week. One month after surgery, mice were tested on the reversal water maze task. Deficits in neurogenesis-sensitive strategy flexibility were observed in CCI-vehicle treated mice whereas mice treated with diazepam immediately after CCI had no deficits and performed comparable to non-injured mice. These results suggest that neurons born after a moderate TBI have a maladaptive versus beneficial role in hippocampal recovery.

Nanosymposium

512. Traumatic Injury: Repair and Mechanisms

Location: SDCC 31ABC

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 512.08

Topic: C.10. Brain Injury and Trauma

Title: Modelling secondary injuries after traumatic brain injury - in vitro studies and development of neurotherapy.

Authors: *C. A. HALL[1], K. BARANES[1,2], M. J. KILLEN[1], A. HELMY[1], M. R. KOTTER[1,2], K. L. H. CARPENTER[1], P. J. A. HUTCHINSON[1];
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Abstract: Following traumatic brain injury (TBI), downstream cascades, including deranged brain metabolism and inflammation, contribute to secondary injury. Unfavourable patient outcome correlates with a high brain extracellular lactate/pyruvate ratio (LPR), indicating

reduced mitochondrial activity and increased glycolysis. Previous in-vitro studies with rat mixed glia showed that succinate partially protected them against rotenone-induced metabolic dysfunction. Rotenone is an inhibitor of complex I of the mitochondrial electron transport chain (ETC), whilst succinate is an intermediate of the tricarboxylic acid cycle that interacts with complex II of the ETC, bypassing complex I. We studied the metabolic dysfunction induced by rotenone in both human induced neurons (iNs) and human induced astrocytes (iAs) as a model for TBI secondary injuries, investigating disodium succinate for its possible metabolic protective effects on these cells. Using "Optimised Inducible Overexpression", an established cellular reprogramming protocol, we produced iNs and iAs through transcription factor overexpression. Following differentiation, these cell cultures were treated with carefully selected concentrations of rotenone and succinate and evaluated at 12-, 24- and 48-hour time points. Cell viability was quantified using propidium iodide and Hoechst 33342 live cell staining, whilst metabolism was evaluated through analysis of LPR in the extracellular medium by ISCUSflex. We observed significant increases in LPR in both iNs and iAs following treatment with rotenone, indicating the expected metabolic dysfunction. However, succinate did not protect the iNs against rotenone at any of the concentrations or timepoints we tested. The effect of succinate as a putative rescue agent on rotenone-treated iAs is currently being evaluated. We have shown that by using rotenone, neurons' mitochondria are inhibited, emulating some of the secondary injuries of TBI. However, succinate did not protect neurons against rotenone. We postulate a mechanism whereby an interaction between neurons and glia may allow succinate to provide rescue. Further investigation is ongoing.

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Nanosymposium

513. Visual Object and Scene Recognition

Location: SDCC 5

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 513.01

Topic: D.06. Vision

Support: European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant Agreement No. 861423

Title: Using Augmented Reality to Determine the Efficacy of Retinal Implants in a Naturalistic Environment

Authors: *S. HINRICHS[1], L. D. M. PLACIDET[1,2], J. T. THORN[1], C. AUTHIE[3], A. ARLEO[2], D. GHEZZI[1];
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Abstract: Retinal implants have been shown to provide artificial visual percept to blind people in clinical trials. However, due to technical limitations resulting in low spatial and temporal resolution and a small field of view, existing implants failed to provide a vision that proved useful in the daily life of a blind patient. We have shown that among several implant parameters, the field of view has the most significant impact on performance, but its minimum required size remains unclear. Wide-field and high-resolution retinal implants have been developed to improve artificial vision. Here, we aim to determine which field of view is required for a retinal implant to be useful in daily life. Two augmented reality studies were conducted in an artificial street environment. A patient's performance and level of effort were assessed by exposing normally sighted subjects to a simulation of the POLYRETINA implant where the field of view varied between 20° (the widest field of view in current implants) and 45° (POLYRETINA's maximum field of view). In the first study, 29 subjects were required to complete the following set of tasks in less than 10 min: post a letter, retrieve money from the ATM and return home. Performance, time taken, trajectories, and time spent in dangerous locations (i.e., not on footpaths) were used as measures to compare the field of view conditions. In a second experiment, we adapted the study to make it suitable for EEG as an indicator of the effort required to perform daily tasks under the different field of view conditions. For this study, 8 subjects were instructed to identify a digit on an ATM grid and to touch it once they found it. We hypothesised that a 45° field of view would result in better performance and less effort in these naturalistic tasks than a 20° field of view. Preliminary behavioural analyses support this hypothesis. POLYRETINA's 45° field of view allows participants to perform faster, more accurately, and more safely in their environment than the present implants' largest 20° field of view. The combination of simulated prosthetic vision with the naturalistic setup pioneers an approach for the challenge of testing the usefulness of artificial vision in real-life scenarios.

Disclosures: S. Hinrichs: None. **L.D.M. Placidet:** None. **J.T. Thorn:** None. **C. Authie:** None. **A. Arleo:** None. **D. Ghezzi:** None.

Nanosymposium

513. Visual Object and Scene Recognition

Location: SDCC 5

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 513.02

Topic: D.06. Vision

Title: Contextual representations of natural scenes in monkey V1 neurons

Authors: *P. PAPALE[1], F. WANG[1], A. MORGAN[2,3], X. CHEN[1,4], A. GILHUIS[1], L. S. PETRO[3], L. MUCKLI[3], P. R. ROELFSEMA[1,5,6,7], M. W. SELF[1];
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Abstract: Neuronal activity in the primary visual cortex (V1) is governed by feedforward information processing in each neuron's receptive field (RF) and by contextual information from surrounding regions. Ongoing feedforward responses are rapidly influenced by contextual inputs, so it is challenging to dissociate their functional roles. We used full and occluded views of natural scenes to reveal the impact of contextual mechanisms on V1 representations in the absence of information in the RF. We presented partially occluded and full (non-occluded) natural scenes while recording V1 activity from monkeys using electrophysiology (Fig.1A: 24 images per condition; 211 sites in two macaques). In the absence of visual stimulation in their RFs, V1 neurons were modulated by contextual influences that encoded the stimulus identity from ~85ms after stimulus onset (Fig. 1B). We successfully decoded stimulus identity from V1 activity without visual stimulation within neuronal RFs. The representation of contextual influences was similar when feedforward information was either available or absent, but the latency of modulation was longer when feedforward input was absent. We compared V1 representations of occluded scenes between electrophysiology in monkeys and fMRI in 18 human subjects and found a strong correlation between the two species in the sustained phase of the response (Fig. 1C). These results reveal the presence of contextual influences on V1 spiking activity and lay the groundwork for future research on the precise contextual features that contribute to the complexity of this extra-classical RF response.
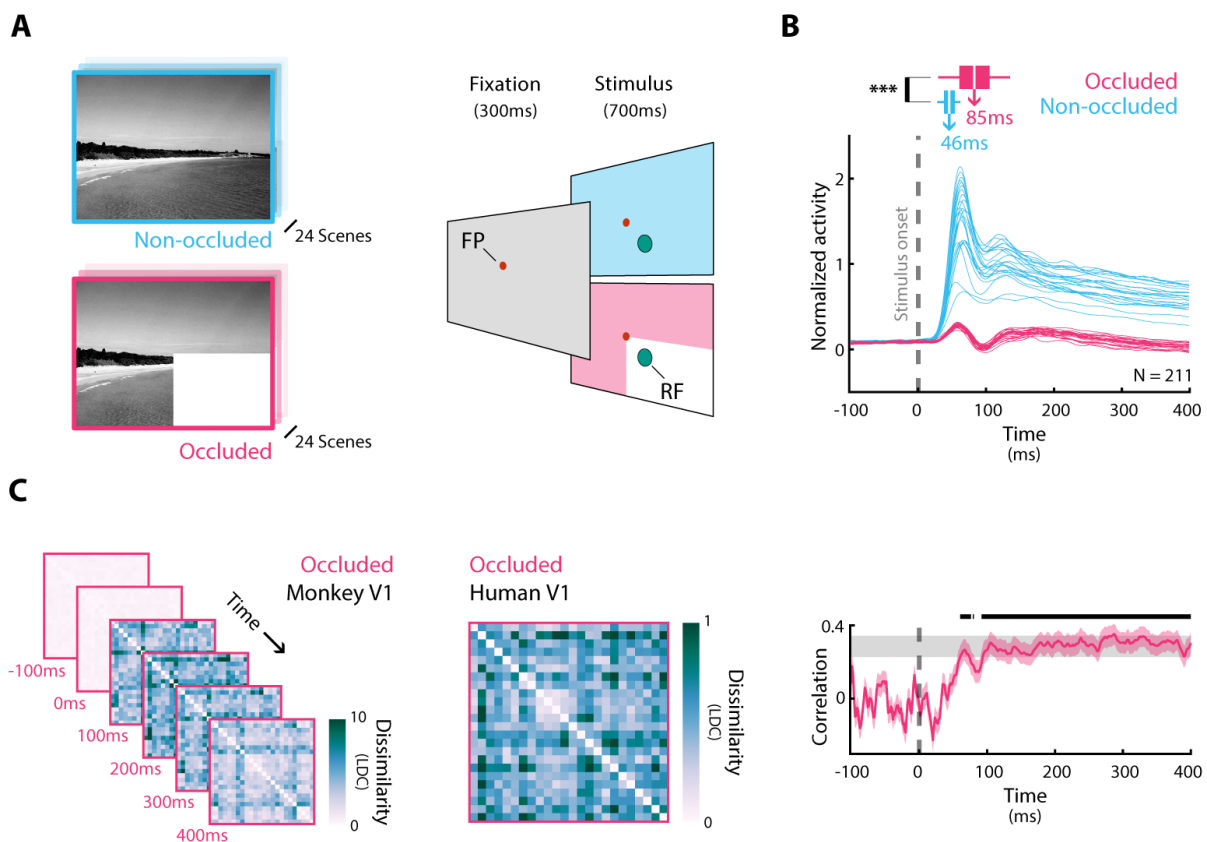
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Nanosymposium

513. Visual Object and Scene Recognition

Location: SDCC 5

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 513.03

Topic: D.06. Vision

Support: Grossman-Kavli Scholar
Sloan Research Fellowship
Klingenstein-Simons Fellowship Award in Neuroscience

Title: Representational straightening of natural movies in robust feedforward neural networks

Authors: *T. TOOSI, E. ISSA;
Columbia Univ., New York, NY

Abstract: The idea of temporal straightening was proposed as a way to make prediction of the next frame possible in natural movie sequences, thus contiguous movie frames should ideally be represented by a linear trajectory in the underlying neural feature space. Prior work established straightening in neural representations of the primate primary visual cortex (V1) and perceptual straightening in human behavior relative to trajectories of movies in the intensity domain. In contrast to biological vision, artificial feedforward neural networks (ANNs) did not demonstrate this phenomenon as they were not explicitly optimized to produce smooth representations over time and are typically not trained on natural movies. Thus, it remained unclear whether and how such straightened representations could be produced in computational models and whether this would indeed lead to models that better predict brain data. Here, we show that standard feedforward ANNs can indeed produce straightened representations of natural movies under certain forms of training for robustness to input noise - using static images without any natural movie exposure. Furthermore, these improvements in a model's representational straightening metric correlated with increased predictivity of neural data in primate V1 whereas other previously proposed metrics for brainlike representations, such as adversarial robustness, were not as strongly correlated with V1 neural predictivity. Thus, this work demonstrates that a proposed hallmark of biological vision, temporal straightening, is particularly diagnostic of the most brain-like models of early visual cortex and perhaps surprisingly, straightening of movie sequences comparable to primate V1 can be realized in ANN models without fundamentally changing their architecture or direct training on natural movie statistics; rather, a simple bio-plausible constraint such as robustness to input noise can lead to learning a manifold geometry for natural stimuli which exhibits brain-like behavior.

Nanosymposium

513. Visual Object and Scene Recognition

Location: SDCC 5

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 513.04

Topic: D.06. Vision

Title: Neurons in macaque V4 prefer natural images to scrambled textures

Authors: *J. D. LIEBER, T. D. OLESKIW, E. P. SIMONCELLI, J. A. MOVSHON;
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Abstract: Humans and monkeys can effortlessly recognize objects in natural scenes. This ability relies on neural computations in the ventral stream of visual cortex. Neurons in primary visual cortex (V1) do not distinguish natural images from synthetic ones with the same spatial frequency content, while neurons in temporal visual areas (IT) respond selectively to natural scenes and objects. The intermediate computations in V2 and V4 that lead to IT object selectivity are not well understood, but previous studies in both macaque neurophysiology (Rust & DiCarlo, 2010, JNeurosci) and human fMRI (Movshon & Simoncelli, 2014, CSHL) implicate V4 as an early site of selectivity for object shape. To explore the mechanisms of this selectivity, we generated "scrambled" textures from natural images using techniques that preserve the local statistics of original natural images while discarding information about scene and shape (Portilla & Simoncelli, 2000, IJCV). To create a continuum of images that smoothly vary between scrambled textures and natural images, we varied the size of scrambling regions from the whole image to small fractions of the image; the scrambling regions always tessellated the whole image and abutted each other seamlessly. We measured the responses of single units in awake macaque V4 to these images. On average, V4 neurons responded more vigorously to natural than scrambled images. In some cases responses were proportional to the size of the scrambling regions, such that images which more closely approximate natural images (smaller regions) evoked progressively stronger responses. Even though images with the smallest pooling regions (~1 deg, or ~¼ the diameter of the typical V4 receptive field) appear similar to the original natural images, V4 cells reliably preferred unscrambled images. That is, even perceptually subtle alterations to natural images can strongly modulate firing rates in V4. We wondered whether response dynamics might help uncover the neural circuits responsible. Selectivity for natural images emerged roughly 80 ms after the onset of visual response. This suggests that selectivity does not simply depend on feedforward pooling of signals in V4, but relies instead on recurrent neural circuits throughout the ventral stream whose computations are more extended in time.

Nanosymposium

513. Visual Object and Scene Recognition

Location: SDCC 5

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 513.05

Topic: D.06. Vision

Title: Persistent object signals in primate visual area V4

Authors: *T. P. FRANKEN, J. H. REYNOLDS;
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Abstract: Object perception is remarkably stable, even though saccades move visual input across the retina several times per second. O'Herron and von der Heydt (2009, 2013) discovered a potential neural correlate of this stability: the assignment of a border in the classical receptive field (cRF) to a foreground object (border ownership, BO) in visual cortical area V2 persists for more than a second even after removing the contextual information that determined BO, and these persistent signals can transfer to other BO neurons across saccades. The grouping cell hypothesis proposes that these different phenomena are due to cortico-cortical feedback from grouping cells in a higher area. Such cells would show persistent object signals in response to an ambiguous border if it used to be owned by an object on the side of the cRF center prior to the loss of context (in contrast to BO neurons, whose preferred side of ownership does not depend on the position of the cRF center). In addition, these same cells would also inherit such persistent object signals after saccades. Here we tested whether area V4, the main source of cortico-cortical feedback to V2, contains such persistent object neurons (PONs). Using 32-channel laminar electrodes we recorded single units during fixation, while presenting a central border at different positions in the cRF. BO of this border was initially indicated by a contour outlining a square object (typically 4º x 4º), but then we removed this contour except for the central border, rendering BO ambiguous. In a subset of trials the border was only positioned in the cRF after a saccade that occurred following displacement of the fixation point (250 ms after removal of the object contour). Spike count differences (>200 ms after the removal of contextual cues in non-saccade trials, or >100 ms after the saccade in saccade trials) were evaluated between trials with an identical central border in the ambiguous phase but an opposite location of the initial square

object relative to that border. On non-saccade trials, we find that the number of neurons that spike more to an ambiguous border if that border used to be owned by the side closest to the cRF center (PONs), is larger than expected by chance. On saccade trials, this same population of PONs (classified as such on non-saccade trials) shows a statistically significant object persistence signal after the saccade brings the ambiguous border in their cRF, as opposed to the neurons that do not classify as PONs in non-saccade trials. Together, our data are consistent with the predictions of the grouping cell model. PONs in V4 may underlie persistent BOS in V2 through cortico-cortical feedback.

Disclosures: T.P. Franken: None. **J.H. Reynolds:** None.

Nanosymposium

513. Visual Object and Scene Recognition

Location: SDCC 5

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 513.06

Topic: D.06. Vision

Title: Distributed population activity in the macaque inferior temporal cortex reflects perceived not retinal object size

Authors: *V. C. PAULUN[1], K. ZHENG[1], K. KAR[2,1];
[1]MIT, Cambridge, MA; [2]Biol., York Univ., Toronto, ON, Canada

Abstract: To interact with various objects in the environment, primates must accurately estimate their sizes. Results from Hong, Yamins, Majaj & DiCarlo (2016) support a linear readout of object size from the population activity across the macaque inferior temporal (IT) cortex. Importantly, object size estimates from macaque IT-based neural decodes, as well as from specific artificial neural network (ANN) models of primate IT were consistent with human behavioral size estimates. However, in their study perceived and retinal sizes were highly correlated. Does the linear IT-readout model predict perceived or retinal size? In the real world, perceived and retinal size can diverge drastically. This phenomenon becomes apparent in the Ponzo illusion, in which two objects with identical retinal sizes differ in perceived size when embedded at different locations along a linear perspective. Here, we exploit the Ponzo illusion to test whether the population activity in IT reflects the retinal or perceived size of objects and whether artificial neural networks (ANNs) as state-of-the-art models of the ventral stream reflect the same size. For this purpose, we created a stimulus set of 200 images in which objects were either placed near, far, or randomly along a linear perspective. In a corresponding set of 200

control images, identical objects were placed at the same image position but on a background without linear perspective. A behavioral experiment with 34 human participants confirmed a discrepancy between the retinal and perceived size in our stimulus set even for brief image presentation times (100 ms). Next, to probe the neural mechanisms, we performed large-scale multi-electrode recordings across the macaque V4 (96 sites, 1 monkey), IT (288 sites, 2 monkeys), and ventrolateral prefrontal cortices (vlPFC; 288 sites, 2 monkeys) while the monkeys fixated the images for 100 ms. Preliminary analyses show that the population activity in IT (and not V4 and vlPFC) reflects perceived size rather than retinal size. Therefore, their neural responses can account for the Ponzo illusion effect as observed in humans. In contrast, we found that the linear readout from the several object-recognition pretrained ANNs (AlexNet, ResNet50, CORnet-S/Z/RT) does not predict the Ponzo illusion. Instead, ANN responses better correspond to the retinal rather than the perceived size of objects. Furthermore, we found that ANNs pretrained on depth estimation and fusion models (that combine features of object recognition and depth estimation models) do not predict the Ponzo illusion. Thus, our results expose a significant explanatory gap in current ANNs as models of primate vision.

Disclosures: V.C. Paulun: None. **K. Zheng:** None. **K. Kar:** None.

Nanosymposium

513. Visual Object and Scene Recognition

Location: SDCC 5

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 513.07

Topic: D.06. Vision

Title: Representation of object motion in the macaque visual ventral stream

Authors: *K. KAR[1,2];
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Abstract: Primates seamlessly integrate dynamic visual information of moving objects to navigate their daily activities. However, we currently lack a neurally mechanistic understanding of how the brain supports the joint representation of object identity and position across time, leading to a unified perception of a moving object. Building on previous reports of behaviorally explicit object identity (Majaj et al., 2015; Kar et al., 2019) and object position information (Hong et al., 2016) in the macaque inferior temporal (IT) cortex, here we first explicitly tested whether we could approximate object velocities from the distributed IT population activity. We showed 600 movies (300ms long) that contained objects (one of ten) moving in specific directions (one of eight), at varying speeds, to monkeys (n=3) that passively fixated a central dot.

We simultaneously measured large-scale neural activity (using chronic multielectrode arrays) from areas V4 (155 sites), IT (212 sites), and ventrolateral PFC (174 sites) across these monkeys. First, we observed that a nonlinear temporal integration model could dynamically transform V4, IT, and vlPFC population activity into object-velocity readouts. Interestingly, however, unlike V4 and vlPFC-based decodes, object-velocity could also be decoded linearly from instantaneous (~10ms) IT population activity (peaking ~300ms post-movie onset), supporting the notion of a pre-computed velocity signal in the IT population activity pattern. Consistent with previous studies, the corresponding object identity decodes from IT significantly preceded (~150ms) these motion signals. To gather correlational evidence on whether the motion information available in IT is used for object-motion related behaviors, we trained two monkeys on an object-motion velocity discrimination task. Preliminary results show that IT responses can significantly predict the movie-by-movie behavioral error patterns of the monkeys, implicating IT in these behaviors. In addition, we observed that IT-like layers from two-stream convolutional neural network models (of action recognition) also support linear readouts of object identity and velocity - establishing these as good baseline hypotheses to model object motion processing. These results challenge the common functional segregation of primate visual processing into the ventral ("what") and dorsal ("where") pathways and motivate the development of integrated (dorsal+ventral) models to study dynamic scene perception.

Disclosures: K. Kar: None.

Nanosymposium

513. Visual Object and Scene Recognition

Location: SDCC 5

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 513.08

Topic: D.06. Vision

Title: A distributed network for object-based attentionin the monkey brain

Authors: *P. C. KLINK[1,2,3], K. WAGHMARE[1], J. R. WILLIFORD[1], P. R. ROELFSEMA[1,2,4,5];
[1]Netherlands Inst. for Neurosci., Netherlands Inst. For Neurosci., Amsterdam, Netherlands; [2]Lab. of Visual Brain Therapy, Inst. de la Vision, Sorbonne Université, INSERM-CNRS, Paris, France; [3]Exptl. Psychology, Helmholtz Inst., Utrecht Univ., Utrecht, Netherlands; [4]Dept. of Integrative Neurophysiology, Ctr. for Neurogenomics and Cognitive Res., VU Univ., Amsterdam, Netherlands; [5]Dept. of Psychiatry, Academic Med. Center, Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: The visual system groups image elements into objects, segregates those objects from their background, and employs selective attention to highlight behaviorally relevant objects for preferential neural processing. The mechanisms underlying selective visual attention are thought to involve frontoparietal control areas modulating the neuronal representation of visual objects in more posterior areas of the brain through feedback connections. Here we used a curve-tracing paradigm combined with fMRI in awake behaving macaque monkeys to localize the full distributed network of brain regions involved in the attentional selection of objects. In the curve-tracing paradigm, monkeys mentally trace a target curve from a central fixation dot to a response indicator while ignoring distractor curves. Previous electrophysiological studies from our lab have shown enhanced neuronal responses for the attended target curve relative to the ignored distractor curve in early visual areas. With whole-brain fMRI, we now reveal a distributed network of brain areas involved in this type of object-based attentional selection that stretches beyond these posterior visual areas. Significant attention-related increases in the BOLD response were observed in posterior visual areas like V1, V2 and V4, as well as frontoparietal areas like the frontal eye fields (FEF) and the lateral intraparietal cortex (LIP). Interestingly, we also identified a distinct region in the temporal cortex (mid-STS) where activity was significantly modulated by attention. This area has previously been implicated in spatial attention and prioritization in macaques. With population receptive field mapping techniques, we were able to reconstruct not only the visual stimulus from the BOLD responses, but also the spatial profile of selective visual attention in several nodes of the distributed object-based attention network. These reconstruction results suggest a functional specialization within the attention network that could be further investigated at the neuronal level and causally probed with perturbation methods in future experiments.

Disclosures: P.C. Klink: None. **K. Waghmare:** None. **J.R. Williford:** None. **P.R. Roelfsema:** None.

Nanosymposium

514. Post-Lesion Cortical Dynamics During Reaching

Location: SDCC 7

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 514.01

Topic: E.04. Voluntary Movements

Title: Neuron population state reflects reaching impairment after brain injury

Authors: *S. E. CLARKE[1], I. E. BRAY[2], P. NUYUJUKIAN[1,3,2,4,5];
[1]Bioengineering, [2]Electrical Engin., [3]Neurosurg., [4]Wu Tsai Neurosciences Inst., [5]Bio-X Inst., Stanford Univ., Stanford, CA

Abstract: It is widely accepted that the coordinated activity of neuron populations in motor cortex generates voluntary movement, yet much remains unknown about which aspects are truly necessary to plan and produce the desired behavior. Insightful experiments using temporary manipulations of recorded neuron activity (e.g., microstimulation and optogenetics) suggest that low-dimensional structure in the activity of large populations plays a meaningful role. Although compelling, these reversible manipulations are difficult to sustain over long timescales; thus, the field of motor systems neuroscience still lacks the complementary evidence provided by permanent neuronal inactivation (i.e., termination of neurons). To this end, we performed fourteen electrolytic lesions through chronically implanted microelectrode arrays in the motor cortex of two adult male rhesus macaques (11-14 years of age). Since no surgical procedure is required, recordings of the same population can continue after repeated millimetre-scale lesions that target the neuronal correlates of behavior.
During daily recording sessions, animals participated in a reaching task with a sham lesion performed during a short break. On lesion day, a targeted 150uA of steady direct current of was passed through two array electrodes for 30 or 45 seconds. Up to 72 measures of reach performance were examined using a false detection rate analysis that rigorously protects against false positives without sacrificing statistical sensitivity. Tracking these measures over the days following a lesion demonstrated impaired reaching that largely recovered alongside changes in the recorded local field potential and spiking activity of the remaining population. Despite repeated termination of individual task-responsive neurons, low-dimensional structure in the population re-emerged alongside behavior. Although different measures of brain activity clearly reflected the injury, only changes in the neuronal state provided a reliable biomarker of reaching deficit. Given the injury's histological likeness to salvageable human tissue, we argue that lesioning cortex through implanted microelectrode arrays offers unique translational opportunities while enabling new causal insight into the neuronal basis of behavior.

Disclosures: S.E. Clarke: None. **I.E. Bray:** None. **P. Nuyujukian:** None.

Nanosymposium

514. Post-Lesion Cortical Dynamics During Reaching

Location: SDCC 7

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 514.02

Topic: E.04. Voluntary Movements

Title: Electrolytic lesioning through a microelectrode array and associated neuronal loss

Authors: *I. E. BRAY[1], S. E. CLARKE[2], P. NUYUJUKIAN[2,3,1,4,5];
[1]Electrical Engin., [2]Bioengineering, [3]Neurosurg., [4]Wu Tsai Neurosciences Inst., [5]Bio-X, Stanford Univ., Stanford, CA

Abstract: While lesion studies have proven key in establishing causal connections between brain and behavior, they stand to provide additional insight if integrated with multielectrode techniques from systems neuroscience. Here we present a platform for creating electrolytic lesions in awake-behaving animals through chronically implanted, intracortical microelectrode arrays without compromising electrophysiology. Stable current is delivered to cortex through two electrodes of the array, using a custom direct-current source that allows for controlled, repeatable lesions. Device parameters were calibrated through *ex vivo* and *in vivo* testing in sheep and pigs (61 lesions total). Histology from one lesion (150µA of DC current, 45 seconds) in an adult male rhesus macaque shows an ablated and necrotic core surrounded by rarefied tissue (visible damage contained within 1.2mm^3). Fourteen subsequent lesions (targeting 150µA of DC current, 30 or 45 seconds) were performed over a year and a half in two awake-behaving adult male rhesus macaques, resulting in a behavioral deficit during reaching from which the animals recover typically within days. Stable current traces confirm the delivery of controlled direct current from the lesioning device. Comparisons of recorded electrophysiology before and after lesioning demonstrate that the microelectrode arrays continue to record stable neuronal activity. Although the majority of waveforms were unaltered, suggesting that an acute damage response did not affect the stability of the electrophysiological recording, a small percentage of the waveforms did appear to change significantly, either through neuron damage or through an adaptive response of the neuronal circuit to lesioning. We compared the relative change in daily turnover of recorded neurons in the days following a lesion. The percentage of neuron waveforms that matched before and after lesioning was significantly lower than the percentage of matching waveforms across the pre lesion days (**, $p = 0.0002$), providing a proxy measure of neuron loss by electrolytic lesioning. The percentage of matching neurons trends back towards the expected percentage of matching neurons in the pre-lesion period with behavioral recovery, despite still being significantly decreased (*, $p = 0.002$). It is possible that the recorded electrophysiology could be used to decode a neuronal recovery timecourse distinct from that of behavioral recovery, providing insight into local reorganization after neuronal loss to support known motor system dynamics.

Disclosures: I.E. Bray: None. **S.E. Clarke:** None. **P. Nuyujukian:** None.

Nanosymposium

514. Post-Lesion Cortical Dynamics During Reaching

Location: SDCC 7

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 514.03

Topic: E.04. Voluntary Movements

Support: R01NS112424

Title: Changes in somatosensory and premotor cortex neurophysiology during recovery of reach-to-grasp control following motor cortex stroke

Authors: *I. S. HEIMBUCH[1], **P. KHANNA**[1], **L. NOVIK**[2], **R. J. MORECRAFT**[3], **K. GANGULY**[1];
[1]Neurol., UCSF, San Francisco, CA; [2]California Natl. Primate Res. Ctr., UC Davis, Davis, CA; [3]Univ. of South Dakota, Vermillion, SD

Abstract: Somatosensory cortex plays an important role in motor function, with inactivation of primary somatosensory cortex (S1) leading to motor deficits [1, 2]. Its role in motor function is also highlighted in the case of stroke. Non-human primates that retain S1 following primary motor cortex (M1) stroke show reduced deficits compared to animals with stroke in both S1 and M1 [3]. Translationally, middle cerebral artery stroke is a common site of stroke in humans and leads to M1 & S1 injury. Stroke patients often demonstrate impaired somatosensation. Anatomically, it is known that there is extensive cortical rewiring, including across distant areas of the sensorimotor network [4]. This anatomical rewiring likely facilitates partial recovery of motor dexterity, even following the loss of a crucial node like M1. However, how the somatomotor network changes functionally to facilitate recovery of precise hand control is poorly understood. / We present electrophysiology data showing changes in somatosensory cortex responses in non-human primates (rhesus macaque) over the course of recovery after a M1 stroke. These cortical responses were measured using a chronic 64-channel microwire electrode array (Tucker-Davis Technologies) implanted in area 2 of S1 while subjects performed a reach-to-grasp task that is likely dependent on touch and proprioception of the hand and arm. By binning sessions by subject performance relative to pre-stroke metrics, we can compare cortical responses for days when the subject was still in the early phase of behavioral recovery to days when the animal had behaviorally recovered from stroke. We report changes in physical contact-related responses in somatosensory cortex over the course of recovery. We also examined changes in coupling to premotor areas with recovery of prehension. / These data illuminate post-stroke dynamics in the somatosensory cortex and its communication with premotor areas that could potentially be indicative of, or directly involved in, systems-level restructuring of somatomotor processing that underlies recovery of dexterity following cortical injury. / References: 1) Hikosaka et al., 1985. Deficits in manipulative behaviors induced by local injections of muscimol in the first somatosensory cortex of the conscious monkey. 2) Brochier et al., 1999. The effects of muscimol inactivation of small regions of motor and somatosensory cortex on independent finger movements and force control in the precision grip. 3) Darling et al., 2016. Sensorimotor cortex injury effects on recovery of contralesional dexterous movements in Macaca mulatta. 4) Dancause et al., 2005. Extensive cortical rewiring after brain injury.

Disclosures: I.S. Heimbuch: None. **P. Khanna:** None. **L. Novik:** None. **R.J. Morecraft:** None. **K. Ganguly:** None.

Nanosymposium

514. Post-Lesion Cortical Dynamics During Reaching

Location: SDCC 7

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 514.04

Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS112424
NIH Grant K99NS124748

Title: Cortical-subcortical beta band interactions with motor recovery from stroke

Authors: P. KHANNA[1], I. S. HEIMBUCH[1], H. CHOI[1], L. NOVIK[2], K. THIESEN[2], R. J. MORECRAFT[3], K. GANGULY[1];
[1]Neurol., Univ. of California, San Francisco, San Francisco, CA; [2]California Natl. Primate Res. Ctr., Davis, CA; [3]Univ. of South Dakota, Vermillion, SD

Abstract: Dexterous movements rely on coordinated cortical and subcortical networks, but how these networks supports recovery of movement after stroke is unknown. Many studies have focused on the role of perilesional motor cortex (pMoCtx) in vicariation, but little is known about how pMoCtx regains coordination with perilesional subcortical networks (pSubCtx). This process is likely critical as recent work across hundreds of patients demonstrates that atrophy to subcortical gray matter structures is strongly associated with poor sensorimotor outcomes (Liew et al., 2021). Uncovering how functional cortical-subcortical interactions re-emerge following recovery from stroke may lay the foundation for novel approaches to multi-area neuromodulation to improve movement control after stroke. To investigate how cortical-subcortical interactions are re-established in pMoCtx and pSubCtx to support hand control following stroke, we trained rhesus macaques to perform a reach-to-grasp task. To model a stroke, aspiration was used to remove the forelimb region of primary motor cortex unilaterally after cauterization of the primary arterial supply. In the same surgery, chronic microwire electrodes were implanted into ventral premotor cortex (pMoCtx) and a chronic linear microelectrode probe was implanted subcortically targeting motor thalamus (pSubCtx). Here, we report on how pMoCtx - pSubCtx dynamics change as animals recover dexterity following the lesion. We find that immediately following the lesion, pMoCtx and pSubCtx exhibit neural oscillations in 13-30 Hz range (beta band oscillations) that are remarkably coordinated. With behavioral recovery, beta band oscillations in pMoCtx becomes less coherent spatially, and less coherent with pSubCtx activity. These results may reflect cortical-subcortical interactions that are pathologically synchronized early after stroke, and become flexibly coupled or uncoupled depending on behavioral phase after recovery from stroke. Further, these observations may support a role for spatially and temporally segregated bursts of beta activity rather than a single synchronous sensorimotor beta oscillation in healthy movement control.

Disclosures: P. Khanna: None. **I.S. Heimbuch:** None. **H. Choi:** None. **L. Novik:** None. **K. Thiesen:** None. **R.J. Morecraft:** None. **K. Ganguly:** None.

Nanosymposium

514. Post-Lesion Cortical Dynamics During Reaching

Location: SDCC 7

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 514.05

Topic: E.04. Voluntary Movements

Title: Investigating the neuroprotective effects of electrical stimulation following acute ischemic stroke in non-human primates

Authors: *J. ZHOU[1], **K. KHATEEB**[1], **A. GALA**[2], **M. RAHIMI**[3], **A. YAZDAN-SHAHMORAD**[4];
[1]Bioengineering, [2]Neurosci., [3]Electrical Engin., [4]Bioengineering and Electrical Engin., Univ. of Washington, Seattle, WA

Abstract: Brain stimulation has emerged as a novel therapy for ischemic stroke, a major cause of brain injury that often results in lifelong disability. Although previous *in vitro* and rodent studies have demonstrated neuroprotection using electrical or sensory stimulation acutely after stroke, few of these results have been replicated in humans due to significant scale and anatomical differences, in addition to a limited understanding of stimulation-induced network changes. Therefore, we combined electrophysiology and histology to study the effects of electrical stimulation following cortical ischemic stroke in non-human primates (NHPs). To produce controlled focal lesions, we used the photothrombotic method to induce targeted vasculature damage in the sensorimotor cortices of two macaques while collecting electrocorticography (ECoG) signals bilaterally. In another two macaques, we followed the same lesioning procedures and applied repeated electrical stimulation via an ECoG electrode medial to the lesion. We investigated the protective effects of stimulation on neural dynamics using a variety of electrophysiological markers such as ECoG signal power and coherence. In addition, we performed histological analysis including Nissl and immunohistochemistry staining to evaluate the differences in lesion volume, neuronal death, and neuroinflammatory response. In comparison to controls, the ECoG signals showed decreased gamma power across the sensorimotor cortex in stimulated animals. Meanwhile, histology revealed smaller lesion volumes for the stimulated group, suggesting that electrical stimulation may exert neuroprotection by suppressing post-ischemic neural activity and reducing excitotoxicity. With the similarity between NHP and human brains, this study paves the path for developing effective stimulation-based therapy for acute stroke in clinical studies.

Disclosures: J. Zhou: None. **K. Khateeb:** None. **A. Gala:** None. **M. Rahimi:** None. **A. Yazdan-Shahmorad:** None.

Nanosymposium

514. Post-Lesion Cortical Dynamics During Reaching

Location: SDCC 7

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 514.06

Topic: E.04. Voluntary Movements

Title: Acute network neurophysiological dynamics following cortical lesioning and stimulation

Authors: *K. KHATEEB[1], J. ZHOU[1], M. RAHIMI[2], A. YAZDAN-SHAHMORAD[3];
[1]Bioengineering, [2]Electrical and Computer Engin., [3]Bioengineering and Electrical Engin., Univ. of Washington, Seattle, WA

Abstract: Stroke is a leading cause of adult long-term disability in the United States. Despite its prevalence, little progress has been made in recent decades to develop treatments addressing the multitude of functional deficits following stroke. Electrical stimulation near the site of injury is one proposed novel treatment method to capitalize on increased plasticity observed in perilesional areas and promote functional recovery. However, previous unsuccessful efforts to translate preclinical successes of rodent studies to the clinic have revealed the importance of non-human primate preclinical studies in developing effective novel treatments for complex neurological disorders such as stroke. Here, we address these issues by utilizing our previously published toolbox (Khateeb et al. 2022) to investigate network physiological dynamics following cortical lesioning and subsequent electrical stimulation in four adult macaques. With our toolbox, we induced unilateral targeted focal ischemic lesions in sensorimotor cortex using the photothrombotic method while bilaterally recording electrocorticographic (ECoG) signals before, during, and up to three hours after lesion induction with our implanted ECoG array. In two of the four monkeys, we tested the effects of approximately one hour of perilesional electrical stimulation one hour after lesion induction using our ECoG array. To investigate network neurophysiological dynamics over the time course of lesion induction and subsequent lesioning, we analyzed changes in signal power, current source density, and phase-amplitude coupling. Through power analysis, we observed differential changes in post-lesion neural activity across the network in non-stimulated animals while neural activity levels were consistently reduced in stimulated animals. Current source density analysis revealed stabilizing effects of stimulation on the network. Additionally, through phase-amplitude coupling analysis we observed frequency-dependent changes in network coordination. By enhancing our understanding of acute network dynamics following injury and the effects of subsequent

stimulation on the network in an animal model with high clinical relevance, we can drive the development of effective therapies for neurological disorders such as stroke.

Nanosymposium

514. Post-Lesion Cortical Dynamics During Reaching

Location: SDCC 7

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 514.07

Topic: E.04. Voluntary Movements

Title: Bistability of beta oscillations and movement-related population spiking in motor areas

Authors: *H. CHOI[1], J. KIM[1], P. KHANNA[1], L. NOVIK[2], K. THIESEN[2], R. J. MORECRAFT[3], K. GANGULY[1];
[1]Neurol., Univ. of California, San Francisco, San Francisco, CA; [2]California Natl. Primate Res. Ctr., Univ. of California, Davis, Davis, CA; [3]Lab. of Neurolog. Sci., Univ. of South Dakota, Vermillion, SD

Abstract: Beta oscillations are a well-known feature of movement preparation and have been clinically used to track motor recovery. Moreover, it is increasingly clear that the movement-related spatial and temporal dynamics of population spiking activity in the motor cortex are important for movement control. Changes in population spiking are also known to track motor recovery and can be a target for neuromodulation using electrical stimulation. However, it remains unclear how beta oscillations and movement-related population spiking activity interact. The goal of this study was to characterize how beta oscillations and neural population dynamics interact and to develop a model for neuromodulation that accounts for their interaction. We measured local field potentials (LFP) and population spiking activity in the motor cortex of non-human primates performing reach and grasp tasks. We also performed such recordings from the premotor cortex in animals recovering from an M1 lesion. We tracked spiking activity and behavior throughout the recovery process. We then analyzed the interactions between changes in beta power and spiking activity during movements In general, we found that in expert animals there were rapid and sharp transitions from population spiking to changes in beta power. We characterized this process using a model of bistability, which is characterized as the presence of two states that can rapidly transition from one to the other. We also found that the reemergence of bistability correlated closely with the recovery of reach and grasp function. Notably, we found that low frequency alternating current electrical stimulation could modulate the bistable relationship between beta and population spiking. We also developed a computational model that could inform how best to deliver stimulation for a bistable process. Overall, our results indicate a close relationship between cortical beta oscillations and population spiking activity during

skilled (recovered) reach and grasp behaviors. The bistable nature of this relationship suggests rapid transitions between states that are important to consider for movement control and for neuromodulation.

Nanosymposium

515. Central and Peripheral Mechanisms of Energy Metabolism

Location: SDCC 24

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 515.01

Topic: F.08. Food and Water Intake and Energy Balance

Title: The coding of visceral senses in the brainstem

Authors: *C. RAN, J. C. BOETTCHER, J. A. KAYE, C. GALLORI, S. LIBERLES;
Harvard Med. Sch. and HHMI, Boston, MA

Abstract: Our external senses of sight, smell, sound, touch, and taste enable us to perceive the external world. In addition, our internal sensory system monitors the physiological states of peripheral organs, such as mechanical and chemical cues from ingested food, irritants in the airway that induce cough, and inflammatory cues that signal tissue damage. In comparison to external sensory systems, the principles that define visceral sensory processing remain poorly defined. Here, we developed an *in vivo* two-photon calcium imaging preparation to understand internal organ representations in the nucleus of the solitary tract (NTS), a sensory gateway in the brainstem that receives vagus and other inputs from the body. Combining the imaging platform with stimulation of visceral organs, we uncover diverse neuronal responses to internal stimuli, while functionally defined cell types are highly organized within the NTS. Combining functional imaging with pharmacogenetic manipulations and viral tracing from genetically defined vagal sensory cell types, we show that the highly organized representations of internal senses are generated by vagal axon sorting and higher-order sensory processing within the NTS. Ongoing work will connect the response profiles of NTS neurons to the connectivity to downstream target areas. Together, our study reveals basic coding principles used by the brain to process visceral inputs and may shed light on the treatment of viscerosensory and autonomic dysfunctions.

Nanosymposium

515. Central and Peripheral Mechanisms of Energy Metabolism

Location: SDCC 24

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 515.02

Topic: F.08. Food and Water Intake and Energy Balance

Title: Diverse brainstem cell types coordinating energy balance

Authors: *A. R. NECTOW;
Columbia Univ., Columbia Univ., New York, NY

Abstract: The central nervous system (CNS) plays the key role in the sense-and-respond system responsible for regulating energy balance. However, the molecular, cellular, and circuit mechanisms through which the CNS achieves this goal is incompletely understood. We recently identified the dorsal raphe nucleus (DRN) as an important node within the CNS, that is capable of regulating numerous aspects of energy balance. Diverse cell types within the DRN potently regulate food intake, and various aspects of energy expenditure, such as thermogenesis and locomotor activity. Critically, prolonged changes in the activity of these neurons can ultimately drive changes in body weight. Until recently, the marked molecular heterogeneity of the DRN had limited our understanding of how its component cell types regulate energy balance. Here, we describe new molecular profiling studies, in which we dissect the role of distinct subpopulations of glutamatergic and GABAergic neurons in the DRN to regulate food intake and thermogenesis. We find that these subpopulations are capable of regulating various aspects of energy homeostasis, and together begin to explain the complex and parallel mechanisms through which this nucleus potently controls body weight. Together, this work represents an important step towards understanding the principles through which the brain regulates peripheral metabolism.

Nanosymposium

515. Central and Peripheral Mechanisms of Energy Metabolism

Location: SDCC 24

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 515.03

Topic: F.08. Food and Water Intake and Energy Balance

Title: Neurovascular control of body weight

Authors: *M. SCHNEEBERGER PANE[1], N. RENIER[2], D. MESSEGUER[1];
[1]Cell. and Mol. Physiol., Yale Univ., New Haven, CT; [2]Paris Brain Inst., ICM, Paris, France

Abstract: The CNS is strongly reliant on a continuous supply of blood for proper function. Its high metabolic needs at the cellular level and its inability to properly store sufficient amounts of energy necessitate tightly coupled neuronal and vascular functions. Alterations in blood flow compromise neuronal function/survival, while changes in neuronal activity profoundly curb hemodynamics. Consequently, many neurological disorders have a vascular component. It is thus imperative to use large-scale, integrative, and quantitative analysis of both brain vasculature and neuronal function to understand the impact of vascular topology on neuronal circuits. Here, I applied this rationale to a specific pathophysiologic state with reported alterations in both central neuronal and vascular components, obesity. Obesity is reaching pandemic levels in western societies and its incidence is increasing at alarming rates worldwide. Obesity is directly associated with Type 2 diabetes (insulin resistance (IR)). Moreover, obesity and its associated metabolic impairments are primary factors for neurological disorders in both the CNS and peripheral nervous systems. Interestingly, obesity and IR are underlying causes of stroke and different forms of dementia, exhibiting thus neurovascular alterations associated to canonical neurovascular impairments. However, while obesity research has extensively studied the central circuits regulating energy balance and has identified many molecular components impaired upon dietary shifts; it has missed an explanation for how different metabolic environments upon obesity can underlie defects in the neurovascular unit. We combined tissue clearing methods with whole-mount IHC and computational mapping to a registered atlas (Allen Brain Institute) for unbiased high-throughput mapping between different biological conditions. Using such methods, we were able to screen for changes in neuronal activity using early activated genes (Fos) (Fos-Maps), neuronal projections (Projection-Maps), and the fine-penetrating vascular network of brain capillaries and arteries (Vascular-Maps). As a result, these sets of studies resulted in static pictures of either the structure/activity of the neuronal/glial/vascular network. Those studies revealed that the LHA undergoes structure-activity impairments suggestive of reduced vascular density while the DRN reveals angiogenic radial vessel growth, confirming nutritionally driven neurovascular plasticity in adult states. Both centers have a key role in energy balance control. Thus, these new findings will revisit the canonical circuitry regulating weight.

Disclosures: M. Schneeberger Pane: None. **N. Renier:** None. **D. Messeguer:** None.

Nanosymposium

515. Central and Peripheral Mechanisms of Energy Metabolism

Location: SDCC 24

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 515.04

Topic: F.08. Food and Water Intake and Energy Balance

Title: Transient cAMP production directly influences PBN neuronal activity to suppress motivated behaviors

Authors: J. SINGH ALVARADO, A. LUTAS, J. ISAAC, J. C. MADARA, M. L. ANDERMANN;
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Abstract: Neurons transmit electrical signals at millisecond timescales, yet behavioral states such as hunger, nausea, and chronic pain persist for minutes to hours. Such states are likely driven by longer timescale changes in neural activity, yet how these persistent shifts in activity occur remains poorly understood. G protein-coupled receptors (GPCRs) are a potential mechanism by which neurotransmitters can cause longer lasting changes in neuronal activity. Recent work from our lab suggests that activation of Gs-coupled GPCRs can result in persistent changes in behavior via the production of cyclic adenosine monophosphate (cAMP). Here, we examine cAMP dynamics and their relationship to intracellular calcium levels in the parabrachial nucleus (PBN), a brain region known to express a wide variety of GPCRs and to be crucial in regulating pain and ingestive behavior. Using optical tools to measure and manipulate cAMP levels, we show that photostimulation of cAMP production results in rapid increases in neuronal calcium activity that can persist for multiple minutes. The increase in calcium activity reflected increased action potential firing and did not depend on protein kinase A (PKA) or hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Moreover, cAMP-driven activity did not require synaptic transmission, suggesting that cAMP functions via a cell-autonomous mechanism. Finally, brief photostimulation of cAMP production in the PBN was sufficient to suppress feeding for up to 45 seconds, whereas constitutive reduction of PBN cAMP levels led to weight gain. Taken together, our results describe a direct role for PBN cAMP in controlling feeding behaviors and neural circuit activity over the timescale of minutes.

Disclosures: J. Singh Alvarado: None. **A. Lutas:** None. **J. Isaac:** None. **J.C. Madara:** None. **M.L. Andermann:** None.

Nanosymposium

515. Central and Peripheral Mechanisms of Energy Metabolism

Location: SDCC 24

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 515.05

Topic: F.08. Food and Water Intake and Energy Balance

Title: Decreases in DNA 5-hydroxymethylation within the hypothalamus control weight gain in a sex-specific manner during the development of obesity

Authors: *T. MCFADDEN[1], M. MUSAUS[2], K. FARRELL[3], I. CARUCCI[2], T. J. JAROME[4];
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Abstract: Obesity affects about 34% of the U.S. population and is a major contributing factor associated with health risks such as cancer, heart disease, and diabetes. Understanding the neurobiology of obesity is crucial for responding to the etiology of this disease. The hypothalamus is responsible for controlling food intake and numerous studies have observed altered hypothalamic gene regulation in obesity models. However, little is known about the mechanisms controlling persistent changes in gene expression in the hypothalamus during obesity. Epigenetic mechanisms, such as DNA methylation, serves as powerful mechanisms of persistently controlling gene transcription throughout the lifetime of the individual. Yet little is known about whether hypothalamic DNA methylation changes contribute to the development of obesity. Here, we found that rats fed a high fat diet over the course of 7 weeks gained more bodyweight compared to rats that were fed standard rat chow, which was associated with reduced DNA 5-hydroxymethylation (5-hmC), a potent transcriptional activator, in the hypothalamus of males, but not females. Correlational analyses revealed that within the hypothalamus of the male, but not female, rats fed a high fat diet there was an inverse relationship between body weight and DNA 5-hmC levels whereas the latter decreased, bodyweight increased. Consistent with this, only 3 weeks of exposure to the high fat diet, which was not sufficient to cause abnormal weight gain, decreased 5-hmC levels in the hypothalamus of male rats, suggesting that the decreases in DNA 5-hmC preceded significant weight gain. CRISPR-dCas9-VP64 mediated increases in expression of the DNA 5-hmC enzymes (*Tet1/2/3*) in the hypothalamus slowed weight gain in male, but not female, rats fed a high fat diet relative to control injected animals, which resulted in a lower percentage of bodyweight gained over 7 weeks on the high fat diet. However, CRISPR-dCas9-KRAB-MECP2 mediated reductions in the expression of DNA 5-hmC enzymes in the hypothalamus of males did not facilitate abnormal weight gain in rats fed a standard chow diet, suggesting that reductions in DNA 5-hmC are not sufficient to induce significant weight gain. Collectively, these results suggest that decreases in hypothalamic DNA 5-hmC are an important factor in facilitating weight gain during the development of obesity in a sex-specific manner. Current experiments are underway to determine whether altered DNA 5-hmC occurs at genes that specifically regulate appetite.

Disclosures: T. McFadden: None. **M. Musaus:** None. **K. Farrell:** None. **I. Carucci:** None. **T.J. Jarome:** None.

Nanosymposium

515. Central and Peripheral Mechanisms of Energy Metabolism

Location: SDCC 24

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 515.06

Topic: F.08. Food and Water Intake and Energy Balance

Title: Brain glucagon-like peptide-1 receptor (GLP-1R) and beta-klotho are required for fibroblast growth factor 21 to mediate weight loss induced by the GLP-1R agonist liraglutide

Authors: *T. D. V. LE[1], N. BOZADJIEVA KRAMER[2], R. J. SEELEY[2], J. E. AYALA[1];
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Abstract: Glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) agonists and fibroblast growth factor 21 (FGF21) both act on the central nervous system to regulate energy balance. GLP-1R agonists activate GLP-1R in several regions of the brain to induce anorexia and weight loss while central nervous system (CNS) expression of beta-klotho (KLB), an obligate co-receptor of FGF21, is required for the suppressive effect of FGF21 on carbohydrate consumption. Prior studies reported that GLP-1R agonists induce FGF21. We show that the GLP-1R agonist liraglutide increases FGF21 independently of reduced food intake, a driver for FGF21 production, and that FGF21 contributes to the weight loss induced by liraglutide when mice are fed carbohydrate-rich diets. Given the metabolic benefits of GLP-1R agonists and FGF21, the therapeutic potential of a GLP-1R-FGF21 axis merits investigation. While FGF21 is primarily produced in the liver, hepatic expression of the GLP-1R has not been consistently demonstrated. Given the known actions of GLP-1R agonists and FGF21 in the brain, we hypothesize that (1) liraglutide acts on GLP-1R in the brain to stimulate hepatic FGF21 production and (2) liraglutide-induced FGF21 engages CNS KLB to mediate the food intake and weight lowering effects of liraglutide. To test hypothesis (1), we administered vehicle and liraglutide to fasted control mice and mice lacking the GLP-1R in glutamatergic neurons (VGLUT2+ neuron-*Glp1r* knockout), which have been shown to be resistant to Liraglutide-induced anorexia and weight loss. Liraglutide failed to induce fasting FGF21 in VGLUT2+ neuron-*Glp1r* knockout mice, suggesting that GLP-1R expression in glutamatergic neurons is required for liraglutide to stimulate FGF21. To address hypothesis (2), we chronically administered vehicle or liraglutide to chow-fed control and mice lacking *Klb* expression in neurons targeted by *CamK2a*-Cre. CAMK2A+ neuron-*Klb* knockout mice show partial resistance to the weight-lowering actions of liraglutide. In sum, our studies demonstrate that liraglutide acts centrally to induce circulating FGF21, which in turn signals through KLB expressed in CAMK2A+ neurons to facilitate the weight lowering effect of liraglutide. These findings support a novel role for a GLP-1R-FGF21 axis in mediating carbohydrate consumption via its actions in the CNS.

Disclosures: T.D.V. Le: None. **N. Bozadjieva Kramer:** None. **R.J. Seeley:** None. **J.E. Ayala:** None.

Nanosymposium

515. Central and Peripheral Mechanisms of Energy Metabolism

Location: SDCC 24

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 515.07

Topic: F.08. Food and Water Intake and Energy Balance

Title: Parasympathetic signaling controls biosynthesis of endocannabinoids in the small-intestinal epithelium in diet-induced obesity and drives hyperphagia via local CB_1Rs

Authors: *C. P. WOOD, N. V. DIPATRIZIO;
Div. of Biomed. Sci., Univ. of California, Riverside, Riverside, CA

Abstract: The endocannabinoid (eCB) system is an endogenous lipid signaling system that controls food intake and energy balance. In diet-induced obese (DIO) mice, overactivation of cannabinoid receptor subtype-1 (CB_1R) in the small-intestinal epithelium (SI) inhibits nutrient-induced release of satiation peptides and promotes hyperphagia. We tested the hypothesis that parasympathetic signaling at muscarinic acetylcholine receptors (mAChRs) leads to increased biosynthesis of the eCB 2-arachidonoyl-*sn*-glycerol (2-AG) in the SI epithelium in DIO, which drives overeating via local CB_1Rs. Male mice were maintained on a high-fat/high-sucrose western-style diet (WD) for 60 days to induce DIO. Mice received IP injections of methylhomatropine bromide (ATR, a peripheralized mAChR antagonist), DAU5884 (DAU, a selective m_3 mAChR antagonist), or pirenzepine (PIR, a selective m_1 mAChR antagonist) 30 minutes prior to tissue harvest. Levels of 2-AG and its precursor, 1-stearoyl-2-arachidonoyl-*sn*-glycerol (SAG), in the SI were quantitated by UPLC-MS/MS. Ex-vivo activity of the synthetic and degradative enzymes for 2-AG, diacylglycerol lipase (DGL) and monoacylglycerol lipase (MGL), respectively, in the SI were also analyzed. Food intake, water intake, and ambulation were recorded with automated feeding chambers. DIO mice exhibited elevated levels of SAG, 2-AG, and DGL activity in the SI, when compared to lean controls maintained on standard chow. These effects were blocked by ATR, DAU, or PIR. Furthermore, ATR, DAU, or AM6545 (a peripheralized CB_1R neutral antagonist) reduced caloric intake in DIO mice to levels found in lean mice during a 24 h test. A second group of male mice conditionally lacking CB_1Rs in the SI (IntCB$_1$-/-) and controls (IntCB$_1$+/+) were maintained on WD for 60 days. Mice received single IP injections of ATR or AM6545 and caloric intakes were recorded for 24 h. ATR and AM6545, independently and combined, reduced caloric intake in IntCB$_1$+/+ DIO mice for up to 24 h but had no effect on intake in IntCB$_1$-/- mice. These results suggest that in DIO, hyperactivity at G_q-coupled mAChRs in the periphery increases the PLC-dependent generation of SAG, which is then converted to 2-AG by DGL in the SI and activates local CB_1Rs to drive hyperphagia.

Disclosures: C.P. Wood: None. **N.V. DiPatrizio:** None.

Nanosymposium

515. Central and Peripheral Mechanisms of Energy Metabolism

Location: SDCC 24

Time: Tuesday, November 15, 2022, 1:00 PM - 3:00 PM

Presentation Number: 515.08

Topic: F.08. Food and Water Intake and Energy Balance

Title: Therapeutic targeting of stress metabolism in obesity and metabolic disease

Authors: *Z. KRUMM[1], H. FUTCH[2], K. MCFARLAND[1], B. MOORE[1], Y. LEVITES[1], T. GOLDE[1];
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Abstract: While sufficient acute stress responses are crucial to learning, development, and adaptation to the environment in both model animal systems and humans, severe acute and prolonged chronic stress drive maladaptive changes in an organism that drive the progression of several chronic diseases, including obesity, diabetes, vascular disease, neurodegenerative dementias, aging, substance use disorders, and depression, among several other disease processes. Stress metabolism is largely governed by two cascades. Corticotropin-Releasing Factor (CRF), synthesized and released from several brain nuclei in response to stressful stimulus, signals directly to neuronal circuits that directly impact behavior, cognition, metabolism, and several other processes. In parallel, CRF synthesis and release is greatest in the Paraventricular Nucleus (PVN) of the hypothalamus, where it travels along descending neurons that stimulate Adrenocorticotropic Hormone (ACTH) release from the pituitary gland, which enters circulation to stimulate cortisol/corticosterone synthesis and release from the adrenal gland. While the relationship between stressors, stress metabolism, and several disease processes has been characterized, attempts to develop therapeutics that intervene in or interrupt the progressive contributions of stress to clinical disease have been underwhelming. This may be due to the fact that small molecule therapeutics have often had only a modest impact basal stress metabolite levels. Additionally, candidates have had relatively short half-lives. We have developed a picomolar-affinity monoclonal antibody against CRF that suppresses both basal and stress-induced glucocorticoid levels, demonstrating a half-life of approximately 7 days. Using metabolic phenotyping paired with transcriptomic/proteomic analysis, we have demonstrated that longitudinal treatment of multiple animal models with this antibody has demonstrated durable therapeutic effect in diet and age-induced metabolic dysfunction, resulting in reduced body weight, reduced food intake, improved glucose tolerance, and improved body composition. This results in a 30-50% reduction in the weight and fat gained in multiple models challenged with diet-induced obesity, with more a favorable distribution of adipose tissue and addition of lean mass observed compared to landmark GLP1RA candidates. These effects are especially prominent in older animals. These endpoint targets represent a novel approach to targeting stress pathways in chronic metabolic disease and may represent primary or adjunctive therapies for several pervasive clinical diseases.

Nanosymposium

516. Cortical Basis of Cognitive Control Across Species

Location: SDCC 23

Time: Tuesday, November 15, 2022, 1:00 PM - 3:15 PM

Presentation Number: 516.01

Topic: H.04. Executive Functions

Title: The relationship between task demands and the dimensionality of control representations

Authors: *H. KEGLOVITS, A. BHANDARI, E. CHICKLIS, D. BADRE;
Cognitive Linguistics and Psychological Sci., Brown Univ., Providence, RI

Abstract: Cognitive control enables flexible mapping from diverse inputs to outputs. Theories of control posit a representation in PFC which enables this mapping, but the relationship between task structure and the geometry of control representations is poorly understood. We focus on the geometric property of dimensionality, or the number of unique components required to specify all points in neural firing space across task conditions. Theoretical work has highlighted the computational properties of dimensionality, and studies of non-human primate physiology have observed its relationship with controlled behavior. One hypothesis motivated by these studies is that the PFC will generally favor separable, high dimensional representations independent of task demands in order to enhance flexibility. However, prior experiments have not manipulated the task demands most impactful for dimensionality. This leaves open an alternative hypothesis that the dimensionality of control representations may expand or compress based on task, and evidence from animal studies indicates that dynamic changes in geometry are possible. We tested these alternatives in humans by estimating the geometry of control representations during two tasks which differ in rule structure. Using a within-subject deep sampling (5 days X task X participant) approach to fMRI, we measured brain activity to estimate dimensionality while participants performed the two tasks. Across participants the tasks used the same stimulus sets, stimulus arrangements, and response options, but critically differed in the structure of the rules that map the stimuli to responses. One task used hierarchically structured rules but the other had a non-linear, flat rule mapping. Therefore, while the hierarchical task can be solved with a low dimensional geometry, the flat task requires a high dimensional geometry. To measure dimensionality we utilized a multi-pronged analysis approach, leveraging, in parallel, novel within-voxel repetition suppression effects and across-voxel multivariate pattern similarity. Preliminary results from N=8 (target N=20) provide evidence that task rule structure influences geometry. Specifically, multi-voxel pattern analysis has provided evidence for a more hierarchical representational geometry, i.e., a lower dimensionality, in the right posterior frontal cortex during the hierarchical task relative to the flat task. This study provides preliminary

evidence that task rule structure affects the geometry of control representations in PFC with dimensionality changing in accord with task demands.

Disclosures: H. Keglovits: None. **A. Bhandari:** None. **E. Chicklis:** None. **D. Badre:** None.

Nanosymposium

516. Cortical Basis of Cognitive Control Across Species

Location: SDCC 23

Time: Tuesday, November 15, 2022, 1:00 PM - 3:15 PM

Presentation Number: 516.02

Topic: H.04. Executive Functions

Support: NIH Grant R37 MH066078

Title: Studying neural mechanisms of flexible distractor resistance

Authors: *M. FREUND, J. M. BUGG, T. S. BRAVER;
Psychological and Brain Sci., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: A core aspect of human cognition is our ability to resist taking courses of actions naturally engendered by our behavioral environments, and via control representations encoded within PFC, to instead pursue goals that we have maintained internally. But, as our environments are liable to change, our control strategies must also be flexible enough to adapt. Such flexibility is clearly evident within classical "response conflict" paradigms such as Stroop, as performance depends strongly on the *history of congruency:* that is, people tend to be more effective at resisting the influence of distracting information after having recently performed several incongruent, rather than congruent, trials. While a multitude of neural control strategies could implement this performance enhancement — for example, enhancing prefrontal target representations, enhancing prefrontal distractor representations, or both — the mechanisms remain unclear. Leveraging retrospective RSA and decoding analyses of an extant multi-session color-word Stroop fMRI dataset (N = 73), we attempted to identify putatively mediating neural representations. Although we found that, during *"mostly congruent"* versus "*mostly incongruent"* sessions, neural Stroop effects within prefrontal cortex were enhanced (paralleling the behavioral Stroop effect), as well as distractor representations within early visual cortex (suggesting subjects encoded the word form more strongly), we did not find clear evidence for a systematic impact of congruency history on target or distractor coding in PFC. Results suggest the difficulty in identifying unbiased history-driven enhancements within stimulus-dependent representations (target, distractor) in PFC, as well as in retrofitting fMRI datasets for representational analyses. In an ongoing follow-up study, we are using a tailored Stroop task-switching design and EEG (current N = 8) to examine a more comprehensive spatiotemporal cascade of neural coding, including of pre-trial rules.

Disclosures: M. Freund: None. **J.M. Bugg:** None. **T.S. Braver:** None.

Nanosymposium

516. Cortical Basis of Cognitive Control Across Species

Location: SDCC 23

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Presentation Number: 516.03

Topic: H.04. Executive Functions

Support: NIH Grant MH124849

Title: Orthogonal neural encoding of targets and distractors during cognitive control

Authors: *H. RITZ, A. SHENHAV;
Brown Univ., Brown Univ., Providence, RI

Abstract: People flexibly adapt neural information processing to achieve their goals through an array of different strategies. Our previous research has found robust behavioral markers of independent attentional control over target and distractor processing in a novel task (Ritz & Shenhav, 2021). Here, we examine whether this independent control is mirrored by independent neural representations of targets and distractors. Human participants (N=29) performed a random dot color-motion task during fMRI. In the critical condition, they made a left/right keypress indicating which dot color was in the majority, ignoring dot motion. We parametrically varied target difficulty (% dots in majority color), and distractor congruence (% dots moving in the same or opposite direction as the color response). Consistent with our previous work, participants' task performance depended on both targets and distractors (ps < .0001). We first tested whether task difficulty representations in dorsal anterior cingulate cortex reflect overall difficulty or differentiate targets from distractors. We found a rostrocaudal axis, with caudal encoding of target difficulty and rostral encoding of distractor difficulty. Targets and distractors were encoded orthogonally (patterns were uncorrelated), consistent with the monitoring of multiple control demands. We next tested whether representations of feature strength (absolute coherence) reflected global salience or feature-specific attentional priority. In parietal cortex, we found encoding of both targets and distractors, with a mediolateral dissociation. Whereas target and distractor patterns were negatively correlated in superior parietal lobule (cross-validated), they were orthogonal in intraparietal sulcus (Bayesian t-test). We further tested whether feature strength representations depend on task performance and control demands, as indices of cognitive control. Compared against patterns encoding RT and accuracy, across the frontoparietal network we found target strength patterns were aligned with better performance, whereas distractor strength patterns were aligned with poorer performance. We manipulated control demands by having participants view identical stimuli but respond to motion, which previous work has shown is more automatic. During these more automatic blocks, task-relevant regions similarly encoded response evidence (whether a feature supports a left vs right choice), but no longer encoded feature strength. Together, we find that independent control over target and distractor processing is mirrored by orthogonal neural representations of task difficulty and stimulus information.

Disclosures: H. Ritz: None. **A. Shenhav:** None.

Nanosymposium

516. Cortical Basis of Cognitive Control Across Species

Location: SDCC 23

Time: Tuesday, November 15, 2022, 1:00 PM - 3:15 PM

Presentation Number: 516.04

Topic: H.04. Executive Functions

Title: Neural representation of learning multiple abstract rules in fronto-parietal network and basal ganglia

Authors: *S. TAFAZOLI[1], F. BOUCHACOURT[2], N. T. MARKOV[2], M. UCHIMURA[2], M. G. MATTAR[3], N. D. DAW[2], T. BUSCHMAN[2];
[1]Princeton Univ., [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]Dept. of Cognitive Sci., UCSD, San Diego, CA

Abstract: Intelligent behavior requires adapting to changes in the environment; we must be able to learn new rules and flexibly switch between known rules. To understand the mechanisms underlying this flexibility, we trained monkeys to switch between three different rules. Each rule required the animal to categorize either the shape or the color of the stimulus and then respond by making the associated eye movement along either one of two different response axes (rule 1: respond to shape on axis 1, rule 2: respond to color on axis 2, rule 3: respond to color on axis 1). Monkeys combined both fast and slow learning: they rapidly switched to the correct response axis, while, within each axis, the monkeys slowly relearned the category-response mapping on each block. Behavioral modeling favored a class of hybrid models that included both Bayesian inference and incremental learning: animals used Bayesian inference to infer the current axis of response and incremental learning to continuously re-estimate the stimulus features associated with each response within the axis. These two learning processes are thought to use two systems: inference relies on prior knowledge of the task structure to infer which rule applies while incremental learning relies on feedback to learn associations. While recent evidence has shown that fast inference is associated with dynamics of the prefrontal cortex, slow incremental learning has long been associated with dopamine and the basal ganglia. A complete theory of learning involves an interaction between these two classes of learning modes. To assess how the neural representation of task parameters emerges in these two systems during learning, we used large-scale simultaneous recordings across the fronto-parietal network, basal ganglia and inferior temporal cortex. Preliminary analyses revealed multidimensional encoding of task variables across all three areas: while stimulus features were represented in the inferior temporal cortex (IT), rule and reward information were represented in prefrontal (PFC) and basal ganglia, respectively. Our results show behavioral effects of inferential and incremental learning systems in a single task setting and reveal complementary contributions of fronto-parietal network and basal ganglia in rule learning and rule switching.

Nanosymposium

516. Cortical Basis of Cognitive Control Across Species

Location: SDCC 23

Time: Tuesday, November 15, 2022, 1:00 PM - 3:15 PM

Presentation Number: 516.05

Topic: H.04. Executive Functions

Title: Investigating the effects of control signals on subsequent-trial performance

Authors: *C. TENG[1], **J. M. FULVIO**[2], **M. PIETRELLI**[3], **J. JIANG**[4], **B. R. POSTLE**[5];
[1]Psychiatry, Univ. of Wisconsin–Madison, Madison, WI; [2]Psychology and Psychiatry, Univ. of Wisconsin - Madison, Middleton, WI; [3]Psychiatry, UW-Madison Neurosci. Training Program, Madison, WI; [4]Psychological & Brain Sci., Univ. of Iowa, Iowa City, IA; [5]Psychology and Psychiatry, Univ. Of Wisconsin–Madison, Madison, WI

Abstract: On a working-memory (WM)-plus-visual-discrimination dual task experiment, behavioral performance suggests that conflict between WM and perception triggers two modes of flexible control: a reactive mechanism that suppresses perceptual information to protect the contents of WM (captured by two effects on the subsequent trial: feature-specific impairment of recall precision, and a repulsive serial bias); and a proactive mechanism that increases preparedness for the upcoming trial (captured by a Gratton effect; Teng et al., under revision). To identify the neural correlates of these two control processes, we replicated this behavioral procedure while concurrently recording EEG (N=25). Each trial began with the presentation of an oriented Gabor ("memorandum"), followed by a delay period in which a second Gabor ("discriminandum") required a clockwise/counterclockwise judgment (50% congruent in orientation with the memorandum), followed by recall of the memorandum. We operationalized control using two parameters from the Flexible Control Model (FCM; Jiang et al., 2014, 2015): a control prediction error (control PE) parameter dependent on memorandum-discriminandum congruity; and a predicted conflict parameter that captures the tonic level of proactive control, and is adjusted in response to each trial's control PE. Model-based analyses indicated that, following a string of congruent trials, when trial n's discriminandum was incongruent with its memorandum, the magnitude of the resultant control PE was tracked by a phasic frontal positivity beginning at roughly 175ms. This triggered reactive suppression of the discriminandum, as evidenced by a flipping of its reconstruction with multivariate inverted encoding modeling (IEM). This control PE also triggered an increase in predicted conflict, which was tracked by an adjustment of the level of midline frontal theta power that persisted across the ITI and into trial n+1, leading to enhanced IEM reconstruction of trial n+1's memorandum. One account of repulsive serial bias is that information from trial n is unintentionally reactivated during trial n+1, and exerts an influence on trial n+1's memorandum. Our data do not support

this, showing no evidence for reactivation of the memorandum from trial n at the beginning of trial n+1. Instead, we observed a reinstatement of the flipped IEM reconstruction of the discriminandum from trial n. This supports a control-based account, whereby a residual "negative template" produced by reactive suppression during trial n has spillover consequences for trial n+1: the feature-specific impairment of recall precision, and a repulsive serial bias.

Disclosures: C. Teng: None. **J.M. Fulvio:** None. **M. Pietrelli:** None. **J. Jiang:** None. **B.R. Postle:** None.

Nanosymposium

516. Cortical Basis of Cognitive Control Across Species

Location: SDCC 23

Time: Tuesday, November 15, 2022, 1:00 PM - 3:15 PM

Presentation Number: 516.06

Topic: H.04. Executive Functions

Title: Proximity to Appetitive and Aversive Goals Jointly Determines Parameters of Effortful Control Exertion

Authors: *S. DEVINE[1], M. ROY[2], R. OTTO[3];
[1]McGill Univ., [2]Psychology, [3]McGill Univ., Montreal, QC, Canada

Abstract: The now-classic *goal gradient hypothesis* posits that an organism increases effort expenditure as a function of proximity to a goal—for instance, a rat's tendency to run faster towards a nearby reward (approach) or away from a painful stimulus (avoidance). Despite nearly a century having passed since its original formulation, goal gradient-like behaviour in humans remains poorly understood both in the approach and avoidance domains. Recently, the goal gradient hypothesis has found support in the domain of human cognitive control—individuals are willing to exert higher levels of cognitive control in service of proximal versus distal goals. However, thus far these results have been limited to appetitive (i.e., approach) goals and the computational mechanisms for the effects of goal proximity on effortful control—that is, whether goal proximity affects fidelity of stimulus encoding, evidence accumulation thresholds, or other identifiable mechanisms governing speed and accuracy—are far from clear. Here, in two experiments using an attentionally demanding oddball task, we rigorously examined approach and avoidance goal gradient sensitivity in humans, which, importantly allow us to probe the specific cognitive mechanisms underpinning these goal gradient effects using hierarchical drift-diffusion modelling (HDDM). In Experiment 1 (N = 40), we find that subjects responded more quickly, but not less accurately, when rewards were proximal (fewer correct responses were needed to obtain the reward) than when they were distal. Critically, this effect was only observed when subjects were given information about goal proximity. In Experiment 2 (N = 40), we replicated this pattern of response for aversive stimuli—subjects responded more quickly, but not less accurately, when the threat of physical shock was proximal vs. distal. Fitting HDDMs to behavior, we found that this pattern of behaviour—speeded, but nevertheless accurate, responding as a function of stimulus proximity—was driven by increased drift rates and non-

decision times near the motivating stimulus, be it appetitive (Experiment 1: rewards) or aversive (Experiment 2: pain). Together, our results support and extend classical research on goal gradients documented in animals by empirically testing key postulates of the theory in two incentive domains, as well as propose a basic cognitive mechanism underlying goal gradient adaptations in human behaviour.

Nanosymposium

516. Cortical Basis of Cognitive Control Across Species

Location: SDCC 23

Time: Tuesday, November 15, 2022, 1:00 PM - 3:15 PM

Presentation Number: 516.07

Topic: H.03. Decision Making

Title: Anterior cingulate cortex lesions alter selection of, but not preference for, social stimuli in rhesus monkeys (Macaca mulatta)

Authors: *S. J. WATERS[1,2], P. KUśMIEREK[1], J. PARK[1], B. M. BASILE[1], C. J. WATERS[1], E. A. MURRAY[1];
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Abstract: Primate social behavior depends on a network of brain regions specialized for processing social information. One such region—the anterior cingulate cortex (ACC)—is broadly implicated in social cognition by virtue of its connections with the amygdala, the orbitofrontal cortex, and the temporal-parietal junction, among other regions. Our previous research has demonstrated that ACC damage blunts learning of prosocial preferences in a vicarious reinforcement paradigm (Basile et al., 2020). The present study further investigates the role of the ACC by measuring explicit choice of neutral stimuli that predict the onset of social stimuli in adult male rhesus monkeys (*Macaca mulatta*) with selective, excitotoxic lesions of the ACC ($n = 3$) and unoperated controls ($n = 3$). All monkeys were postoperatively trained to perform a computerized behavioral task that probed their preference for viewing images with social (conspecific faces) or nonsocial (inanimate objects) content. On each trial, the monkeys were required to choose via eye fixation one of two neutral antecedent stimuli that predicted the subsequent presentation of either a social or nonsocial image. All monkeys exhibited a slight but statistically significant preference for choosing the antecedent that predicted social images. ACC lesions did not reduce preferences for viewing social images; however, monkeys with ACC lesions tended to commit more errors in maintaining fixation before making a choice compared to unoperated controls. These findings suggest that monkeys with ACC damage experience greater difficulty than controls evaluating deliberate actions associated with social outcomes but have no difficulty assessing the inherent value of social stimuli *per se*.

Nanosymposium

516. Cortical Basis of Cognitive Control Across Species

Location: SDCC 23

Time: Tuesday, November 15, 2022, 1:00 PM - 3:15 PM

Presentation Number: 516.08

Topic: H.03. Decision Making

Title: The Neural Substrates of Metacognitive Monitoring and Control

Authors: *Y. NANJO[1], T. YAMAMOTO[1], D. AGUILAR-LLEYDA[2], R. AKAISHI[3], N. SADATO[4];
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Abstract: Metacognition allows us to monitor our decisions, mainly by estimating our confidence in them, and then use those estimates to control subsequent decision-making. While some studies have investigated the relationship between metacognitive monitoring and control, little is known about their neural relationship, including the overlapping or independence of the neural substrates behind both processes. To investigate that issue, we used a context in which high low confidence in a first perceptual decision was likely to relax enhance metacognitive control, resulting in less more resources being devoted to process subsequent evidence, with this in turn reducing increasing changes of mind for that decision. We then identified which brain areas represented a metric for metacognitive monitoring, in this case initial confidence, and how this metric influenced activation in a candidate area for metacognitive control. In our functional magnetic resonance imaging (fMRI) experiment, 34 subjects performed a perceptual decision-making task. Within a single trial, subjects saw two circular stimuli on the screen, indicated which stimulus was larger, and rated their confidence in this decision; then they saw the same stimuli again, and again made the size decision followed by a confidence rating in this second decision. Across trials, four difficulty levels (differences in size between the two circles) were presented. For each subject, within each difficulty level all trials were median-split according to confidence in the first decision (high vs low confidence). We then calculated, for each confidence level, the probability of subjects changing their mind (first and second decisions being different). Subjects were more likely to change their mind when confidence in the first decision was low, hinting at metacognitive monitoring influencing subsequent control. The fMRI analyses revealed, during the time of the first decision, higher activity in the medial frontopolar area when confidence for that first decision was also higher. This area representing confidence, and thus reflecting metacognitive monitoring, is consistent

with previous studies (Fleming et al., 2010; 2012). Dorsal anterior cingulate cortex (dACC) was closely related to metacognitive control, as its activation showed the influence of the first decision's confidence, with higher activity when confidence in the first decision was low. Moreover, dACC was more active during the second decision when that decision represented a change of mind. Our results suggest that the neural substrates of metacognitive monitoring and control are anatomically dissociable but closely interacting neural systems.

Nanosymposium

516. Cortical Basis of Cognitive Control Across Species

Location: SDCC 23

Time: Tuesday, November 15, 2022, 1:00 PM - 3:15 PM

Presentation Number: 516.09

Topic: H.03. Decision Making

Title: Macaque prefrontal cortex reflects abstract, not embodied, decision-related activity before representing motor plans

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Abstract: During visually guided behavior, the prefrontal cortex plays a pivotal role in mapping sensory inputs onto appropriate motor plans. When the sensory input is ambiguous, this involves deliberation. It is not known whether the deliberation concerns the most likely interpretation of the stimulus, or the motor response that is most likely to be appropriate. To distinguish between these hypotheses, we used multi-electrode arrays to record neural population activity in the prearcuate gyrus (PAG) of two macaque monkeys while the animals performed perceptual inference in a 2-AFC discrimination task. The monkeys judged the orientation of a stimulus presented in the near-periphery and communicated their judgment by means of a saccadic eye movement towards one of two choice targets. The meaning of the response options was not derived from their spatial position (one was placed within the neurons' motor response field, the other on the opposite side of the fixation mark), but from a visual feature (one was oriented clockwise, the other counterclockwise). This dissociation of the meaning of the choice targets from the direction of the saccade the monkey used to indicate his choice enabled us to distinguish the neural correlates of perceptual inference from those of action selection. Importantly, the choice targets were presented to the monkeys 250 ms before the onset of the stimulus and remained on until the end of the trial. Stimulus-focused deliberation and response-focused deliberation were therefore both viable decision-making strategies. We then built a

population decoder optimized to predict the monkey's perceptual choice and corresponding motor action. We found that the resulting decision variable (DV) trajectories exhibited an initial excursion along the perceptual dimension, followed by an excursion along the motor dimension. This suggests that choice formation took place in an abstract perceptual space, and was then mapped onto a motor response. Consistent with this interpretation, we found that all task variables that influenced the choice behavior influenced the perceptual DV, but not the motor DV. Specifically, stimulus orientation, contrast, and frequency impacted the perceptual DV, but not the motor DV. Together, our results provide strong support for the hypothesis that neural activity in the prefrontal cortex can reflect the evaluation of evidence that bears on a decision in a task-specific abstract representation space. The subsequent representation of a motor plan reflects the outcome of this process, not the deliberation itself.

Disclosures: J. Charlton: None. **R.L. Goris:** None.

Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.01

Topic: H.08. Learning and Memory

Title: Hippocampal slow theta oscillations support memory formation in children

Authors: R. LU[1], **Q. YIN**[2], **W. K. CHANG**[4], **E. M. B. RAU**[5], **O. KIM MCMANUS**[6], **S. SATTAR**[6], **J. J. LIN**[7], **E. F. CHANG**[8], **R. T. KNIGHT**[4], **E. ASANO**[9], **N. OFEN**[3], ***E. L. JOHNSON**[1];
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Abstract: Understanding complex human brain functions is critically informed by studying such functions during development. Our research addresses gaps in human memory models by leveraging rare direct electrophysiological recordings from children, adolescents, and young adults. Here, we investigated medial temporal lobe (MTL) mechanisms in 13 female and 15 male neurosurgical patients aged 6-30 years as they performed an established scene memory task. MTL is crucial for memory and is posited to support memory in children, but the neurophysiological mechanisms are largely unknown. To address this unknown, we analyzed data from the study phase, during which patients viewed scenes (3 s each) and indicated whether each was indoor/outdoor, as a function of subsequent recognition performance (i.e., subsequent

memory effects in hit vs. miss trials). Previous studies in adults suggest a regional division of labor, such that cortical regions involved in information encoding exhibit negative low-frequency power effects (hit < miss) and the hippocampus and other regions involved in binding information into episodes exhibit positive effects (hit > miss). However, different brain regions mature at different rates, suggesting that this division of labor may manifest across a protracted maturational trajectory, even within MTL. First, we present published data from subdural recordings of parahippocampal and rhinal cortices (80 electrodes; Johnson et al., 2022, Current Biology), which reveal: 1) distinct slow and fast theta oscillations that separate in frequency across development and 2) negative theta power effects around the indoor/outdoor response, consistent with information encoding. Second, we present new data from stereotactic recordings of the hippocampus (28 electrodes) in a non-overlapping subsample. Analyses suggest: 1) age-invariant slow theta oscillations and two slow theta signatures of memory formation, 2) one replicating the negative power effect observed in parahippocampal and rhinal cortices and, the other, 3) a distinct set of positive power and phase resetting effects beginning before scene onset. In addition, we demonstrate: 4) positive effects in the coupling of local population activity (indexed by high-frequency broadband amplitude) to slow theta phase between scene onset and the indoor/outdoor response, consistent with information binding. Our findings reveal spatiotemporally precise signatures of memory formation in MTL subregions and suggest that hippocampal slow theta mechanisms are mature in children.

Disclosures: R. Lu: None. **Q. Yin:** None. **W.K. Chang:** None. **E.M.B. Rau:** None. **O. Kim McManus:** None. **S. Sattar:** None. **J.J. Lin:** None. **E.F. Chang:** None. **R.T. Knight:** None. **E. Asano:** None. **N. Ofen:** None. **E.L. Johnson:** None.

Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.02

Topic: H.08. Learning and Memory

Title: Beta oscillations involvement in action feedback processing in human brain

Authors: *I. SKELIN[1,2], H. ZHANG[4], M. R. PAFF[6], S. VADERA[5], O. KIM MCMANUS[7,8], E. A. BUFFALO[9,10], R. T. KNIGHT[11,12], J. J. LIN[3,2];
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Abstract: Beta range oscillations (20-30 Hz) are proposed to mediate positive feedback signaling in the human brain. This proposal is based on increased scalp EEG beta power following the correct trials in a variety of behavioral paradigms. We analyzed the anatomical and mechanistic underpinnings of beta feedback signaling during rapid rule acquisition and switching. We recorded intracranial EEG in subjects undergoing pre-surgical evaluation for treatment of pharmacologically resistant epilepsy, during performance on Wisconsin Card Sorting Task (WCST; 16 subjects, 32 sessions, 977 electrodes in frontal and temporal lobes). WCST requires the subjects to find the correct rule out of 12 possible rules, across 3 different dimensions (any of the 4 colors, shapes or textures), with unpredictable rule switching. Rule search is guided by the 'correct' or 'incorrect' feedback message displayed on the screen during feedback period, immediately following the choice. Feedback tuning of local neuronal populations was measured using the high frequency activity (HFA; 70-200 Hz) as a proxy for local cortical activity. Increased beta power and beta-HFA phase-amplitude coupling (PAC) on individual electrodes predicted feedback tuning ($p<0.05$; linear mixed effect model, 100 permutations). This pattern of results supports beta activity in organizing the feedback representation at the neuronal level. Next, we identified the incidence of beta bursts - brief periods of beta oscillatory activity (<150 ms) - during feedback period, selectively on feedback-tuned electrodes. During the periods of increased beta bursts, gamma burst incidence was decreased, similar to antagonistic beta-gamma bursts dynamics observed during working memory maintenance periods in non-human primates. This finding extends the importance of beta/gamma burst dynamics beyond the working memory domain, highlighting their role in feedback information processing, critical for adaptive behavior.

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Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

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Presentation Number: 517.03

Topic: H.08. Learning and Memory

Support: NIH NIMH R01MH128187

Title: Human neuronal population encoding of temporal difference learning variables during risky choices

Authors: E. H. SMITH[1], R. COWAN[2], T. DAVIS[1], B. KUNDU[3], J. D. ROLSTON[4], S. RAHIMPOUR[3], Z. KURTH-NELSON[5], M. M. BOTVINICK[7], S. W. KENNERLEY[6], T. MULLER[8];

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Abstract: Recent research in artificial intelligence showed that learning to predict the full distribution of potential rewards, rather than a central estimate of that distribution, leads to better performance, especially on risky tasks. Correlates of distributional reinforcement learning (distRL) have since been discovered in dopamine neurons in the rodent ventral tegmental area. In this nanosymposium presentation, I will discuss recent work from direct brain recordings in neurosurgical patients undergoing monitoring for treatment of medically refractory epilepsy who performed a risky decision-making task called the Balloon Analog Risk Task (BART). Results from two studies will be presented: In the first study, we examined neuronal population recordings (157 neurons) from microelectrodes implanted in the anterior cingulate, orbitofrontal and temporal cortices (15 participants), finding that human prefrontal and mesial temporal neurons exhibited signatures of distRL: diverse optimism in reward coding, diverse asymmetric scaling of reward prediction error, and correlation between optimism and asymmetric scaling. In the second study, we examined broadband high-frequency local field potentials (an established correlate of population neuronal firing) while 37 participants made risky choices during BART. We found differences in which brain areas (3199 stereoelectroencephalography or electrocorticography contacts sampling frontal, temporal, and parietal lobes) encoded value estimates and reward prediction errors between participants who were more or less risk averse in their choices during BART. These areas included the left dorsolateral prefrontal, anterior cingulate, and orbitofrontal cortices. The results from these studies shed light on the neural underpinnings of human value learning in uncertain environments.

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Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.04

Topic: H.08. Learning and Memory

Support: NIH Grant 2R01-MH104606

Title: Neuronal activity in the human amygdala and hippocampus enhances emotional memory encoding

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Disorders and Stroke, Washington D.C., DC; [3]Univ. of Pennsylvania, Philadelphia, PA; [4]Columbia Univ., Columbia Univ., New York, NY

Abstract: Emotional events comprise our strongest and most valuable memories, yet it is unknown how the brain prioritizes emotional information for storage. Here, we examined the neural basis of this prioritization using direct brain recording, deep brain stimulation, and psychometric assessment in a large cohort of human subjects with electrodes temporarily implanted in the brain for epileptic seizure monitoring. These subjects performed an episodic memory task in which they showed improved performance for emotional stimuli (n=365). During the task, high-frequency activity (HFA), a correlate of neuronal spiking activity, increased in both the hippocampus and amygdala when subjects (n=138) successfully encoded emotional stimuli. Applying inhibitory electrical stimulation to the hippocampus decreased HFA and specifically reversed the enhancement of memory for emotional stimuli, indicating that neuronal activity in this region has a direct role in prioritizing emotional memories. Finally, we found altered patterns of HFA in depressed individuals which correlated with a bias for negative memories in these subjects (n=51). Going forward, targeted modulation that upregulates neuronal excitation in the amygdalohippocampal circuit may have a causal and translational role in modulating emotional memory.

Disclosures: S.E. Qasim: None. **U.R. Mohan:** None. **J.M. Stein:** None. **J. Jacobs:** None.

Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.05

Topic: H.08. Learning and Memory

Title: A week in the life of the brain: stable states punctuated by chaotic-like transitions

Authors: *M. WANG[1], M. G'SELL[2], M. RICHARDSON[3], A. S. GHUMAN[4];
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Abstract: The vast majority of what we know about human brain dynamics is on the scale of milliseconds to seconds while subjects perform artificial, well-controlled tasks, typically inside an imaging machine. Many important neurocognitive processes take place over minutes to days in chaotic, ever-changing ``real'' environments. While some studies look at neural states over

minutes, they still do so in an artificial environment while at rest or presented stimuli. As a result, it remains mostly unknown what rules and patterns govern how the brain physiologically changes over hours to days in ecologically realistic situations. In this project, we analyzed neural recordings collected from neurosurgically embedded devices in twenty humans for roughly a week-long period each (between 75-283 hours). During this week, subjects naturally interacted with friends and family, staff, watched TV, slept, etc. The functional networks that emerged possessed simple, conserved rules that governed their individual temporal dynamics as well as their synchrony/anti-synchrony with one another. These features were linked to physiological phenomenon such as circadian rhythm and arousal. Global brain network dynamics assessed as mixtures of individual networks were complex and would show patterns of punctuated equilibrium: periods where networks would remain in stable states that were interrupted by volatile state transitions that were unique, difficult to predict, and showed patterns associated with chaotic systems. We then show that these dynamic transitions are distributed in a way that is consistent with self-organized critical systems, a concept that has been used to explain how systems that possess simple, local interaction rules can generate emergent complex behavior such as earthquakes, solar flares, financial market fluctuations, and biological cascades. Together, these results show that complex and flexible intra-network dynamics are an emergent property arising from mixtures of simple and stable internetwork dynamics that result in punctuated equilibrium.
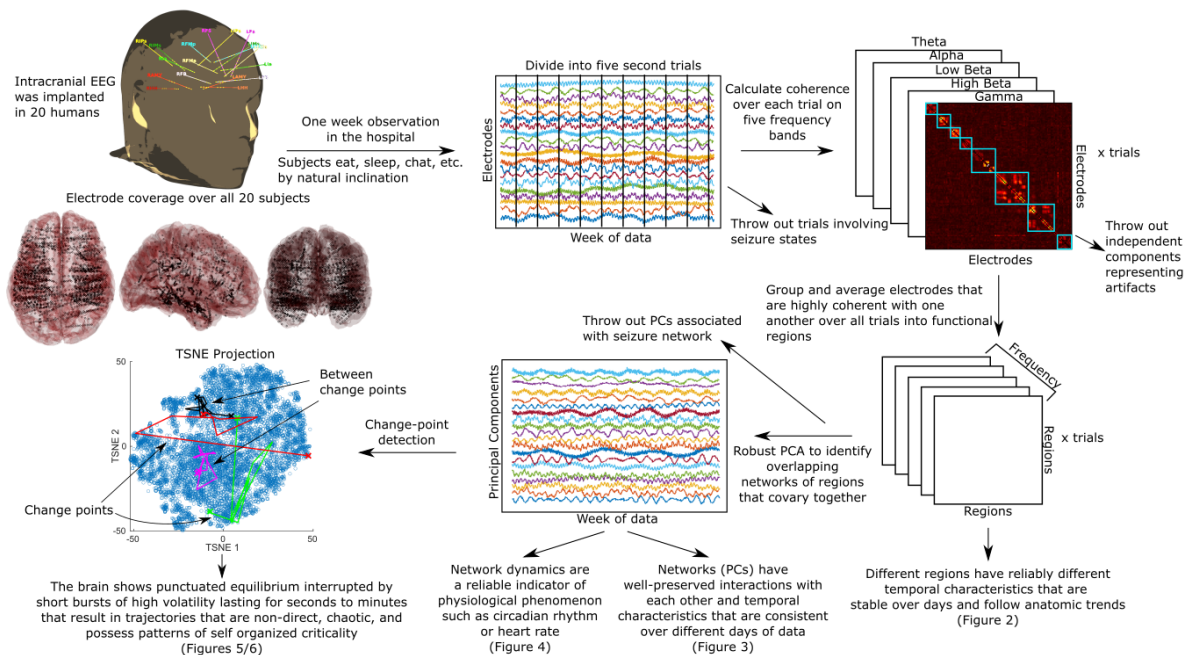
Disclosures: M. Wang: None. **M. G'sell:** None. **M. Richardson:** None. **A.S. Ghuman:** None.

Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.06

Topic: H.08. Learning and Memory

Support: NIH U01NS117839

Title: Phase Precession Supports Memory Formation of Continous Experience In Humans

Authors: *J. ZHENG[1], **M. YEBRA**[2], **S. KALIA**[3], **T. A. VALIANTE**[4], **A. N. MAMELAK**[5], **G. KREIMAN**[6], **U. RUTISHAUSER**[2];
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Abstract: The ability to construct temporal associations among sequential events is essential to episodic memory. Theta phase precession, in which hippocampal place cells represent sequences of positions by firing at progressively earlier phases of theta rhythms, is thought to be a critical mechanism for binding and representing sequential events for learning and memory. However, whether theta phase precession occurs during the continuous experience in humans and how theta phase precession influences subsequent memory performance remain unclear. To address these questions, we investigated theta phase precession in humans during video clip watching to mimic the memory formation of realistic ongoing experience. We recorded single neuron activity (985 neurons) and local field potentials (LFPs) from 20 drug-resistant epilepsy patients during their stays at the hospital under seizure monitoring. Subjects first watched 90 silent video clips embedded with different types of cognitive boundaries - episodic transitions between contextually related/unrelated events. Their memory for each clip was later evaluated using scene recognition and time discrimination tasks. During scene recognition, participants were instructed to decide whether an extract frame from an encoded or novel clip is old (have seen) or new (have not seen). During time discrimination, participants were asked to determine which of the two frames occurs first in the watched clip. We found that in contrast to the rhythmic and continuous theta oscillations observed in rodents, theta rhythms in the human LFPs were transient and non-sinusoidal. Theta rhythms tend to occur within the 1-second time window after cognitive boundaries (i.e., boundary window), with higher frequencies and higher rise-decay asymmetry than other theta rhythms detected outside the boundary windows. Theta phase precession was evident at cognitive boundaries, with significant circular-linear correlations between spiking phases and time and progressive advancement of spiking phases across consecutive theta cycles. Further, cells demonstrating such phase precession largely overlapped with previously reported boundary-responsive cells, whose firing rates increased at cognitive boundaries (*Zheng et al., Nature Neuroscience, 2022*). The magnitude of phase precession (i.e., circular-linear correlation coefficients) at cognitive boundaries during clip watching reflects subjects' subsequent recognition and order memory outcomes. In sum, our findings demonstrate that theta phase precession is not merely circumscribed to spatial navigation tasks but also underlies the processing of episodic sequential information.

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Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

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Presentation Number: 517.07

Topic: H.08. Learning and Memory

Support: NIH

Title: Intracranial Electrophysiological Signature of Cross regional Interplay in the Human Brain During Autobiographical Memory Retrieval

Authors: J. STIEGER[1], ***J. PARVIZI**[2];
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Abstract: Remembering personal events from our past (autobiographical memories, AM) requires the dynamic coordination of activity across a network of brain regions including not only the hippocampus (HPC) and its cortical counterparts, but also subcortical structures such as the anterior thalamus (ANT). Paucity of direct recordings from subcortical structures in the human brain have limited our understanding of the importance of these structures in higher cognitive functions such as the retrieval of past personal experiences. Here, we simultaneously recorded from the ANT along with the HPC and orbitofrontal cortex (OFC) and posteromedial cortex (PMC), two cortical regions known from imaging and lesion studies to be involved in AM processing, while participants engaged in experimental tasks of AM processing. Our findings revealed a distinct signature of functional relationship between the human ANT and its hippocampal and cortical counterparts during AM retrieval. Notably, we documented increased neuronal population activity in the ANT (i.e., increase in gamma (30-50Hz) power) during the retrieval stage of AM processing at a time when sites in the HPC, OFC, and PMC showed increased higher frequency (HF: 70-170Hz) activity. Moreover, the ANT gamma power was highly correlated with the simultaneous power of HF activity in the OFC and PMC while the ANT and HPC sites exhibited an increased phase coherence at low frequency (LF; 1-6Hz) at the individual brain level when subjects were actively retrieving AM events. Furthermore, using repeated single-pulse causal electrical perturbation of sites of interest revealed a directional connectivity across the four regions of interest. Specifically, the strength of causal effective connectivity measured between a pair of distinct neuronal populations across the ANT and HPC or ANT and OFC correlated with the degree of their co-activation during AM retrieval. Our findings provide basic information about the profile of electrophysiological activity across the human ANT, HPC, OFC, and PMC during AM retrieval which can inform future mechanistic models and theories of how neuronal populations across these regions interact to support the remembering of past events.

Disclosures: J. Stieger: None. **J. Parvizi:** None.

Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.08

Topic: H.08. Learning and Memory

Title: Cholinergic modulation of human hippocampal and entorhinal oscillations during memory

Authors: *T. GEDANKIEN[1], R. TAN[2], S. E. QASIM[3], J. JACOBS[1], B. C. LEGA[2];
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Abstract: Cholinergic synapses are essential for memory. While Alzheimer's disease and other age-related dementia entail loss of cholinergic pathways, injection of cholinergic drugs can directly modulate memory formation and its neural correlates. In rodents, injection of the anticholinergic drug scopolamine impairs memory and disrupts hippocampal slow theta oscillations. However, the neurophysiological mechanisms behind the effects of cholinergic modulation on human memory remain elusive. Here, using rare intracranial brain recordings, we describe the effects of scopolamine in the human medial temporal lobe (MTL) during a verbal memory task. We found that the episodic memory impairment induced by scopolamine was associated with decreased slow theta power during encoding as compared to placebo. Scopolamine also disrupted the phase reset of theta oscillations, and the magnitude of that disruption correlated with the degree of induced memory impairment. Lastly, we found that scopolamine significantly decreased oscillatory synchrony within the MTL. Our findings show that the cholinergic system significantly modulates theta power and phase dynamics in the MTL, providing strong evidence that the oscillatory synchronization of neuronal assemblies is functionally relevant for memory formation and associated disorders.

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Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.09

Topic: H.08. Learning and Memory

Title: Decoding depression severity from human intracranial recordings

Authors: *J. XIAO[1], N. R. PROVENZA[1], J. ASFOURI[2], J. MYERS[1], R. MATHURA[1], B. A. METZGER[1], J. ADKINSON[1], A. ALLAWALA[3], V. PIRTLE[1], D. OSWALT[1], B. SHOFTY[1], M. ROBINSON[1], S. J. MATHEW[1], W. K. GOODMAN[1], N. POURATIAN[4], P. R. SCHRATER[5], A. PATEL[1], A. S. TOLIAS[1], K. R. BIJANKI[1], X. S. PITKOW[1], S. A. SHETH[1];
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Abstract: The high degree of heterogeneity in depression symptom type and severity has made finding reliable biomarkers a challenging problem. In this study, we conducted intracranial neural recordings in three patients with severe depression to investigate the neural substrates of this disorder. Stereo-EEG electrodes were placed in brain regions involved in mood regulation and cognition such as anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC). Throughout each patient's nine-day stay in the inpatient monitoring unit after implant, we continuously recorded neural activity while frequently assessing depression severity. Across prefrontal channels, we found that reduced depression severity is associated with decreased low-frequency and increased high-frequency neural activity. The strength of the correlations suggested that depression severity may be reliably predicted from spectral features. Even with unusually frequent sampling using a novel adaptive testing tool, depression measurements were sparse relative to the high dimensionality of neural features. We therefore began by greatly reducing the dimensionality of the neural data. Using a model incorporating automatic region selection and regularized regression, we were able to select a subset of the most informative recording channels. After the model was fitted and selected using the training data, we used it to predict depression severity score in a held-out test set. Our models achieved reliable predictions using both leave-one-out and 5-fold cross-validation. There was a strong and significant correlation between predicted and measured symptom scores ($p < 10^{-4}$ for all participants). Our model selected ACC across most folds of the cross-validation process in all participants, indicating that ACC was the most informative region in predicting depression severity. Relaxing the single-region constraint revealed network involvement beyond ACC. We found that unique, individual-specific sets of spatio-spectral features were predictive of symptom severity, reflecting the heterogeneous nature of depression. The ability to decode depression severity from neural activity increases our fundamental understanding of the neurophysiological basis of depression and provides a target neural signature for personalized neuromodulation therapies.

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Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.10

Topic: H.08. Learning and Memory

Title: Amygdala and hippocampus interactions determining emotional memory formation and retrieval

Authors: *M. COSTA[1], D. LOZANO SOLDEVILLA[1], A. GIL NAGEL[2], R. TOLEDANO[2], C. R. OEHRN[3], L. KUNZ[4], M. YEBRA[5], C. MENDEZ-BERTOLO[6], L. STIEGLITZ[7], J. SARNTHEIN[8], N. AXMACHER[9], S. MORATTI[10], B. A. STRANGE[1];
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Abstract: Although important for survival, memory for aversive events can become maladaptive in psychiatric disorders. Communication between the amygdala and the hippocampus has been proposed to support emotional, relative to neutral, memory but it is still unknown how these two structures interact during successful encoding and retrieval of unpleasant information. We used simultaneous intracranial recordings from both structures in human patients, while they encoded and later retrieved (after 24 h) unpleasant and neutral scenes from the IAPS database.
At encoding, high gamma activity in the amygdala was enhanced at 310ms after stimulus presentation with a greater amplitude for aversive scenes that were later remembered. Subsequently, theta oscillations in the amygdala unidirectionally influenced hippocampus theta responses to aversive stimuli, but not neutral. At around 500ms gamma power increased in the hippocampus for stimuli that were later recalled irrespective of their valence. Critically, we found successful emotional memory encoding depends on the precise amygdala theta phase to which hippocampal broadband gamma activity and neuronal firing couple. We observed a consistent phase difference between successful and unsuccessful encoding of aversive scenes of ~1.67 radians, corresponding to approximately 30-45ms. Crucially, this time difference also led to a transient lagged coherence between gamma activity in the two structures that predicts subsequent memory for aversive stimuli.At retrieval, amygdala gamma activity was higher for aversive scenes compared to neutral ones and at around 400ms gamma responses increased for aversive scenes that were correctly remembered. By contrast, hippocampus gamma activity was

boosted for all correctly remembered compared to correctly rejected scenes. Correctly rejected scenes further enhanced theta activity starting from around 900ms. We further tested whether amygdala and hippocampus discriminate between old and new scenes and found that this effect was significant only in the hippocampus with higher gamma activity for old scenes compared to new ones. Overall, results at encoding reveal a mechanism whereby amygdala theta phase coordinates transient coherence between amygdala and hippocampal gamma activity to facilitate the encoding of aversive memories in humans. Results at retrieval suggest that this process may rather rely on hippocampus-centered circuits pointing to a different neuronal dynamic between the two structures.

Disclosures: M. Costa: None. **D. Lozano Soldevilla:** None. **A. Gil Nagel:** None. **R. Toledano:** None. **C.R. Oehrn:** None. **L. Kunz:** None. **M. Yebra:** None. **C. Mendez-Bertolo:** None. **L. Stieglitz:** None. **J. Sarnthein:** None. **N. Axmacher:** None. **S. Moratti:** None. **B.A. Strange:** None.

Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.11

Topic: H.08. Learning and Memory

Title: Human intracranial electrophysiology reveals the dynamics of salience network in threat processing

Authors: *M. S. SANDHU[1], A. ALJISHI[1], N. SHAIKH[1], L. LAMSAM[1], A. P. KAYE[2], E. C. DAMISAH[1];
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Abstract: Anxiety disorders are associated with excessive perception of threat. However, our understanding of pathophysiological mechanisms underlying this phenomenon is limited. Identification of neural networks involved can provide unique mechanistic insights and facilitate the development of circuitry therapeutics for anxiety and anxiety-related disorders. Amygdala-cingulate-insula (salience network) hyperreactivity to aversive events is observed in neuroimaging studies of anxiety disorders, in particular, habituation of amygdala has been observed as a correlate of PTSD severity. Understanding how spatiotemporal interactions in the salience network function to sustain threat perception is thus an important question. Here, we use intracranial electroencephalography (icEEG) to localize and observe salience network dynamics during threat processing. To do so, we utilized a recently developed anxiogenic spatial avoidance task to elicit threat processing at multiple timescales. We enrolled 18 subjects undergoing

intracranial icEEG for seizure onset localization performed a task involving spatial navigation to avoid a threat. We recorded 11 discrete regions, including the salience network (cingulate cortex, insula, and amygdala) and other regions, which comprised orbitofrontal, dorsolateral prefrontal cortex (PFC), dorsomedial PFC, ventromedial PFC, hippocampus, and entorhinal cortex. We observed widespread activation, i.e., increase in event-related potential (ERP) of limbic structures during the task, but salience network preferentially responded to aversive outcomes. However, the salience network exhibited ERP habituation to aversive outcomes over the course of task. This habituation to aversive outcomes over time correlated with increasing gamma power in the salience network. Our results suggest that accumulation of aversive outcomes is associated with habituation of the acute evoked response but an increase in gamma oscillations. Understanding the precise spatiotemporal patterns of the salience networks during the threat processing may identify critical nodes for anxiety-related circuit modulation.

Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.12

Topic: H.08. Learning and Memory

Title: Modulation of emotion and memory via direct brain stimulation in humans

Authors: *C. S. INMAN[1], J. R. MANNS[3], L. BLANPAIN[4], K. BIJANKI[7], M. S. E. SENDI[8], S. B. HAMANN[3], R. E. GROSS[9], D. L. DRANE[5], K. L. WAHLSTROM[1], M. HOLLEARN[1], J. CAMPBELL[2], B. MAHMOUDI[6], J. T. WILLIE[10];
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Abstract: The experience of emotion shapes our memories of the past, decisions during the present, and predictions of the future based on our significant past experiences. Recent work has examined the effects of direct electrical stimulation to the human amygdala on the autonomic nervous system, emotional experience, and long-term declarative memory. In this presentation, we describe a study examining the safety, physiological, and subjective effects of human amygdala stimulation. We found that amygdala stimulation in epilepsy patients undergoing monitoring of seizures via intracranial depth electrodes elicited immediate and substantial dose-dependent increases in electrodermal activity and decelerations of heart rate, most often without eliciting any subjective emotional response. We also show that high amplitude, direct amygdala

stimulation can provoke a reliable change in the subjective experience of emotion, although this modulation of subjective experience is rare and may be related to stimulation location near amygdalar projections to the hypothalamus via the central nucleus. In a subsequent study, we describe work showing that brief, low-amplitude, direct electrical stimulation of the human amygdala enhances long-term declarative memory without eliciting an emotional response. We describe the variety of stimulation parameters we've explored to test for further memory enhancement effects including stimulation amplitude, duration, and timing relative to an image event. We also show that neuronal oscillations in the amygdala, hippocampus, and perirhinal cortex during encoding and a next-day memory test indicated that a neural correlate of the memory enhancement was increased theta and gamma oscillatory interactions between these regions. This finding is consistent with the idea that the amygdala prioritizes consolidation by engaging other memory-related regions like the hippocampus. Taken together, these results show that emotion-related circuitry in the human brain can provoke autonomic and subjective changes in emotion and initiate endogenous memory prioritization processes in the absence of emotional input, addressing a fundamental question and opening a path to future therapies.

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Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.13

Topic: H.08. Learning and Memory

Support: NIH Grant R01MH110311

Title: Network dynamics of hierarchical processing in the prefontal cortex during task abstraction

Authors: *D. M. CLEVELAND[1], J. M. PHILLIPS[1], S. CHEN[3], S. MOHANTA[1], M. BOLY[2], L. WANG[4], Y. B. SAALMANN[1];
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Abstract: When performing tasks in our everyday lives, we must retain our overarching goal while simultaneously performing the concrete steps necessary to achieve that goal. This behavior, referred to as task abstraction, allows us to control our behavior across time and flexibly respond to different contexts. These behaviors are linked to the lateral prefrontal cortex (PFC), with anterior regions active during abstract goal processing and posterior regions active during concrete processing and motor responses, generating a rostro-caudal hierarchy. However,

the exact specification of this hierarchy is disputed, with theories placing either anterior PFC or dorsolateral PFC (DLPFC) at the top of the hierarchy. Additionally, different specifications ascribe different functions to the top of the hierarchy, alternately emphasizing the role of processing and maintaining abstract information, sending information to downstream prefrontal regions, or specifying the relevant context to maintain.

To clarify the role of the PFC in hierarchical processing, we collected stereotactic electroencephalography data from patients (n=10) with refractory epilepsy performing a task abstraction paradigm. Patients were asked to selectively respond to different features of a concrete rule cue, with the attended feature governing behavior determined by a prior abstract rule cue. We recorded from electrodes across the rostral and caudal PFC and examined each hypothesis of hierarchical processing across prefrontal areas. We used high gamma power (60-200Hz) to assess which regions respond preferentially to the abstract or concrete cue, non-parametric Granger causality to determine causal influences between PFC areas, and changes in power between abstract and concrete rules to test which regions perform task-relevant computations.

Preliminary data indicate anterior PFC electrodes show an evoked response in high gamma power during the abstract cue, while DLPFC electrodes show a later evolving response. However, anterior PFC electrodes show a similarly robust response to the concrete cue, suggesting anterior PFC does not solely respond to abstract processes. Additionally, anterior PFC modulates DLPFC activity from 4-10 Hz throughout the task more than the reverse. However, connectivity changes are not strictly rostro-caudal, as some areas caudal to DLPFC influence DLPFC more than the reverse. Finally, abstract rule selectivity in high gamma power is earlier and more robust in anterior PFC compared to DLPFC.

While our data provide more support for anterior PFC as the top of the hierarchy during task abstraction than DLPFC, we also reveal key differences from previous hierarchies.

Nanosymposium

517. Human Intracranial Recording: Memory, Cognition, and Emotion

Location: SDCC 33

Time: Tuesday, November 15, 2022, 1:00 PM - 4:30 PM

Presentation Number: 517.14

Topic: H.08. Learning and Memory

Title: Cingulum bundle stimulation to modulate affect, anxiety, and pain

Authors: *K. R. BIJANKI[1], B. A. METZGER[2], J. MANNS[3], C. S. INMAN[5], K. CHOI[6], D. L. DRANE[4], J. T. WILLIE[7];
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Abstract: ⸏SEP⸎**Introduction** The anterior cingulate cortex (ACC) and the underlying cingulum bundle have been ascribed putative roles in a wide variety of emotional and cognitive functions, including modulation of affective responses, error detection and conflict monitoring, attention to physical and psychic pain, and the motivation to persevere. The cingulum bundle is being evaluated as a possible neuromodulation target for the treatment of anxiety, pain, and mood disorders.
Methods Patients underwent stereotactic depth electrode implantation for epileptic seizure focus localization. Clinical post-implantation T1-weighted MRI scans were examined for electrode localization relative to the anatomical structure of interest; the cingulum bundle. Stimulation testing occurred extra-operatively in the epilepsy monitoring unit. Stimulation did not cause after-discharges or epileptiform activity. Effects of stimulation were evaluated across 37 contact pairs in 17 patients. Subjective effects were tracked using structured visual analog scale ratings of instantaneous happiness, relaxation, and contemporaneous pain. Objective effects were quantified in terms of local field potentials and shifts in affective bias, a cognitive proxy for emotional state.
Results Stimulation was accompanied by robust positive shifts in emotional bias (p=0.023), and electrophysiology showed significant stimulation-evoked reductions in endogenous 6-11hz power and coherence following active stimulation ($ps < 0.0001$) in the index patient. Across the study group, stimulation evoked a statistically significant positive shift in emotional biasing (t(7)=2.7296, p=0.0294), laughter, smiling, patient-reported happiness, relaxation, and/or analgesia in the majority of cases. Systematic examination of neuroimaging and behavioral responses across our 17-patient sample revealed spatial clustering of the behavioral components of response. Stimulation orientation (parallel > orthogonal to the cingulum bundle) predicts increased self-reported happiness (t=2.87, *p=.00742)*, and increased self-reported relaxation (t=3.536, p=0.00144). Stimulation orientation does not significantly predict self-reported reduction of pain (t=1.632, p=0.115). These findings suggest bipolar stimulation parallel to the cingulum bundle is most likely to evoke desirable responses.
Conclusions The current findings suggest the cingulum bundle as a target for mood and anxiety disorders, as well as chronic pain syndromes, and provides unique insights into the mechanism of DBS-induced effects in organized singular fiber bundles.

Disclosures: K.R. Bijanki: None. **B.A. Metzger:** None. **J. Manns:** None. **C.S. Inman:** None. **K. Choi:** None. **D.L. Drane:** None. **J.T. Willie:** None.

Nanosymposium

518. Networks: Functional Connectivity and Computation

Location: SDCC 25

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 518.01

Topic: I.06. Computation, Modeling, and Simulation

Support: 1RF1MH125933-01A1

Title: Surrogate models of neuroimaging data based on hierarchical modularity

Authors: *A. ABBASI[1], A. NANDA[1], Y. MU[2], J. MANLEY[3], A. VAZIRI[3], M. B. AHRENS[4], M. RUBINOV[1];
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Abstract: It is challenging to test the significance of new hypotheses in systems and network neuroscience. One promising approach to such tests involves building statistical models that constrain a relatively small number of neurobiological features and that have high explanatory power. An important set of such features concern the notion of hierarchical modularity, i.e., the nested subdivision of brain networks into systems, sub-systems, and regions. Here, we used this set of features to build models that incorporate empirically estimated hierarchically modular information from functional MRI data. We defined five hierarchical levels and sampled data in a way that preserved two types of constraints in each level. First, we preserved the module-average time series at every level. Second, we preserved covariances between pairs of modules nested within larger modules. In this second case, we either preserved within-module covariances ("within-module" model), or within- and between-module covariances at each level ("full" model). We tested these models using functional MRI data acquired from 90 subjects during movie watching and at rest (data made available by the Human Connectome Project). We found that our models accurately explained several aspects of empirical data, including specific features such as average regional correlations. Collectively, our framework generates strong null models of brain organization and is considerably faster than simple randomization approaches. In this way, our methods ultimately have the potential to advance rigorous explanatory modeling of large neuroimaging and neurophysiological datasets.
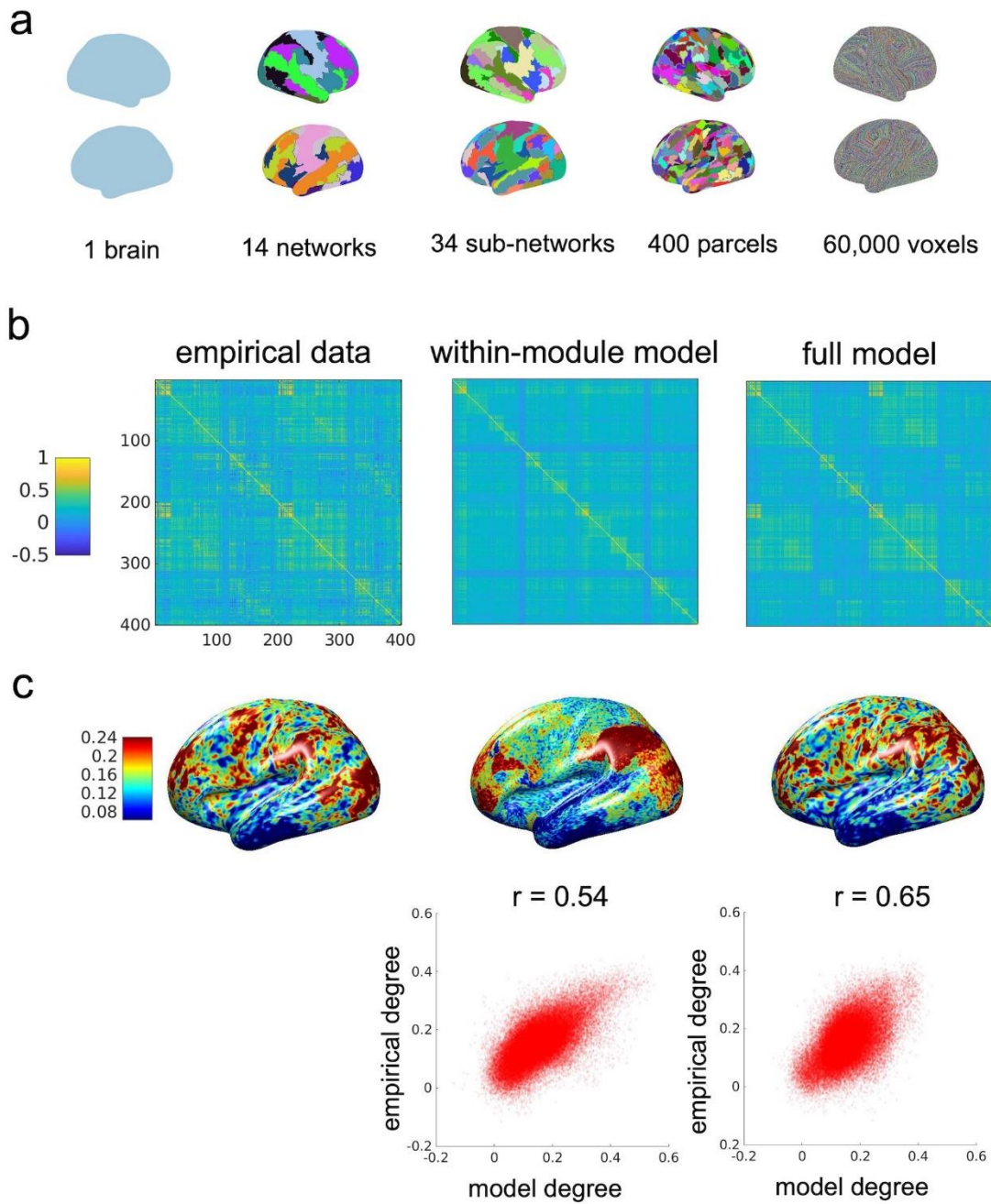
Fig. 1. Hierarchical sampling framework and experimental results. a) Hierarchical modular organization across five layers. b) Correlation matrices for the data and models. c) Degree maps of empirical data and each of the models, and degree model-data scatter plots, illustrated for a representative single subject.

Nanosymposium

518. Networks: Functional Connectivity and Computation

Location: SDCC 25

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 518.02

Topic: I.06. Computation, Modeling, and Simulation

Title: Extended Brain Sources Estimation via Model Based Deep Learning Framework

Authors: *F. LIU[1], M. JIAO[1], G. WAN[2], J. XIANG[3];
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Abstract: Background: Electroencephalography (EEG)/Magnetoencephalography (MEG) source imaging aims to seek an estimation of underlying activated brain sources to explain the observed EEG/MEG recording. Due to the ill-posed nature of inverse problem, solving EEG/MEG Source Imaging (ESI) requires design of regularization or prior terms to guarantee a unique solution. Method: Most of the recently developed algorithms requires an iterative procedure to reach the final solution, which can be time consuming. Inspired by the recent advancement of unrolled optimization in solving the inverse problem, we attempt to use the model based deep learning framework, termed as unrolled optimization neural network (UONN) to solve the ESI problem to improve the accuracy and efficiency solving ESI problem. The advantage of using the unrolled optimization framework is to learn a data-driven regularization instead of using hand-crafted one such as total variation, and also replace the iterative procedure with neural network modules, thus improving the online reconstruction efficiency significantly. It can also learn the hyperparameter automatically in a data-driven manner. The proposed new learning framework is the first one that use the unrolled optimization neural network to solve the ESI problem. The proposed network architecture is illustrated in Fig. 1.
Fig 1: Illustration of proposed network architecture.
Result: We validated our proposed framework on both synthetic EEG data under different signal to noise ratio (SNR) as well as clinical epilepsy EEG data. We tested the performance of the proposed framework against other benchmark algorithms such as sLORETA, MNE, dSPM etc. We used Localization Error (LE) and Area Under Curve (AUC) to evaluate the performance. Our proposed model can achieve above 0.95 AUC and much less LE than the benchmark algorithms, under different SNR conditions and different size of source extent. For clinical epilepsy data, we used the high frequency oscillations to localize the seizure onset region and achieved good concordance with the gold standard.

Nanosymposium

518. Networks: Functional Connectivity and Computation

Location: SDCC 25

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 518.03

Topic: I.06. Computation, Modeling, and Simulation

Title: Estimating Brain Functional Connectivity from Common Subspace Mapping Between Structural and Functional Connectomes

Authors: *S. GHOSH, A. RAJ, S. NAGARAJAN;
Radiology and Biomed. Imaging, Univ. of California San Francisco, San Francisco, CA

Abstract: Understanding the connection between the brain's structural connectivity and its functional connectivity is of immense interest in computational neuroscience. Although some studies have suggested that whole brain functional connectivity is shaped by the underlying structure, the rule by which anatomy constrains brain dynamics remains an open question. In this work, we introduce a computational framework that identifies a joint subspace of eigenmodes for both functional and structural connectomes. We found that a small number of those eigenmodes are sufficient to reconstruct functional connectivity from the structural connectome, thus serving as low-dimensional basis function set. We then develop an algorithm that can estimate the functional eigenspectrum in this joint space from the structural eigenspectrum. By concurrently estimating the joint eigenmodes and the functional eigenspectrum, we can reconstruct a given subject's functional connectivity from their structural connectome. We perform elaborate experiments and demonstrate that the proposed algorithm for estimating function connectivity from the structural connectome using joint space eigenmodes is superior to all other existing benchmark methods.

Disclosures: S. Ghosh: None. **A. Raj:** None. **S. Nagarajan:** None.

Nanosymposium

518. Networks: Functional Connectivity and Computation

Location: SDCC 25

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 518.04

Topic: I.06. Computation, Modeling, and Simulation

Support: Simons Collaboration on the Global Brain 521921

Title: Switching Functional Network Models of Multiple Rhythms During Anesthesia

Authors: *W.-C. HSIN, U. EDEN, E. STEPHEN;
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Abstract: Oscillations in brain activity are critical for information processing, and changes in brain-wide oscillatory coupling networks underlie many cognitive processes. Classical methods for estimating rhythmic functional networks such as pairwise coherence analysis and more recent methods such as global coherence are limited in their statistical efficiency and temporal resolution for dynamical functional networks. Our proposed solution to this challenge exploits the assumption that specific cognitive states and behavioral tasks are mediated by a small number of network modes whose expression changes dynamically. In this work, we introduce a state space modeling framework that uses the full data to estimate a set of discrete functional network modes and uses local data over short time scales to estimate which network modes are being expressed at each moment. We construct the model with two sets of latent states, one set of switching states that represents transitions between different network modes and one set of latent oscillators where each oscillator is characterized by an estimated mean oscillation frequency and an instantaneous phase at each time point. An observation model is constructed to relate the observed activity at each electrode to a linear combination of the latent oscillators, whose dynamics are determined by the switching state variable. A switching Kalman filter and smoother are used to estimate the instantaneous phase of each oscillator and the probability of each switching state at each moment. The estimated switching state characterizes which of the network modes is being expressed at each time point. We demonstrate the application of this model in an analysis of EEG data from a subject undergoing propofol-induced general anesthesia. During loss of consciousness, we observe the appearance of a frontal alpha oscillation (8-12 Hz) network and the loss of an occipital alpha oscillation network, consistent with previous literature (Purdon et al., 2013). The model can also capture anesthesia-induced slow oscillations at frequencies below 1 Hz. These results show that we are able to capture network structures across multiple rhythms and transitions between these rhythmic modes across the brain.

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Nanosymposium

518. Networks: Functional Connectivity and Computation

Location: SDCC 25

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Presentation Number: 518.05

Topic: I.06. Computation, Modeling, and Simulation

Title: Formation dynamics of hippocampal place cells is explained by distributed distributional codes of uncertainty

Authors: *M. SALMASI, M. SAHANI;
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Abstract: Animals navigate in an environment by combining sensory and path integration signals. The incomplete sensory information and noisy path integration lead to positional uncertainty. A series of experiments suggests that the size of hippocampal place fields is correlated with the level of navigational uncertainty and increases by lowering the contrast of visual cues, in darkness, by increasing the size of the arena and the distance to the walls, in virtual reality and novel environments, and by decreasing the richness of tactile or odor cues. These findings suggest that hippocampal place cells may encode positional uncertainty. However, it remains unclear how the brain represents positional beliefs and how the hippocampus employs these representations to perform probabilistic localisation and planning. We hypothesize that distributional beliefs about location are encoded in the hippocampus by distributed distributional codes (DDC), where the probabilities are represented by the expected values of a set of encoding functions. To perform probabilistic localization and planning, we introduce two classes of DDC neurons. The "filtering DDC" neurons represent the posterior over the current location, and "predictive DDC" neurons encode the posterior over the next location. We show that hippocampus can conduct probabilistic localisation through recursively updating the DDC values—the animal can accurately estimate its location and its distributional belief about the location. We also demonstrate that hippocampus can learn the structure of the environment and perform planning via DDC values.
Recent experimental findings suggest distinct formation dynamics in CA1 and CA3 regions of the hippocampus: in a new environment, the place fields of CA1 neurons form faster than CA3 neurons (Dong et al., Nature Communications 2021). Interestingly, filtering DDC neurons also show rapid spatial tuning in a new environment, while formation of place fields in predictive DDC neurons is gradual; fields emerge only after learning the structure of the environment. To test whether this correspondence is accidental or not, we studied the other distinct characteristics of CA1 and CA3 cells. The skewness and size of place fields in CA1 is less than CA3 neurons (Lee, Rao, and Knierim, Neuron 2004). Our analysis reveals that the tuning functions of both filtering and predictive DDC neurons are skewed backwards after enough exploration, with larger skewness and field size in predictive DDC neurons. These experimental findings suggest that CA1 and CA3 neurons may respectively correspond to filtering and predictive DDC neurons.

Disclosures: M. Salmasi: None. **M. Sahani:** None.

Nanosymposium

518. Networks: Functional Connectivity and Computation

Location: SDCC 25

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 518.06

Topic: I.06. Computation, Modeling, and Simulation

Title: Why do networks need negative weights?

Authors: *Q. WANG[1], **M. POWELL**[2], **A. GEISA**[3], **J. T. VOGELSTEIN**[4];
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Abstract: Artificial intelligence (AI) has achieved supra (-human) level performance in certain AI settings, typically dependent on a prohibitive amount of computational power and training samples. In contrast, natural intelligences (NIs) thrive in a dynamic world - they learn quickly, sometimes with only a few samples. How can we leverage attributes of NIs to inspire better AI - one that is more computationally efficient and statistically efficient? At the same time, how can we use AI frameworks to improve our understanding of NIs? Here, we propose a research avenue based on a simple observation from NIs: brains have excitatory and inhibitory neurons, and post-development they seldom switch their polarities. Why do networks have negative weights at all? The answer is: to learn more functions. We mathematically prove that deep neural networks with all non-negative weights are not universal approximators. Why do brains have fixed neuron polarity? The answer is: to learn more quickly. We mathematically prove that if weight polarities are adequately set *a priori* then networks learn more quickly. We substantiate these results via empirical simulations, further illustrating that if weight polarities are adequately set *a priori* then networks learn with less time, space, power, and data. We also explicitly illustrate situations in which *a priori* setting the weight polarities is disadvantageous for networks. Our work illustrates the value of negative weights, from the perspective of statistical and computational efficiency, for both NI and AI.

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Nanosymposium

518. Networks: Functional Connectivity and Computation

Location: SDCC 25

Time: Tuesday, November 15, 2022, 1:00 PM - 2:45 PM

Presentation Number: 518.07

Topic: I.06. Computation, Modeling, and Simulation

Title: A theory of single-trial inference in high-dimensional neural data

Authors: *I. D. LANDAU, G. C. MEL, M. J. SCHNITZER, S. GANGULI;
Stanford Univ., Stanford, CA

Abstract: Contemporary neuroscience has witnessed an impressive expansion in the number of neurons whose activity can be recorded simultaneously, from mere hundreds ten years ago to tens of thousands in recent years.
In classical statistics, the number of repeated measurements is generally assumed to far exceed the number of free variables to be estimated. This means that with today's experimental capabilities, directly estimating some basic statistical measures, such as pairwise correlation between neural activities, is unfeasible. Our work presents a fundamental theory analyzing the trade-off between the numbers of neurons and experimental trials in the era of large-scale neural recordings.
By employing the theories of random matrices and free probability, we show that despite the numbers of recorded neurons exceeding the numbers of trials, even by large amounts, it is still possible to draw conclusions about the correlation structure of network activity, and even to infer underlying latent states on a single-trial basis. In contrast to conventional wisdom that analyses of more neurons require more trials, we show that in fact, for a fixed number of trials additional neurons enable improved latent-space inference. Furthermore, additional neurons improve the accuracy of estimating the high-dimensional embedding structure in neural space, despite the fact that this estimation grows more difficult, by definition, with each neuron.
In order to test our theory we use previously acquired data from two-photon Ca2+ imaging studies using a 16-beam microscope. We imaged between 1,000 and 2,000 neurons simultaneously from V1 of awake mice, and explored the structure of network activity by examining the eigenvectors and eigenvalues of the noise-correlation matrix. Our theory predicts how a number of important statistical measures of the structure of neural activity, such as the leading principle components and the inferred dimensionality, behave under subsampling of both neurons and trials. We directly confirm these predictions, showing that iso-contours of constant performance form hyperbolas in the space of neurons and trials - performance increases as the product of neurons and trials - indicating a non-intuitive but very real compensation between two very different experimental resources.
Given rough estimates of single-neuron signal-to-noise ratios together with latent dimensionality, our theory provides a concrete prescription for numbers of neurons and trials necessary to infer latent states and structures. Our work lays a theoretical foundation for experimental design in contemporary neuroscience.

Nanosymposium

590. Rett Syndrome

Location: SDCC 1

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 590.01

Topic: A.07. Developmental Disorders

Title: Timed molecular, behavioral, and physiological characterization of mice after adult depletion of MeCP2

Authors: *S. S. BAJIKAR[1,4], J. ZHOU[1,4], M. A. DURHAM[2,4], A. J. TROSTLE[3,4], Y.-W. WAN[1,4], Z. LIU[3,4], H. Y. ZOGHBI[1,5,4];
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Abstract: Rett syndrome is a severe neurological disorder caused by loss-of-function mutations in methyl-CpG-binding protein 2 (*MECP2*). Loss of MeCP2 protein function causes the subtle misregulation of hundreds to thousands of genes. However, it is unknown whether these transcriptional changes are proximally regulated by MeCP2 or are secondary consequences of diminishing neuronal health. To address this challenge, we genetically deleted *Mecp2* in adult mice to circumvent any contributions of development and secondary pathogenesis. We then performed RNA-sequencing at multiple timepoints after loss of MeCP2. We first found that the transcriptome becomes progressively more misregulated over time. Furthermore, we observed significant overlap of genes misregulated upon adult loss of MeCP2 and genes misregulated across multiple published transcriptomes in germline *Mecp2*-null males, suggesting that adult loss of MeCP2 converges upon a similar transcriptional signature as the germline knockout. Next, we identified sets of genes that were misregulated early after of loss of MeCP2 and continued further misregulation over time, demonstrating that in absence of MeCP2 the expression of a core set of genes continues to worsen. To further understand the regulatory relationship between MeCP2 and the proximal early altered genes, we performed CUT&RUN for MeCP2 and a panel of histone modifications. Strikingly, we found that MeCP2 was preferentially depleted from the genes that were first to be altered. For the down-regulated genes, we observed a lower accumulation of histone acetyl marks beginning at one week after loss of MeCP2. For up-regulated genes, we observed the higher accumulation of histone acetyl marks beginning at four weeks after loss of MeCP2. These data suggest MeCP2 may proximally regulate the chromatin landscape of down-regulated genes, while it may act as a direct repressor of up-regulated genes. Lastly, we performed physiological and behavioral characterization at matched timepoints to the RNA-sequencing and CUT&RUN experiments. We found that overt behavioral deficits were observed starting at four weeks after loss of MeCP2, suggesting that molecular cascades triggered by loss of MeCP2 precede behavioral changes. Taken together, our approach demonstrates that acute perturbation of MeCP2 function revealed some of the most proximal changes driving pathogenesis.

Nanosymposium

590. Rett Syndrome

Location: SDCC 1

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 590.02

Topic: A.07. Developmental Disorders

Title: Potentiation of the M_1 muscarinic acetylcholine receptor modulates neurophysiological features in a mouse model of Rett syndrome

Authors: *H.-W. DONG[1,2], **K. WEISS**[3], **K. BAUGH**[1], **J. L. NEUL**[1,2], **C. M. NISWENDER**[3,2];
[1]Pediatrics, Neurolog. Div., Vanderbilt Univ. Med. Ctr., Nashville, TN; [2]Vanderbilt Kennedy Ctr., Nashville, TN; [3]Pharmacol. and Vanderbilt Ctr. for Neurosci. Drug Discovery, Vanderbilt Univ., Nashville, TN

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder (NDD) that is caused by loss-of-function mutations in the X chromosome-linked Methyl-CpG Binding Protein 2 (MECP2) gene. In addition to well-characterized symptoms such as the loss of acquired spoken language, repetitive hand movements, gait problems, seizures, and breathing irregularities, parallel changes in neurophysiological features, including basal EEG power and event-related potentials, have also been identified in RTT patients and animal models. These neurophysiological abnormalities represent potential pharmacodynamic or treatment-responsive biomarkers that could serve as translational outcome measures. Recent results have shown that the muscarinic acetylcholine subtype 1 receptor (M_1) is reduced in brain autopsy samples from people with RTT, and treatment of RTT mice with a compound that acts as a positive allosteric modulator (PAM) of M_1 activity improves phenotypes, suggesting a novel therapeutic approach for the disease. In the current study, we investigated whether M_1 PAM treatment could alter neurophysiological assessments in RTT animals to evaluate their potential to serve as biomarkers. The M_1 PAM VU0486846 (VU846) was administered acutely via an intraperitoneal route at doses of 3, 10 and 30 mg/kg in heterozygous female mutant mice and auditory event related potentials (AEPs) were recorded via electroencephalography. Our results demonstrate that acute dosing of VU846 at 3 mg/kg in wildtype female mice did not have any effect on AEP waveform or peak amplitudes. However, acute injection of this same dose of VU846 improved the AEP waveform and increased AEP N1 and P2 peak amplitudes in Mecp2[null/+] animals when compared to vehicle treatment. Interestingly, higher doses of VU846 (10 and 30 mg/kg) did not improve AEPs in mutant mice, and actually reduced P2 amplitude at 30 mg/kg, suggestive of a bell-shaped response. These findings suggest that potentiation of M_1 can functionally improve neural circuit synchrony to auditory stimuli in RTT animals, but that the effect is limited to low doses. We

anticipate that this acute response could represent a pharmacodynamic biomarker useful for dose finding and guiding future pre-clinical or clinical trials.

Nanosymposium

590. Rett Syndrome

Location: SDCC 1

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 590.03

Topic: A.07. Developmental Disorders

Title: The role of the TCF20 complex in neurodevelopment and MECP2-related disease pathogenesis

Authors: *J. ZHOU[1], H. HAMDAN[5], H. YALAMANCHILI[2], S. S. BAJIKAR[3], Z. LIU[2], M. N. RASBAND[6], H. Y. ZOGHBI[4];
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Abstract: Loss of function mutations in *MECP2* cause Rett syndrome (RTT) while duplications spanning the gene cause *MECP2* duplication syndrome (MDS). While both disorders share some phenotypes with other neurodevelopmental disorders (NDDs), the precise molecular mechanism driving pathogenesis remains unclear. MeCP2 binds methylated DNA and recruits chromatin modifying proteins but the relationship between these proteins and gene expression changes is not clear. Therefore, it is crucial to identify and characterize MeCP2 interactors to fully understand the molecular function of MeCP2 and the pathogenesis of *MECP2*-associated disorders. To this end, we performed proximity-dependent biotin identification (BioID) in cultured rat primary neurons using a biotin ligase fused to MeCP2. In addition, we used two mutant alleles, *MECP2^{R111G}* and *MECP2^{ΔNLS}*, which disrupt DNA binding and nuclear localization of MeCP2, respectively, to filter out non-chromatin associated MeCP2 interactors. Our unbiased approach identified a novel MeCP2-interacting complex which includes TCF20, encoded by a known NDD-causing gene, and three other transcriptional regulators: RAI1, PHF14, and HMG20A. We found MeCP2 interacts with the TCF20 complex via PHF14 and that several RTT-causing *MECP2* mutations reduce MeCP2's binding with the TCF20 complex. Next, we found that *Tcf20* modulates *MECP2*-mediated synaptogenesis in cultured primary

neurons by co-regulating the key neuronal gene *Bdnf*. Further, reducing *Tcf20* partially rescued behavioral deficits caused by *MECP2* overexpression in mice, underscoring a functional relationship between MeCP2 and TCF20 in MDS pathogenesis. We next assessed global gene expression changes in mouse models and found a significant proportion (33%) of differentially expressed genes in *Tcf20*^{+/−} mice were also altered in *Mecp2*[−/y] mice; a majority (72%) of these genes changed in the same direction and with similar magnitude. Next, we performed CUT&RUN experiments and found that in absence of MeCP2, there is a significant reduction of TCF20 binding to genes differentially expressed in both *Tcf20*^{+/−} and *Mecp2*[−/y] brains, suggesting that MeCP2 recruits TCF20 complex to chromatin to co-regulate gene expression. Notably, we identified one de novo *PHF14* missense mutation in a patient displaying clumsy gait, speech delay, and mild regression in gross motor skills and found that this mutation disrupts MeCP2-PHF14-TCF20 interaction. Our data demonstrate the critical role of a novel MeCP2-TCF20 complex for brain function and reveal a converging molecular mechanism whereby mutations of genes encoding several subunits in the same complex contribute to shared NDD symptoms.

Disclosures: J. Zhou: None. **H. Hamdan:** None. **H. Yalamanchili:** None. **S.S. Bajikar:** None. **Z. Liu:** None. **M.N. Rasband:** None. **H.Y. Zoghbi:** None.

Nanosymposium

590. Rett Syndrome

Location: SDCC 1

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 590.04

Topic: A.07. Developmental Disorders

Support: Brain/MINDS project of AMED JP15dm0207001
        Rett Syndrome Supporting Organization

Title: Generation and analysis of MECP2 mutant marmosets

Authors: *N. KISHI[1,2], K. SATO[3], J. HATA[1,4], M. OKUNO[1], T. ITOU[1], J. OKAHARA[1,3], H. J. OKANO[1,4], E. SASAKI[1,3], H. OKANO[1,2];
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Abstract: Rett syndrome is a neurodevelopmental disorder presenting almost exclusively in girls, with a prevalence rate of one in 10,000. Children with Rett syndrome develop relatively normally for 6-18 months, after which they undergo a period of rapid regression, with loss of purposeful hand use, deceleration of head growth, and autistic behaviors. Mutations of the *MECP2* gene on the X chromosome are found in over 95% of cases of classic Rett syndrome, and *MECP2* has been implicated in several other neurodevelopmental disorders, including autism, childhood schizophrenia, and X-linked cognitive disability. Although it is evident that symptoms of Rett syndrome are attributable to lack of the *MECP2* gene in the CNS, there is little neuropathological understanding of the abnormalities in the CNS of patients with Rett syndrome.

Although rodent models have been used for these studies, these models do not faithfully recapitulate human neurodevelopmental processes. The establishment of nonhuman primate (NHP) models that are similar to humans in many aspects is very important to understand the pathogenesis of Rett syndrome.To add to our understanding of pathological mechanisms of Rett syndrome, we created *MECP2* mutant marmosets suitable for research on Rett syndrome, using genome editing technology, and we have successfully generated two *MECP2* heterozygous marmosets and eight *MECP2*-null marmosets. MRI imaging shows that the brain size of *MECP2* heterozygous marmoset was smaller than wild-type ones by approximately 15% at 24 months of age, and the brain size of *MECP2*-null marmosets becomes smaller than wild-type one by 20% at 3 months of age, mimicking microcephaly of Rett syndrome patients. Furthermore, to investigate what is responsible for the reduction in brain volume in *MECP2*-null marmosets, we analyzed neuronal morphology by Golgi staining. The analysis reveals that loss of *MECP2* causes robust reduction in dendritic arborization of pyramidal neurons. Those results indicate that those *MECP2* mutant marmosets recapitulate symptoms of Rett syndrome, and this new primate model will potentially contribute to the development of future therapeutic strategies for patients with Rett syndrome.

Disclosures: N. Kishi: None. **K. Sato:** None. **J. Hata:** None. **M. Okuno:** None. **T. Itou:** None. **J. Okahara:** None. **H.J. Okano:** None. **E. Sasaki:** None. **H. Okano:** None.

Nanosymposium

590. Rett Syndrome

Location: SDCC 1

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 590.05

Topic: A.07. Developmental Disorders

Title: Identification of a therapeutic candidate for Rett syndrome with a differentiated mechanism of action using a patient-derived cortical organoid screening platform

Authors: *C. CARROMEU[1], N. COUNGERIS[2], N. SODHI[1], H. AHMED[1], M. SEIBEL[2], K. PRUM[2], R. FREMEAU[1], A. LACROIX[2];
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Abstract: Rett syndrome (RTT) is a progressive neurodevelopmental disorder caused by mutations in the X-linked gene *MECP2*. One of the challenges to developing therapeutics for RTT has been the lack of a screening system that recapitulates the underlying disease pathophysiology. Using RTT patient-derived induced pluripotent stem cells (iPSCs), we developed a cortical organoid platform that can efficiently screen large compound libraries in a reproducible fashion. We characterized the RTT disease organoid platform at the molecular level via bulk and single-nuclei RNA transcriptomics and identified novel potential miRNA biomarkers for the disease. Our proprietary organoid platform displays a spontaneous functional network activity that can be recorded in high-throughput, providing a stable foundation for recovery-based screening. Compared to controls, RTT patient-derived organoids exhibit aberrant

functional waveforms characterized by irregular asynchronous bursts of activity that can also be observed via high-resolution calcium imaging and multi-electrode array (MEA). We developed an algorithm to quantify this disease-specific functional phenotype using a clinically translatable endpoint to define compound rescue of the disease phenotype. Here, we describe the use of this platform to identify repurposed and novel potential therapeutic candidates for RTT. Functional screening of the IRSF SMART library (a targeted compound library developed for RTT) identified several known inhibitors of acetylcholinesterase (AChE) and histone deacetylases that rescued the functional RTT disease phenotype. We further explored the rescue potential of donepezil, an FDA-approved compound that we prioritized as a potential repurposing candidate for RTT. We observed that donepezil rescued the RTT disease phenotype at concentrations known to be achieved in the human brain. We further confirmed that rescue of the RTT phenotype after chronic treatment with donepezil correlated with near complete AChE inhibition. These findings are consistent with literature support for cholinergic deficits in RTT patients and donepezil-based rescue in a mouse RTT model (reviewed in Ballinger et al., 2019). Finally, we observed that donepezil appears to exhibit a distinct mechanism of action from the most advanced RTT clinical development candidates. The apparent differentiated mechanism mediated by donepezil may provide the potential for combinatorial therapy for patients suffering from RTT syndrome. Vyant Bio is pursuing the clinical development of donepezil for the treatment of pediatric and adult RTT patients with a mutation in the *MECP2* gene.

Disclosures: C. Carromeu: A. Employment/Salary (full or part-time):; Vyant Bio. **N. Coungeris:** A. Employment/Salary (full or part-time):; Vyant Bio. **N. Sodhi:** A. Employment/Salary (full or part-time):; Vyant Bio. **H. Ahmed:** A. Employment/Salary (full or part-time):; Vyant Bio. **M. Seibel:** A. Employment/Salary (full or part-time):; Vyant Bio. **K. Prum:** A. Employment/Salary (full or part-time):; Vyant Bio. **R. Fremeau:** A. Employment/Salary (full or part-time):; Vyant Bio. **A. LaCroix:** A. Employment/Salary (full or part-time):; Vyant Bio.

Nanosymposium

590. Rett Syndrome

Location: SDCC 1

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 590.06

Topic: A.07. Developmental Disorders

Support: NIH
Associaton Française du syndrome de Rett
Fondation Lejeune

Title: Contribution of the adipocyte hormone leptin in the pathogenesis of Rett syndrome

Authors: *Y. BELAIDOUNI[1], D. DIABIRA[2], J.-C. GRAZIANO[3], C. MENUET[4], G. A. WAYMAN[5], J.-L. GAIARSA[6];
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Marseille University, France; [3]CRCM, Aix-Marseille University, France; [4]Mp3-Respiration, UMR CNRS 6153, Mp3-Respiration, UMR CNRS 6153, Marseille, France; [5]Washington State Univ., Washington State Univ., Pullman, WA; [6]INSERM, INSERM, Marseille, France

Abstract: Rett syndrome is a neurodevelopmental disorder that affects mainly girls and represents the second cause of intellectual disability of genetic origin in women. This syndrome is characterized by normal development until approximately 18 months, followed by a regression manifested by loss of language, loss of walking, hand stereotypies, breathing difficulties and epileptic seizures. Rett syndrome is caused by mutations in the *Methyl-CpG-binding protein 2* (*MECP2*) gene located on the X chromosome, and encoding for a transcriptional regulator, essential for the development and maintenance of synapses. Currently, there is no cure for this disease. Among the various biological and neurochemical alterations observed in this syndrome, clinical and experimental studies have reported abnormally elevated levels of leptin in patients and animal models. Leptin is a hormone released mainly from adipocytes and represents a key factor in maintaining energy balance. In addition to this canonical function, leptin regulates several other functions such as anxiety, breathing or cognitive processes, which are altered in the case of this syndrome. Therefore, abnormal elevated leptin levels could contribute to the alterations observed in Rett syndrome. The objective of my thesis was to determine the role played by leptin in this pathology by targeting the leptinergic system in a mouse model of the pathology (mice deficient in *Mecp2*: *Mecp2-/y*). Treatment aimed at blocking leptin transport across the blood-brain barrier during the symptomatic period restored the excitation/inhibition balance in the hippocampus, prevented weight loss and slowed the progression of respiratory symptoms of *Mecp2-/y* mice. Conversely, leptin of wild-type mice altered the excitation/inhibition balance in the hippocampus, led to weight loss and increased the number of apneas mimicking the phenotype observed in mutant mice. This work has revealed a new role for leptin in the pathogenesis of Rett syndrome, thus offering new therapeutic perspectives.

Disclosures: Y. Belaidouni: None. **D. Diabira:** None. **J. Graziano:** None. **C. Menuet:** None. **G.A. Wayman:** None. **J. Gaiarsa:** None.

Nanosymposium

590. Rett Syndrome

Location: SDCC 1

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 590.07

Topic: A.07. Developmental Disorders

Title: Multiparametric analysis of coordinated network activity reveals target-specific functional rescue in a human iPSC-derived organoid model of Rett syndrome

Authors: *A. LACROIX[1], H. AHMED[2], N. COUNGERIS[1], C. JOSEPH[2], N. SODHI[2], K. PRUM[1], R. FREMEAU[2], C. CARROMEU[2];
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Abstract: High-throughput screening (HTS) utilizes enzymatic and cell-based assays designed to robustly scale to screening thousands of compounds daily. However, the limited complexity of the readout typically requires multiple follow-up experiments with more elaborate systems. Subsequent experiments offer richer data to interrogate target-specific or multiparametric rescue, but often suffer from high variability. Currently, few technologies offer the combination of robustness and complexity required to accelerate drug discovery efforts. Towards this end, we have developed a platform amenable to HTS that combines the complexity of patient-derived induced pluripotent stem cells (iPSCs) with a multiparametric functional readout. Specifically, we focus on modeling Rett syndrome (RTT), a progressive neurodevelopmental disorder caused by mutations in the gene MECP2. Phenotypic characterization of RTT-patient iPSC-derived cortical organoids showed a striking difference in the coordinated neuronal activity compared to control organoids. We developed an algorithm to quantify the count, size, and shape of activity peaks, then engineered additional waveform features to capture the disease-specific biology of RTT organoids. In all, >50 waveform features define the RTT functional phenotype and contribute to global functional rescue. We screened the IRSF SMART library (compounds curated for treating RTT) and identified acetylcholine esterase (AChE) inhibitors and histone deacetylase (HDAC) inhibitors as the most promising therapeutic targets based on global functional rescue. While both compound classes exhibited global rescue, AChE inhibitors rescued a distinct set of features compared to both HDAC inhibitors and top clinical candidates Anavex 2-73 and trofinetide with distinct biological targets. Specifically, at equivalent levels of AChE inhibition (enzymatic assay) and acetylcholine media concentration (HPLC), three distinct AChE inhibitors rescued the same 5 parameters (in the same order), providing strong evidence of target-specific functional rescue. While we saw strong dose-dependent rescue of waveform features by multiple compounds, only donepezil, a lead repurposing candidate, was able to fully rescue the functional deficit in RTT organoids; based on global functional rescue, >90% of parameters were statistically indistinguishable from control organoids. Overall, we establish how combining patient-derived cortical organoids with advanced multiparametric functional readouts can be used to interrogate target-specific disease biology, formulate therapeutic hypotheses, and drive pre-clinical drug discovery efforts.

Disclosures: A. LaCroix: A. Employment/Salary (full or part-time):; Vyant Bio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vyant Bio. **H. Ahmed:** A. Employment/Salary (full or part-time):; Vyant Bio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vyant Bio. **N. Coungeris:** A. Employment/Salary (full or part-time):; Vyant Bio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vyant Bio. **C. Joseph:** A. Employment/Salary (full or part-time):; Vyant Bio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vyant Bio. **N. Sodhi:** A. Employment/Salary (full or part-time):; Vyant Bio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vyant Bio. **K. Prum:** A. Employment/Salary (full or part-time):; Vyant Bio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vyant Bio. **R. Fremeau:** A. Employment/Salary (full or part-time):; Vyant Bio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vyant Bio. **C. Carromeu:** A. Employment/Salary (full or

Nanosymposium

591. Signaling Mechanisms in Long-Term Plasticity II

Location: SDCC 5

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 591.01

Topic: B.05. Synaptic Plasticity

Support: NIH Grant 1R01MH120300-01A1

Title: Tug of war between ERK phosphorylation and dephosphorylation encodes number, strength, and order of stimuli inducing long-term synaptic facilitation

Authors: *N. KUKUSHKIN, T. TABASSUM, T. J. CAREW;
New York Univ., New York, NY

Abstract: Two-trial learning in Aplysia reveals non-linear interactions between training trials: a single trial has no effect, but two precisely spaced trials induce long-term memory. ERK activity is essential for intertrial interactions, but the mechanism remains unresolved. A new combination of immunochemical and optogenetic tools reveals unexpected complexity of ERK signaling during the induction of long-term synaptic facilitation by two spaced pulses of serotonin (5HT). Specifically, dual ERK phosphorylation at its activating TxY motif is accompanied by dephosphorylation at the pT position, leading to a build-up of inactive, singly phosphorylated pY-ERK. Phosphorylation and dephosphorylation occur concurrently, but scale differently with varying 5HT concentrations, predicting that mixed two-trial protocols involving both "strong" and "weak" 5HT pulses should be sensitive to the precise order and timing of trials. Indeed, LTF is induced only when "weak" pulses precede "strong", not vice versa. This may represent a physiological mechanism to prioritize memory of escalating threats.

Title: ELAV-mediated mRNA translocation to the synapse: a candidate molecular mechanism for savings memory

Authors: *A. A. MIRISIS, J. Y. ZHAO, A. SERRANO LUGO, T. TABASSUM, T. J. CAREW;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Savings memory is a phenomenon in which the molecular remnants of a "forgotten" memory are able to prime a neural system to re-express the memory following a reminder event (which alone is insufficient to induce a *de novo* memory). In the marine mollusk *Aplysia californica*, behavioral long-term memory (LTM) for the tail-elicited siphon withdrawal reflex requires: (i) ERK activation, (ii) protein synthesis, and (iii) *de novo* transcription in the pleural sensory neurons (SNs) that mediate the reflex. Following the natural decay of LTM (forgetting), savings LTM can be established by a reminder stimulus which alone does not establish LTM in naïve animals. This process is dependent on protein synthesis but is unaffected by ERK inhibition (Menges et al., 2015). In our current work we have studied the *Aplysia* sensory neuron-motor neuron (SN-MN) system in both culture and *ex vivo* pleural-pedal ganglia. We found that, following LTM training (4 5HT pulses separated by 15 min), the RNA binding protein ApELAV1 translocates in a microtubule-dependent manner from the SN nucleus into synaptic/dendritic processes. We have further determined that the mRNA encoding the transcription factor ApC/EBP, an ELAV-target RNA (Mirisis et al., 2021), is localized to distal SN compartments (including SN-MN synapses) after LTM training. Translation of this mRNA by subsequent savings training could provide a molecular mechanism for the re-establishment of LTM following its natural decay. In support of this hypothesis, we have found that savings training induces expression of ApC/EBP protein in previously trained, but not in naïve animals. The mechanisms underlying this expression of savings memory are distinct from the mechanisms underlying its expression in initial LTM training. Our findings thus identify a set of novel molecular mechanisms which can facilitate relearning, and interestingly, are distinct from those mechanisms that are required to establish original LTM.

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Nanosymposium

591. Signaling Mechanisms in Long-Term Plasticity II

Location: SDCC 5

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM
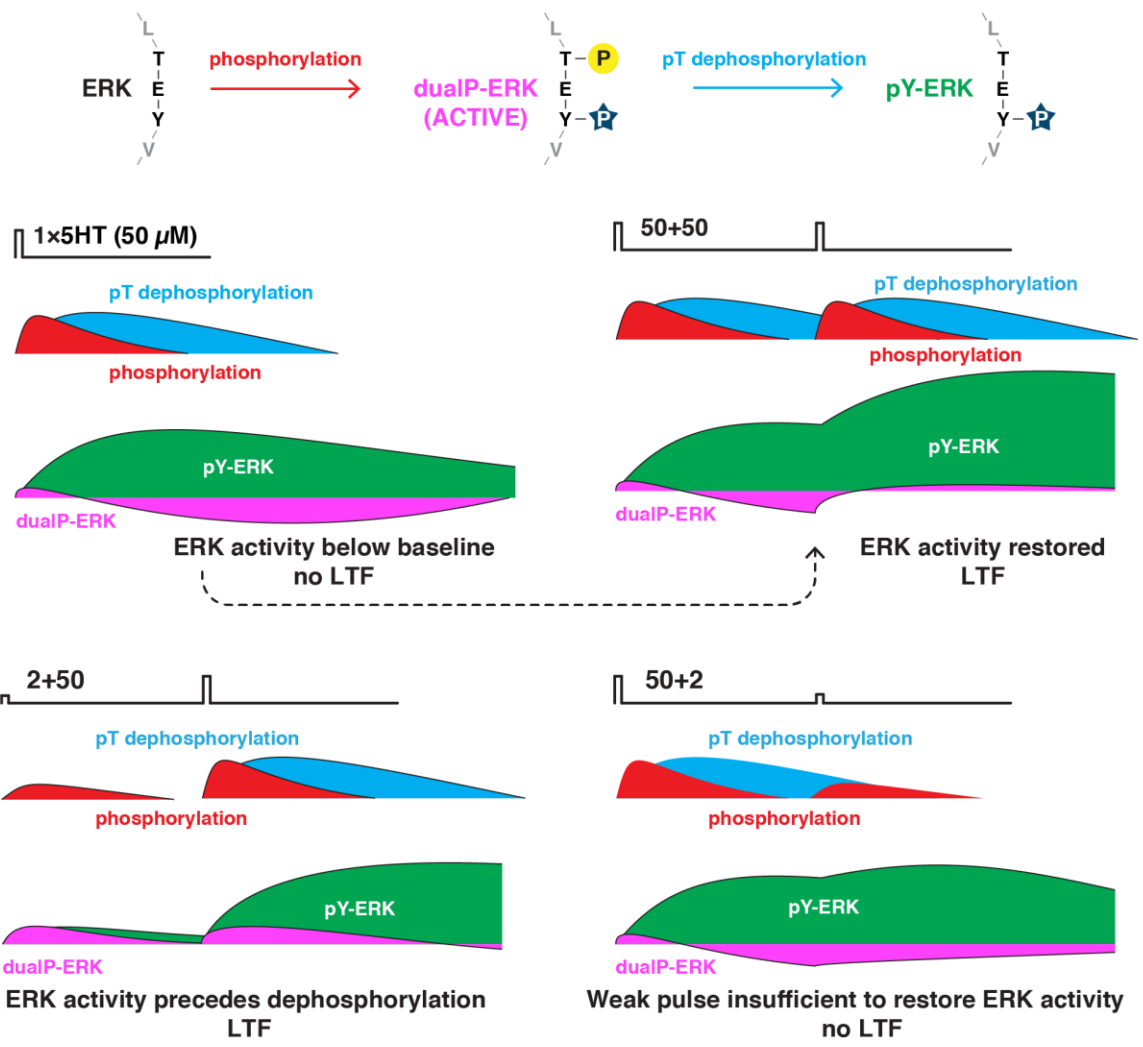
Presentation Number: 591.03

Topic: B.05. Synaptic Plasticity

Title: Intracellular Signaling by Norepinephrine via the b[2]-Adrenergic Receptor inLTP induced by Prolonged Theta Tetanus

Authors: B. LEE[1], ***X. XING**[1], E. A. HAMMES[1], K. KIM[1], K. E. IRETON[1], K. N. M. MAN[1], A. JACOBI[1], J. WANG[2], P. J. GASSER[3], C.-Y. CHEN[1], M. HORNE[1], J. W. HELL[1];
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Abstract: Signaling by norepinephrine (NE) via adrenergic receptors (ARs) is critical for arousal and attention yet the underlying molecular mechanisms are largely unknown. The β_2 AR and its downstream effectors, the trimeric G_s protein, adenylyl cyclase, and PKA form a unique, dedicated signaling complex with AMPA-type glutamate receptors to augment phosphorylation and thereby surface expression of its GluA1 subunit (Joiner et al., 2010: EMBO J. 29, 482-495). We show that intracellular β_2 AR signaling mediates this phosphorylation and long-term potentiation (LTP) induced by a prolonged theta-tetanus (PTT-LTP, 900 stimuli at 5 Hz) in the presence of NE. Accordingly, inhibitors and knock out of the organic cation transporter 3 (OCT3) and plasma membrane monoamine transporter (PMAT) impair GluA1 phosphorylation on S845 by PKA, its surface insertion, and PTT-LTP with membrane impermeant NE but not with the membrane permeant β AR agonist isoproterenol (ISO).

Disclosures: B. Lee: None. **X. Xing:** None. **E.A. Hammes:** None. **K. Kim:** None. **K.E. Ireton:** None. **K.N.M. Man:** None. **A. Jacobi:** None. **J. Wang:** None. **P.J. Gasser:** None. **C. Chen:** None. **M. Horne:** None. **J.W. Hell:** None.

Nanosymposium

591. Signaling Mechanisms in Long-Term Plasticity II

Location: SDCC 5

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 591.04

Topic: B.05. Synaptic Plasticity

Title: Functional specialization of PI3K isoforms for neuronal architecture, synaptic plasticity and cognition

Authors: C. SANCHEZ-CASTILLO[1,2], M. I. CUARTERO[1,3], A. FERNANDEZ-RODRIGO[1], S. LOPEZ-GARCIA[1], V. BRIZ[1], R. JIMENEZ-SANCHEZ[1], J. A. LOPEZ[3], M. GRAUPERA[4], ***J. A. ESTEBAN**[1,5];
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Abstract: Neuronal connectivity and activity-dependent synaptic plasticity are fundamental properties that support brain function and cognitive performance. PI3K intracellular signaling controls multiple mechanisms mediating neuronal growth, synaptic structure and plasticity. However, it is still unclear how these pleiotropic functions are integrated at molecular and cellular levels. To address this issue, we have used neuron-specific virally delivered Cre expression to delete either PIK3CA (coding for p110alpha) or PIK3CB (coding for p110beta), the two major catalytic isoforms of type I PI3K, from the hippocampus of adult mice. In this manner, gene deletion was spatially and temporally restricted. We then carried out a comprehensive electrophysiological, morphological and behavioral analysis of these animals, together with proteomics. Unexpectedly, p110alpha and p110beta control synaptic strength in opposite directions, and also contribute to opposing forms of synaptic plasticity (LTP versus LTD). We also found that postsynaptic structures are almost exclusively supported by p110alpha activity, whereas p110β controls neurotransmitter release and synaptic vesicle recruitment. These molecular and functional specializations were reflected in different proteomes controlled by each isoform, and in distinct behavioral alterations for learning/memory and sociability in mice lacking p110alpha or p110beta. In conclusion, this study is revealing a precise task distribution between the major PI3K catalytic isoforms, and helps to organize the repertoire of synaptic and structural effects that had been ascribed to this pathway.

Disclosures: C. Sanchez-Castillo: None. **M.I. Cuartero:** None. **A. Fernandez-Rodrigo:** None. **S. Lopez-Garcia:** None. **V. Briz:** None. **R. Jimenez-Sanchez:** None. **J.A. Lopez:** None. **M. Graupera:** None. **J.A. Esteban:** None.

Nanosymposium

591. Signaling Mechanisms in Long-Term Plasticity II

Location: SDCC 5

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 591.05

Topic: B.05. Synaptic Plasticity

Title: Fret-based quantitative optical measurements reveal aberrant molecular phenotypes of de novo CAMK2A mutations linked to intellectual disability.

Authors: *H. FUJII[1], H. KIDOKORO[2], Y. KONDO[1], M. KAWAGUCHI[2], S.-I. HORIGANE[3,4], J. NATSUME[2,5], S. TAKEMOTO-KIMURA[3,4], H. BITO[1];
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Abstract: Intellectual disabilities (ID) are prevalent in approximately 1% of world population. Recently, de novo mutations in a key synaptic enzyme CaMKIIalpha (Ca^{2+}/calmodulin (CaM)-dependent protein kinase II alpha), have been shown to be associated with ID. Consistently, previous biochemical studies examining threonine 286 autophosphorylation states of heterologously expressed ID-related CAMK2A mutant proteins suggested that some mutants' autophosphorylation was upregulated, while others' was downregulated. However, whether these mutations affected the central molecular phenotypes of the CaMKIIalpha enzyme, namely Ca2+/CaM-dependent activation and frequency-tuning, has not been tested. With a view to achieving a mechanistic understanding of ID, and to begin to identify novel disease-modifying therapeutic strategies, in this study, we developed a quantitative molecular phenotyping pipeline of ID-related de novo CAMK2A mutations. First, we identified an ID patient with a de novo CAMK2A mutation. To investigate the genotype-phenotype relation of the ID pathology, we built an analysis pipeline to quantitate the activation of CaMKIIalpha in vitro and then uncover possible alteration in its frequency-tuning in primary hippocampal neurons. First, we introduced various ID-related mutations to an optical FRET sensor based on the full-length CaMK2alpha enzyme, and developed a fluorescence resonance energy transfer (FRET)-based optical molecular phenotyping system. Mutant CaMKIIalpha activities were investigated via high-throughput fluorometric assays of purified enzymes from heterologous cell lysates. This was combined then after with multiplex imaging in primary cultures of hippocampal neurons interrogated with serial rounds of high and low frequency glutamate UV-caging. These interrogations showed that a dominant majority of the ID-linked CaMKIIalpha mutations showed aberrant molecular phenotypes in vitro and in living neurons. Pharmacological reversal of a discovered phenotype was achieved in a specific mutant, by repurposing a compound that is well-tolerated in children, thus providing a rationale for future development and testing of tailored therapeutics in CaMKII-linked disorders. Taken together, an optical CaMKIIalpha molecular phenotyping pipeline was developed in this study, and unveiled hitherto unappreciated molecular phenotypes of ID-related de novo CAMK2A mutations. Our study paves the way towards further discovery of genotype-phenotype association in the clinical domain of, and foster the development of disease-modifying therapeutics in CAMK2A-linked ID.

Disclosures: H. Fujii: None. **H. Kidokoro:** None. **Y. Kondo:** None. **M. Kawaguchi:** None. **S. Horigane:** None. **J. Natsume:** None. **S. Takemoto-Kimura:** None. **H. Bito:** None.

Nanosymposium

591. Signaling Mechanisms in Long-Term Plasticity II

Location: SDCC 5

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 591.06

Topic: B.05. Synaptic Plasticity

Support: DFG (SFB/TRR 167)
MOTI-VATE Program

Title: Microglia mediate synaptic plasticity induced by transcranial magnetic stimulation

Authors: *A. EICHLER[1], D. KLEIDONAS[1,2,3], Z. TURI[1], M. KIRSCH[1], D. PFEIFER[4], T. MASUDA[5,6], M. PRINZ[5,7,8], M. LENZ[1], A. VLACHOS[1,8,9];
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Abstract: Microglia are the resident immune cells of the brain. Their role in physiological processes such as the regulation of neural excitability and plasticity is well recognized. Here, we investigated the impact of microglia in synaptic plasticity induced by 10 Hz repetitive magnetic stimulation (rMS), a clinically employed non-invasive brain stimulation technique. Whole-cell patch-clamp recordings, confocal microscopy, immunohistochemistry, protein and transcriptome analyses were used to assess rMS-induced structural and functional plasticity in mouse organotypic tissue cultures prepared from animals of both sexes in the presence or absence of microglia. Microglia were depleted with PLX3397 (Pexidartinib). 10 Hz rMS induced excitatory synaptic plasticity of CA1 pyramidal neurons, while no changes in excitatory neurotransmission were observed in the absence of microglia - both under baseline conditions and following 10 Hz rMS. Although rMS did not alter the morphology or the dynamics of microglia, an increased production and secretion of microglia-related cytokines were observed 3 h after stimulation. Concordantly, substitution of these cytokines in microglia-depleted tissue cultures rescued the expression of rMS-induced synaptic plasticity. We conclude that clinically employed non-invasive electromagnetic brain stimulation affects synaptic plasticity by modulating the production and release of microglial cytokines.

Disclosures: A. Eichler: None. **D. Kleidonas:** None. **Z. Turi:** None. **M. Kirsch:** None. **D. Pfeifer:** None. **T. Masuda:** None. **M. Prinz:** None. **M. Lenz:** None. **A. Vlachos:** None.

Nanosymposium

591. Signaling Mechanisms in Long-Term Plasticity II

Location: SDCC 5

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 591.07

Topic: B.05. Synaptic Plasticity

Support: NIH Grant 1R15DA049260-01A1

Title: Differential Activation of CB1-Dependent Long-Term Depression in Ventral Tegmental Area GABA Neurons in Adult versus Adolescent Mice

Authors: *M. VON GUNTEN[1], I. OSTLUND[2], S. HOFFMAN[1], J. G. EDWARDS[3];
[2]Physiol. and Developmental Biol., [3]PDBio, [1]Brigham Young Univ., Provo, UT

Abstract: Ventral tegmental area (VTA) dopamine (DA) signaling plays a key role in reward and drug dependence. DA cells in part are regulated by glutamate and GABA cells. Using whole-cell electrophysiology in GAD67-GFP knock-in mice, we examined glutamatergic plasticity at excitatory inputs onto VTA GABA cells. We previously identified a cannabinoid type 1 receptor (CB1R)-dependent form of long-term depression (LTD) at this synapse. This LTD was dependent on metabotropic glutamate receptor 5 (mGluR5) activation and 2-AG production. Chronic Δ9-tetrahydrocannabinol (THC) injections eliminated this LTD. Currently, we are examining the mechanism through which THC exposure eliminates LTD, as well as the presence of adult plasticity and effect of THC on this plasticity, as adults often are less susceptible to the negative cognitive impact of THC. First, we noted that after chronic (7-10 days) THC exposure, CB1 agonist WIN55,212-2 no longer induced synaptic depression in adolescent mice, illustrating that CB1 is either desensitized or removed from synapses after chronic THC exposure. As reversal of drug-induced effects following withdrawal of a drug is an important consideration, we examined this as well. Following 7 days of withdrawal after chronic THC exposure, LTD was restored. Interestingly, adults did not exhibit LTD using the same conditioning stimulus that induces LTD in adolescent mice. However, adults continued to express both functional CB1 receptors and mGluR5-mediated synaptic depression. Therefore, we examined the quantitative nature of this plasticity by doubling the conditioning induction protocol for adult mice, which was significant enough to then induce LTD ($p < 0.05$; n=5). This LTD was also blocked by CB1 antagonist AM-251 as in adolescent mice ($p > 0.05$; n=6). This reveals that this form of LTD is qualitatively the same as adolescents compared to adults, but differs quantitatively. A possible explanation for this quantitative difference was a noted decrease in synaptic NMDA receptor current in adult GABA cells versus adolescents, which may cause a decrease in calcium influx required for LTD induction. Lastly, we also demonstrate some significant changes in mRNA levels of CB1, DAG-lipase and FAAH in chronic THC exposure compared to vehicle controls or versus adults ($p < 0.05$; n= 7-10) using quantitative PCR. Collectively, this data illustrates for the first time, differences in GABA cell plasticity in adolescent versus adults animals, and that THC-induced impact of brain plasticity is likely via desensitizing CB1, which is reversible following withdrawal.

Disclosures: M. Von Gunten: None. **I. Ostlund:** None. **S. Hoffman:** None. **J.G. Edwards:** None.

Nanosymposium

591. Signaling Mechanisms in Long-Term Plasticity II

Location: SDCC 5

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 591.08

Topic: B.05. Synaptic Plasticity

Title: Activity-dependent plasticity of synaptic inhibition from somatostatin-expressing GABAergic neurons mediated by endocannabinoid signaling in the prefrontal cortex

Authors: N. SANGUINETTI[1], R. MEZA[2], M. J. HIGLEY[3], E. DELPIRE[4], A. E. CHAVEZ[5], *C. CHIU[1];
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Abstract: GABAergic interneurons that co-express the neuropeptide somatostatin (SOM-INs) play an essential role in controlling cortical activity by forming inhibitory synapses on dendrites of principal pyramidal cells. However, the mechanisms that regulate inhibition from SOM-INs are less understood. Given commonalities between SOM-INs and the endocannabinoid (eCB) system in influencing cognitive and emotional processing, we examine the possibility that GABAergic synapses from SOM-INs can be modulated by eCB signaling in the prefrontal cortex (PFC). Using optogenetic tools to selectively activate SOM-INs in whole-cell patch experiments, we found that postsynaptic inhibitory currents in layer II/III pyramidal cells evoked by photostimulation of SOM-INs (SOM-IPSCs) depressed following bath application of WIN 55,212-2, a potent agonist of cannabinoid type 1 receptors (CB1Rs). Supporting a role of presynaptic CB1Rs, WIN depression of SOM-IPSCs was accompanied by changes in paired-pulse ratio and is absent in mice lacking CB1Rs specifically in SOM-INs (SOM-CB1R KOs). Importantly, a brief depolarization step induced suppression of inhibition transiently (DSI), an effect that was eliminated by the CB1R antagonist AM251. Moreover, theta-burst stimulation through an extracellular electrode in PFC layer 1 triggered long-term depression of SOM-IPSCs (TBS-iLTD) that was blocked by AM251 and was absent in SOM-CB1R KO animals. Consistent with a presynaptic expression mechanism, we observed a decrease in paired-pulse depression of SOM-IPSCs following the induction of TBS-iLTD. In addition, WIN and TBS has no effect on IPSCs mediated by photostimulating parvalbumin-expressing interneurons (PV-INs), supporting the idea that inhibition from PV-INs is not regulated by eCB signaling. Altogether, these results reveal an input-specific eCB modulation of a major source of dendritic inhibition in the PFC to control information flow from multiple sources to shape associative cognitive processing.

Nanosymposium

591. Signaling Mechanisms in Long-Term Plasticity II

Location: SDCC 5

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 591.09

Topic: B.05. Synaptic Plasticity

Title: CB_1 receptors in hippocampal mossy cells control spatial memory and seizure activity

Authors: *C. BERTHOUX, K. NASRALLAH, P. E. CASTILLO;
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: The dentate gyrus (DG), a brain region critically involved in performing spatial memory and pattern separation, includes two principal excitatory neurons, dentate granule cells (GCs) and hilar mossy cells (MCs) that connect each other thereby establishing a GC→MC→GC recurrent excitatory circuit. Given the extensive projections of MCs and the large proportion of these cells that are active during exploratory behaviors, MCs have a great potential to destabilize this circuit. Moreover, burst stimulation of MC axons *in vitro* elicits robust, BDNF/TrkB-dependent, presynaptic LTP at MC-GC synapses that increases DG output and facilitates seizure activity *in vivo*. Left unchecked, this MC-GC LTP may interfere with DG-dependent learning, like pattern separation –which relies on sparse GC firing– and may even promote epileptic activity. Remarkably, MC axon terminals express high levels of type-1 cannabinoid receptors (CB_1Rs) whose activation dampens MC-GC synaptic transmission, LTP induction, and GC output. We therefore hypothesized that by controlling MC-GC synaptic function, CB_1Rs contribute to DG-dependent learning and prevent epileptic activity. We first tested whether endocannabinoid signaling, like at many synapses in the brain, could mediate LTD at MC-GC synapses. We found that repetitive burst firing of GCs, a protocol known to trigger both endocannabinoid release and MC-GC LTP, also induced presynaptic MC-GC LTD in the presence of the TrkB inhibitor ANA-12, a manipulation that blocks MC-GC LTP. In addition, CB_1R antagonism blocked MC-GC LTD, whereas transient application of a CB_1R agonist induced "chemical LTD", indicating that CB_1Rs are necessary and sufficient for MC-GC LTD. Furthermore, selective deletion of CB_1Rs from MCs –using a conditional knock out approach– impaired spatial memory and contextual memory, and also increased the severity and susceptibility to experimentally-induced seizures. Altogether, these data indicate that CB_1Rs in MC axon terminals significantly contribute to DG-dependent learning and to prevent runaway activity.

Disclosures: C. Berthoux: None. **K. Nasrallah:** None. **P.E. Castillo:** None.

Nanosymposium

591. Signaling Mechanisms in Long-Term Plasticity II

Location: SDCC 5

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 591.10

Topic: B.05. Synaptic Plasticity

Support: NSF NeuroNex 1 (1707356)
            NSF NeuroNex 2 (2014862)

Title: Regional and LTP-dependent variation of Synaptic Information Storage Capacity in Rat Hippocampus

Authors: *M. SAMAVAT[1], T. M. BARTOL, Jr.[2], C. BROMER[3], J. B. BOWDEN[4], D. D. HUBBARD[4], D. C. HANKA[4], M. KUWAJIMA[4], J. M. MENDENHALL[4], P. H. PARKER[4], W. C. ABRAHAM[5], K. M. HARRIS[4], T. J. SEJNOWSKI[6];
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Abstract: The amount of information that can be stored at a synapse depends on the precision of synaptic plasticity. Signal detection theory was used previously to estimate the number of distinguishable synaptic weights for hippocampal synapses based on dendritic Spine Head Volume (SHV), a measure of synaptic weight that correlates with synapse size (Bartol et al., 2015). We introduce here a new analysis of Synaptic Information Storage Capacity (SISC), based on Shannon Information Theory, that uses a series of novel algorithms to generate a non-overlapping clustering of SHVs as a measure of functionally discrete synaptic weights. Precision was determined from the variance between pairs of spine synapses on the same dendrite from the same axon, which would share a common history of coactivation. Shannon Information per synapse was calculated based on SHVs binned in unique clusters. In adult rats, SHVs in area CA1 occupied 24 clusters (4.1 bits). In contrast, in the middle molecular layer of the dentate gyrus (DG) control SHVs occupied only 5 clusters (2 bits); 30 min following LTP induction this value doubled to 10 clusters (3 bits) an increase that lasted at least 2 hours after induction of LTP. Thus, synapses in different regions of the hippocampus had different SISC values and these were not fixed properties but could be increased by LTP. Finally, measurement of the Kullback-Liebler divergence, namely the distance between the distribution of SHV clusters and the optimal uniform distribution, revealed that the distribution of synaptic states evolved closer to a uniform distribution across 2 hr post-induction of LTP. Thus our new SISC analysis provided more accurate estimates of information capacity of synapses and revealed that the Shannon Information is nearly maximized for the number of distinguishable categories obtained after LTP.

Nanosymposium

591. Signaling Mechanisms in Long-Term Plasticity II

Location: SDCC 5

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 591.11

Topic: B.05. Synaptic Plasticity

Title: Ethanol Blocks a Novel Form of LTD, but not LTP of Inhibitory Inputs toVTA GABA Neurons

Authors: J. G. EDWARDS[1], T. M. NUFER[5], Z. BOYCE[2], S. STEFFENSEN[3], *B. WU[4];
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Abstract: The ventral tegmental area (VTA) is an essential component of the mesocorticolimbic dopamine (DA) circuit that processes reward and motivated behaviors. The VTA contains DA neurons essential in this process, as well as GABAergic inhibitory cells that regulate DA cell activity. In response to drug exposure, synaptic connections of the VTA circuit can be rewired via synaptic plasticity—a phenomenon thought to be responsible for the pathology of addiction. While synaptic plasticity to VTA DA neurons and prefrontal cortex to nucleus accumbens GABA neurons has been well studied, VTA GABA cell plasticity, specifically inhibitory inputs to VTA GABA neurons, is less understood. Therefore, we investigated the plasticity of inhibitory inputs to VTA GABA neurons. Using whole cell electrophysiology in GAD67-GFP mice to identify GABA cells, we observed that these VTA GABA cells experience either inhibitory GABAergic long-term potentiation (iLTP) or inhibitory long-term depression (iLTD) in response to a 5 Hz stimulus. Paired pulse ratios, coefficient of variance, and failure rates suggest a presynaptic mechanism for both plasticity types, where iLTP is NMDA receptor-dependent and iLTD is GABAB receptor-dependent—this being the first report of LTD onto VTA GABA cells. As illicit drug exposure can alter VTA plasticity, we employed chronic intermittent exposure (CIE) to ethanol (EtOH) vapor in male and female mice to examine its potential impact on VTA GABA input plasticity. Chronic EtOH vapor exposure produced measurable behavioral changes illustrating dependence and concomitantly prevented previously observed iLTD, which continued in air-exposed controls, illustrating the impact of EtOH on VTA neurocircuitry and suggesting physiologic mechanisms at play in alcohol use disorder and withdrawal states. Taken together, these novel findings of iLTP and iLTD occurring at the same

synapse within the mesolimbic DA circuit characterize inhibitory VTA plasticity as a malleable, experience-dependent system.

Nanosymposium

592. Alzheimer's Disease: Glial Cells

Location: SDCC 29

Time: Wednesday, November 16, 2022, 8:00 AM - 10:00 AM

Presentation Number: 592.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Enhanced synapse elimination by glial cells precedes tau tangle pathology and predicts cognitive status of individuals with Alzheimer's disease at tau Braak III-IV stages

Authors: *R. N. TADDEI[1,2], R. C. PERBET[1], T. CONNORS[1,3], A. GAONA[1,3], A. MELLONI[1,3], K. DUFF[2], M. FROSCH[4], T. GOMEZ-ISLA[1];
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Abstract: Synaptic loss is a strong predictor of cognitive decline in Alzheimer's disease (AD). Yet, presence and burden of amyloid and tau pathologies alone are insufficient to account for the full extent of synapse loss observed in demented AD brains. We recently showed that increased proinflammatory and decreased homeostatic glial responses are present in the visual cortex of demented but not resilient AD brains at Braak III-IV stages of tau pathology. Moreover, emerging evidence suggests that microglia take up excessive amounts of synapses in human AD brains, and in vitro models show that glial cells are capable of engulfing synapses. Yet, human brain derived evidence of aberrant glial-mediated synapse elimination in AD and the underlying contributors remain widely unknown. We studied human brains of demented and resilient individuals at intermediate (Braak III-IV) stages of tau pathology that harboured similar burdens of amyloid and tau deposits, and healthy controls without AD neuropathology. We evaluated the visual cortex, a Braak stage VI region, that showed no neurofibrillary tau (NFT) deposits in these brains. We applied expansion microscopy, a novel technique that allows synaptic resolution, and IMARIS software analyses to quantify synaptic densities and spatial relationships with glial cells in 3D. Our data show that demented brains display significantly lower pre-, post-, as well as synaptic puncta, and higher proportions of microglial and astrocyte internalized synaptic elements in the visual cortex compared to resilient and controls. These preliminary results

suggest that synaptic density loss precedes NFT deposition in demented human AD brains, serving as a better predictor of cognitive decline than amyloid or tau deposits. Moreover, synapses are excessively internalized by a subset of glial cells in demented compared to resilient or control brains, and this is not only accomplished by microglia but also by a subset of astrocytes. These novel findings favour a model in which astroglial mediated synapse elimination occurs ahead of NFT formation and predicts cognitive impairment in AD. Our findings could have potential implications for development of novel biomarkers and treatment avenues aimed at preventing cognitive decline in early stages of the disease.

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Nanosymposium

592. Alzheimer's Disease: Glial Cells

Location: SDCC 29

Time: Wednesday, November 16, 2022, 8:00 AM - 10:00 AM

Presentation Number: 592.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: GFAP upregulation by astrocytes does not impact tau pathology in a tauopathy mouse model

Authors: *C. MUÑOZ-CASTRO[1,2,3], M. HEALEY[1,2], M. CALVO RODRIGUEZ[1,2,3], S. ALLA[1,2], Z. D. KASHLAN[1,2], A. NOORI[1,2,4], Z. FAN[1,2], E. HUDRY[1,2,3], B. T. HYMAN[1,2,3], A. SERRANO-POZO[1,2,3];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]MassGeneral Inst. for Neurodegenerative Dis., Charlestown, MA; [3]Harvard Med. Sch., Boston, MA; [4]Harvard Col., Boston, MA

Abstract: Introduction: Astrocytes react to both Aβ plaques and tau neurofibrillary tangles in the Alzheimer's disease (AD) brain but their effect on these AD pathological hallmarks remains controversial. A crucial feature of these reactive astrocytes is the upregulation of the intermediate filament glial fibrillary acidic protein (GFAP), which is thought to be necessary for astrocyte process motility and glial scar formation. We asked whether this cytoskeletal remodeling is critical for reactive astrocytes to control the burden of pathological hyperphosphorylated tau species and, more specifically, whether overexpressing GFAP in astrocytes from a tauopathy mouse model could ameliorate neuronal phospho-tau burden. **Methods:** To answer this question, we overexpressed human wild-type GFAP isoform 1 (432 amino acids) with a Myc-tag specifically in the astrocytes (driven by the gfaABC$_1$D promoter) of 4-month-old, sex-balanced ThyTAU22 mice (Thy1.2-$MAPT^{G272V/P301S}$ 1N4R, n=11)—an age in which neurofibrillary

tangles start to appear in the hippocampal CA1 field—using a viral transfer strategy with a single retro-orbital intravenous injection of AAV2/PHP.B (2.5×10^{11} genome copies) and euthanized them at 11 months of age (i.e., prior to the plateau of the tauopathy). Negative control groups consisted of littermates injected with either saline (n=9) or an AAV2/PHP.B vector encoding the enhanced green fluorescent protein (EGFP, n=10). Outcome measures included cortical and hippocampal, soluble and insoluble, phospho-tau (AT8/pTau$^{Ser202/Thr205}$ and AT270/pTau^{Thr181}) and total tau levels as well as AT8-immunoreactive neurofibrillary tangle burden. **Results:** Immunohistochemistry confirmed the astrocyte-specific expression of exogenous (Myc-tagged) GFAP with an apparent integration in their intermediate filament network and a ~4-fold increase in immunoreactive area throughout the cortex, whereas ELISAs revealed a ~10-fold increase in cortical GFAP levels. By contrast, as reported elsewhere, transduction in hippocampal astrocytes was comparatively less efficient. No significant changes were observed across groups in the AT8-immunoreactive neurofibrillary tangle burden, total tau, or pTau levels in either cortex or hippocampus. **Conclusion:** We found that GFAP upregulation by astrocytes—an essential feature of reactive astrogliosis—does not impact neuronal tau burden in a tauopathy mouse model. Further work will determine whether other aspects of reactive astrocytes do accelerate or attenuate neuronal tauopathy.

Disclosures: C. Muñoz-Castro: None. **M. Healey:** None. **M. Calvo Rodriguez:** None. **S. Alla:** None. **Z.D. Kashlan:** None. **A. Noori:** None. **Z. Fan:** None. **E. Hudry:** None. **B.T. Hyman:** None. **A. Serrano-Pozo:** None.

Nanosymposium

592. Alzheimer's Disease: Glial Cells

Location: SDCC 29

Time: Wednesday, November 16, 2022, 8:00 AM - 10:00 AM

Presentation Number: 592.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Bace-1 inhibition in microglia enhances amyloid clearance via activating dam-1 like state with no detrimental effect on synaptic plasticity.

Authors: *N. SINGH, M. BENOIT, B. DAS, J. ZHOU, X. HU, R. YAN;
Neurosci., Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: BACE-1 is required for generating β-amyloid peptides (Aβ) in Alzheimer's disease. Inhibiting BACE-1 and thus blocking Aβ generation is therefore being explored as a logical approach for AD treatment. We identified a novel role of microglial *Bace-1* in regulating its phagocytic function. By utilizing various *Bace-1* knock-out mice models in control and 5xFAD

condition, we demonstrated that microglial Bace-1 regulates the transition of homeostatic to stage 1 disease-associated microglia (DAM-1) signature. Unbiased single-cell RNA sequencing conducted on microglia derived from WT or *Bace-1* deficient microglia revealed that *Bace-1* deficiency elevated a group of transcription factors including *Jun, Jund, Erg1, Junb, Fos*, and *Fosb* while microglia were transitioning from homeostatic to highly phagocytic DAM-1 like state. Likewise, we identified similar transition-state microglia in human AD brains correlated with lowered levels of Bace1 expression. Targeted deletion of *Bace-1* in adult 5xFAD mice microglia elevated these DAM-1 microglia with elevated phagocytic function, which was correlated with the significant reduction in amyloid plaques without altering neuronal APP processing. Silencing or pharmacologically inhibiting BACE-1 in cultured microglia demonstrated higher phagocytic function and amyloid-beta clearance by enhancing autophagolysosomal activity. The mechanistic study demonstrated that BACE-1 regulated Aβ-induced metabolic reprogramming necessary for Aβ degradation by favoring phosphorylation of mTOR at Ser 2448 and modulating the PI3K-mTOR-HIF-1α signaling pathways. Remarkably, unlike memory function impeded by the global deletion of Bace-1, mice with selective Bace-1 deletion in microglia rescued AD-associated reduction in long-term potentiation or memory deficits. Our results suggest that targeted inhibition of BACE-1 in microglia is a superior strategy for AD treatment.

Nanosymposium

592. Alzheimer's Disease: Glial Cells

Location: SDCC 29

Time: Wednesday, November 16, 2022, 8:00 AM - 10:00 AM

Presentation Number: 592.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Identification of Intercellular Pathways in Tissue Repair in Vascular Dementia

Authors: M. TIAN[1], R. KAWAGUCHI[1], Y. SHEN[2], L. DE BIASE[3], J. D. HINMAN[1], H. V. VINTERS[1,4], Y. ZHANG[5], A. J. SILVA[2], S. T. CARMICHAEL[1];
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Abstract: Vascular dementia (VaD) is the second leading cause of dementia; accelerates Alzheimer's disease (AD) and progresses to adjacent white matter. Due to limited knowledge in the mechanisms of VaD recovery, there is currently no direct therapy. It is important to identify novel targets for the treatment of VaD. To achieve this goal, preliminary data was developed with flowcytometry, cell type-specific viral labeling/transgenic mouse strains, and transcriptome profiling of endothelial cells (ECs), pericytes, astrocytes, microglia and oligodendrocyte progenitor cells (OPCs) from the corpus callosum of a VaD mouse model, to identify the molecular systems that communicate among cells of the vascular niche in VaD. Three ligand/receptor (L/R) databases were merged in specific bioinformatic process so that this approach selects the potential cell-cell signaling candidates. Together with our single nucleus RNAseq data from human VaD periventricular white matter, several L-R pairs that are dysregulated in VaD in humans and in mouse models of VaD were identified. Among them, the CD39-A3AR signaling pathway emerges because of the known function of CD39 in depleting extracellular ATP caused by brain injury, which leads to an anti-inflammatory environment, and the effects of A3AR agonists in neuroprotection, anti-inflammation, and large artery stroke models. CD39 (ligand) interacts with A3AR (receptor) as an intercellular signaling pathway through 2 possible patterns: (1) direct binding to A3AR; (2) degrading extracellular ATP to ADP and AMP, and then together with endogenous CD73, converting AMP into adenosine which will bind to A3AR. However, the brain CD39-A3AR expression is not well characterized, and its function in brain tissue repair in VaD has not been studied. Our study found that CD39 is specifically expressed in microglia and endothelial cells, while A3AR is expressed in microglia in corpus callosum. Microglia/EC CD39 and microglia A3AR transcripts are significantly decreased in VaD, indicating an impaired CD39-A3AR signaling pathway among these cell types. Notably, CD39 expression during VaD is reduced by aging as shown in immunostaining. These together indicate that the CD39-A3AR signaling pathway might be an endogenous mechanism which can mediate VaD recovery. In further study, long-term treatment with a specific A3AR agonist in mouse VaD model significantly reduced the lesion size and promoted the axonal regrowth. Therefore, CD39-A3AR is an endogenous intracellular signaling pathway that can be developed as a novel target for the treatment of VaD in terms of tissue and behavior repair.

Disclosures: M. Tian: None. **R. Kawaguchi:** None. **Y. Shen:** None. **L. De Biase:** None. **J.D. Hinman:** None. **H.V. Vinters:** None. **Y. Zhang:** None. **S.T. Carmichael:** None.

Nanosymposium

592. Alzheimer's Disease: Glial Cells

Location: SDCC 29

Time: Wednesday, November 16, 2022, 8:00 AM - 10:00 AM

Presentation Number: 592.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: P30AG072972

Title: Single-cell RNA-sequencing of microglia from a transgenic rat model of Alzheimer's disease

Authors: *C. J. FINNO, S. GHOSH, V. RODRIGUEZ, A. E. VALENZUELA, P. ANDREW, P. J. LEIN;
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Abstract: Single-cell transcriptomics have identified that Alzheimer's disease (AD) pathology is associated with loss of homeostatic microglia and activation of disease-associated microglia (DAM). This shift to DAM has been observed in various mouse models of AD and human AD samples. However, the inflammatory response of rats is more similar to humans than mice. Thus, we profiled microglial transcriptomes in the TgF344-AD rat, which overexpresses human mutant *APPSW* and *presenilin 1* (*PS1ΔE9*). Hippocampal microglia were collected via CD45^{low} and CD11b$^+$ gating from four female TgF344-AD rats and four wild-type littermates at 3 and 9 months of age. Two pools from each group underwent single-cell RNA-sequencing with an average ($\pm$SD) 4,268 $\pm$ 629 cells profiled per group and 44,768 $\pm$ 6,308 reads per cell. Cells were >95% microglia and <5% macrophages. At 3 months, 430 transcripts were differentially expressed (71 up; 359 down). By 9 months, the number of differentially expressed transcripts doubled to 934 (400 up; 534 down). When evaluating the interaction of genotype and age, the top downregulated transcripts included homeostatic markers *Tmem119, Cx3cr1, Csf1r, P2ry12, Ctss, Hexb*, *C1qb*, *C1qa* and *Cst3*. At 3 months, the top upregulated transcript in AD rats included the neuroprotective DAM genes *ApoE* and *Trem2*. By 9 months, the profile had shifted, with*Trem2* no longer significantly upregulated, but neurotoxic DAM transcripts, including *Cd9, C3, Tspo* and *Cxcl16,* upregulated. Homeostatic microglial markers are downregulated concurrent with increased expression of DAM markers in the TgF344-AD rat, supporting the value of this model to study intervention strategies for AD.

Disclosures: C.J. Finno: None. **S. Ghosh:** None. **V. Rodriguez:** None. **A.E. Valenzuela:** None. **P. Andrew:** None. **P.J. Lein:** None.

Nanosymposium

592. Alzheimer's Disease: Glial Cells

Location: SDCC 29

Time: Wednesday, November 16, 2022, 8:00 AM - 10:00 AM

Presentation Number: 592.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH PO1 AG062817t

Title: Aging on the carbohydrate-rich diet induces a large induction of microglial and complement inflammation genes in brain which are blunted by the ketogenic diet

Authors: *A. TOMILOV[1], M. STRATTON[2], J. RAMSEY[1], K. KIM[1], I. MAEZAWA[3], L.-W. JIN[4], Z. ZHAO[1], G. CORTOPASSI[5];
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Davis, Sacramento, CA; [4]M.I.N.D. Inst. UC Davis, Davis, CA; [5]Mol. Biosci., Univ. of California, Davis, Davis, CA

Abstract: Background and Rationale. Aging in the transition from mid-to late-life reduces memory and cognitive function. The Ketogenic diet (KD) introduced at mid-life preserves memory and cognition in aged mice (Roberts et al.PMID: 28877457 2017; Newman et al. PMID: 28877458, 2017). Mice were dosed from 14 to 26 months with either an isocaloric standard chow carbohydrate rich diet (CD) AIN95, or the KD exactly as in Roberts et al., and their brain transcriptomic signatures analyzed. **Objectives.** To test which signatures of brain aging on the CD are altered by aging on the KD. **Methods.** C57BL6 brains were taken at 14 and 26 months of age on CD or KD, nucleic acids isolated for RNAseq, selected transcripts were confirmed by QRTPCR. Also we tested the effect of KD mediator Beta-hydroxybutyrate (BHB) in vitro on A-beta inflamed human IPSC microglia. **Results.** Brain aging from 14 to 26 months of age on the standard carbohydrate diet caused significant alteration of 35 transcripts in the Microglial compartment (p<0.0001), and a strong induction of many known genes of Microglial Inflammation (Oas1b, Parp14, Alox8, Zbtb16, Ifi203, Ifit1, Mpeg, Ifit3, Trem2, Cd68, Cd84, Clec7a) was observed. However, brain aging from 14 to 26 months of age on the KD did not significantly alter the microglial compartment (p=0.4). Thus the significant changes in microglial transcription induced by brain aging on the CD were not observed on KD. Said another way, the KD suppresses the the impact of aging on microglia. Several microglial inflammation genes strongly induced by brain aging on CD: Oas1b, Parp14, Alox8, and Zbtb16, were specifically suppressed by the KD. Brain aging from 14 to 26 months of age on the standard carbohydrate diet also caused a strong rise in several individual Complement genes (C4b,C1qb, C1qa, C1qc). The age-related rise in complement has been noted by others and suggested as an underlying primer for Alzheimer's disease (PMID: 27033548 26400934 28566429). We did not find any specific significant reduction in C4b,C1qb, C1qa, C1qc mediated by the ketogenic diet. Thus we observe two strong signatures of 12 months of mouse brain aging on the CD: Microglial inflammation, and complement activation. While the KD significantly suppresses the microglial signature, it doesn't appear to suppress the complement signature. On the *in vitro* level, the KD mediator BHB significantly suppressed A-beta induced multiple markers of human IPSC microglial inflammation. **Interpretation.** While 12 mo. brain aging on CD induces a strong Microglial and Complement, the KD suppresses the Microglial component, and several specific genes, and the KD mediator BHB suppresses microglial inflammation as well.

Disclosures: A. Tomilov: None. **M. Stratton:** None. **J. Ramsey:** None. **K. Kim:** None. **I. Maezawa:** None. **L. Jin:** None. **Z. Zhao:** None. **G. Cortopassi:** None.

Nanosymposium

592. Alzheimer's Disease: Glial Cells

Location: SDCC 29

Time: Wednesday, November 16, 2022, 8:00 AM - 10:00 AM

Presentation Number: 592.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Reactive astrocytic tonic GABA inhibits sleep-regulating neurons leading to progressive sleep deterioration in the 5XFAD mouse model of Alzheimer's disease

Authors: *V. J. DREW, M. PARK, T. KIM;
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Abstract: Sleep disruption is a symptom of Alzheimer's disease **(AD)**. As AD progresses, sleep disturbances become increasingly debilitating. Astrocytes, recognized for performing several critical functions in the brain including waste removal and forming glial scar tissue for injury repair, have recently been shown to modulate sleep pressure accumulation and associated cognitive consequences. After exposure to insult or injury, astrocytes undergo morphological, chemical and functional changes referred to as "reactive astrogliosis". One functional change of reactive astrocytes involves the degradation of Aβ and biosynthesis of GABA, followed by the release of tonic GABA, which has the potential to suppress neighboring cells possessing extrasynaptic GABA receptors. The 5XFAD mouse model overexpresses Aβ at an early age and has been reported to demonstrate altered sleep behaviors. The objective of this study was to determine if sleep deterioration in AD results from the inhibition of key sleep-regulating neurons by tonic GABA released by reactive astrocytes. In this study, the sleep behavior of 3-, 6-, and 10-month-old male 5XFAD mice was examined using encephalography/electromyography (EEG/EMG) After sleep recording, the mice were sacrificed and the brains were harvested for immunohistochemistry. At 3 months, 5XFAD mice displayed largely similar sleep behavior to age-matched wild type mice. By 6 months, 5XFAD mice showed increased average wake proportions (p = 0.045), and decreases in NREM durations (p = 0.023). These changes intensified in 10-month-old 5XFAD mice, accompanied by decreased REM durations (p = 0.002) and longer REM average bout lengths. Furthermore, IHC revealed an increase of reactive astrocytes as well as elevated levels of GABA in proximity to sleep-regulating neurons. Quantities of cortical nNOS neurons, galaninergic neurons of the ventrolateral preoptic area, and cholinergic neurons of the pedunculopontine **(PPT)** and laterodorsal tegmentum **(LDT)** remained unchanged. The changes in sleep behavior appear to correlate with the progression of Aβ distribution, suggesting a potential inhibitory role of reactive astrocytic tonic GABA towards sleep-regulation regions of the brain, particularly cortical nNOS neurons, and cholinergic neurons of the LDT/PPT. To our knowledge, this study provides the most thorough sleep profile of the 5XFAD mouse model to date, while simultaneously elucidating the relationship among the age-related distribution of Aβ, the inhibitory effects of reactive astrocytic tonic GABA in specific sleep-regulating regions of the brain and the corresponding forms and severity of sleep disruptions.

Disclosures: V.J. Drew: None. **M. Park:** None. **T. Kim:** None.

Nanosymposium

592. Alzheimer's Disease: Glial Cells

Location: SDCC 29

Time: Wednesday, November 16, 2022, 8:00 AM - 10:00 AM

Presentation Number: 592.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Human astrocytes and microglia show augmented ingestion of synapses in Alzheimer's disease via disease-specific mechanisms

Authors: *M. TZIORAS[1], M. J. DANIELS[1], C. DAVIES[1], D. KING[1], P. BAXTER[1], C. SMITH[2], V. E. MIRON[2], R. T. KARADOTTIR[3], G. E. HARDINGHAM[1], C. M. HENSTRIDGE[4], P. BRENNAN[2], B. W. MCCOLL[1], T. L. SPIRES-JONES[1];
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Abstract: Introduction: Synapse loss strongly correlates with cognitive decline in Alzheimer's disease (AD). Genetic evidence points to astrocytes and microglia as contributors to disease risk and mouse models suggest their involvement of microglia in aberrant synapse removal. Astrocytes have not previously been implicated in directly contributing to synapse loss in AD models, and direct human evidence for any glial involvement in synapse removal in human AD remains to be established. Similarly, the feasibility of selectively targeting disease-associated synapse loss is unknown.
Methods: We studied human post-mortem tissue from 31 confirmed late-stage AD cases (mean age 76, 18M:13F, 8APOE3/3:23APOEx/4) and 19 age-matched control cases (mean age 73, 11M:8F, 12APOE3/3:7APOE3/4). Tissue was stained for synapsin I, CD68 for, GFAP and Thioflavin S. Twenty confocal images (63x) were taken in the grey matter of each case from the temporal lobe (BA20/21) and occipital lobe (BA17). For *in vitro* experiments, we looked at primary human microglia from biopsies (n=8) were isolated using CD11b immunomagnetic beads, similar to adult murine microglia (n=8), and embryonic and foetal mouse (n=5) and human astrocytes (n=8), respectively. Human synaptoneurosomes were labelled with pHrodo-RED to track synaptic engulfment. Synaptoneurosomes were treated with a control IgG antibody or anti-MFGE-E8 one for the blocking assays. Mixed-effects linear models were used to analyse the data and account for factors like repeated measurements, age, sex, brain area, and effects of plaques.
Results: In this exploratory study, we demonstrate that astrocytes and microglia from human post-mortem brains contain greater amounts of synaptic protein in AD compared to non-disease control tissue, and that proximity to amyloid-β plaques and inheritance of the *APOE4* risk gene exacerbate this effect. *Ex vivo*, mouse and human astrocytes and primary mouse and human adult microglia show a preference for phagocytosing
AD-derived synapses more than synapses from control brains. Inhibiting MFG-E8 function with a blocking antibody rescued the elevated engulfment of AD synapses by astrocytes, without

affecting non-disease synapse uptake, while AD-specific microglial synapse uptake was reversed by IgG treatment blocking high affinity Fc receptors. This is the first quantitative study in human brains with Alzheimer's disease showing that glia ingest more synapses.
Conclusion: Overall, we found that AD promotes increased synapse ingestion by glial cells via MFGE-E8-related mechanisms with the potential for targeted therapeutic manipulation.

Disclosures: M. Tzioras: None. **M.J. Daniels:** None. **C. Davies:** None. **D. King:** None. **P. Baxter:** None. **C. Smith:** None. **V.E. Miron:** None. **R.T. Karadottir:** None. **G.E. Hardingham:** None. **C.M. Henstridge:** None. **P. Brennan:** None. **B.W. McColl:** None. **T.L. Spires-Jones:** F. Consulting Fees (e.g., advisory boards); TS-J is on the Scientific Advisory Board of Cognition Therapeutics and receives collaborative grant funding from 2 industry partners..

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 593.01

Topic: C.03. Parkinson's Disease

Title: A physiological role of a-synuclein Serine 129 phosphorylation in synaptic vesicle recycling

Authors: *L. PARRA-RIVAS[1], K. MADHIVANAN[1], L. WANG[3], N. BOYER[1], D. PRAKASHCHAND[2], Y. TANG[4], U. DAS[1], D. A. SCOTT[4], P. RANGAMANI[2], S. ROY[5];
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Abstract: α-synuclein (α-syn) is a small presynaptic protein linked to the pathogenesis of Parkinson's disease (PD) and related disorders, collectively called synucleinopathies. Phosphorylation of α-syn at Ser-129 is a pathologic hallmark of synucleinopathies and widely considered to be a therapeutic target. Using pHluorin assays in cultured neurons, we and others have shown that modest levels of α-syn overexpression attenuates synaptic vesicle (SV) recycling, suggesting that α-syn is a physiologic attenuator of neurotransmitter release. Furthermore, we have shown that binding of α-syn to VAMP2 is necessary for the α-syn-induced synaptic attenuation seen in the pHluorin assays. In this study, we evaluated the presynaptic targeting of phospho-incompetent (Ser-129A) and phospho-mimic (Ser-129D) α-syn mutants, and also evaluated the effects of Ser-129 phosphorylation on SV-recycling using pHluorins. While mimicking Ser129 phosphorylation augmented synaptic α-syn accumulation and further attenuated SV-recycling as expected, surprisingly, preventing Ser129-P completely blocked the ability of α-syn to attenuate SV-recycling; suggesting a physiologic role for this post-

translational modification at synapses. Mechanistically, we found that phosphorylation at the Ser-129 site was required for α-syn binding to VAMP2 and subsequent synaptic attenuation, explaining the inability of Ser129 phospho-incompetent α-syn to attenuate SV-recycling. Finally, AlphaFold2-based modeling and membrane-binding simulations support a scenario where Ser-129 phosphorylation triggers a conformational change in α-syn that exposes its C-terminus, allowing VAMP2 binding and consequent functionality. Collectively, the data suggest a physiologic role for α-syn S129 phosphorylation and warrant caution in developing therapeutic agents that inhibit this phosphorylation site.

Disclosures: L. Parra-Rivas: None. **K. Madhivanan:** None. **L. Wang:** None. **N. Boyer:** None. **D. Prakashchand:** None. **Y. Tang:** None. **U. Das:** None. **D.A. Scott:** None. **P. Rangamani:** None. **S. Roy:** None.

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 593.02

Topic: C.03. Parkinson's Disease

Title: Paan/mif nuclease inhibition prevents neurodegeneration in parkinson's disease

Authors: *H. PARK[1], T.-I. KAM[2], H. PENG[2], J. O. LIU[2], V. L. DAWSON[2], T. M. DAWSON[2];
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Abstract: Parthanatos Associated AIF (apoptosis-inducing factor) Nuclease (PAAN), also knownas macrophage migration inhibitor factor (MIF) is a member of the PD-D/E(X)Knucleases that acts as a final executioner in parthanatos. PAAN's role in Parkinson'sdisease (PD) and whether it is amenable to chemical inhibition is not known. Here weshow that neurodegeneration induced by pathologic α-synuclein (α-syn) occurs viaPAAN/MIF nuclease activity. Genetic depletion of PAAN/MIF and a mutant lackingnuclease activity prevent the loss of dopaminergic neurons and behavioral deficits inthe α-syn preformed fibril (PFF) mouse model of sporadic PD. Compound screeningled to the identification of PAANIB-1, a first-in class, brain-penetrant PAAN/MIFnuclease inhibitor that prevents neurodegeneration induced by α-syn PFF, AAV-α-synoverexpression or MPTP intoxication in vivo. Our findings could have broad relevancein human pathologies where parthanatos plays a role for development of new celldeath inhibitors targeting the druggable PAAN/MIF nuclease.

Disclosures: H. Park: None. **T. Kam:** None. **H. peng:** None. **J.O. Liu:** None. **V.L. Dawson:** None. **T.M. Dawson:** None.

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 593.03

Topic: C.03. Parkinson's Disease

Title: α-synuclein fibrils explore actin-mediated macropinocytosis for cellular entry into model neuroblastoma neurons

Authors: *P. HIVARE, J. GADHAVI, D. BHATIA, S. GUPTA;
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Abstract: Alpha-synuclein (α-Syn), an intrinsically disordered protein (IDP), is associated with neurodegenerative disorders, including Parkinson's disease (PD or other α-synucleinopathies. Recent investigations propose the transmission of α-Syn protein fibrils, in a prion-like manner, by entering proximal cells to seed further fibrillization in PD. Despite the recent advances, the mechanisms by which extracellular protein aggregates internalize into the cells remain poorly understood. Using a simple cell-based model of human neuroblastoma-derived differentiated neurons, we present the cellular internalization of α-Syn PFF to check cellular uptake and recycling kinetics along with the standard endocytic markers Transferrin (Tf) marking clathrin-mediated endocytosis (CME) and Galectin3 (Gal3) marking clathrin-independent endocytosis (CIE). Specific inhibition of endocytic pathways using chemical inhibitors reveals no significant involvement of CME, CIE and caveolae-mediated endocytosis (CvME). A substantial reduction in cellular uptake was observed after perturbation of actin polymerization and treatment with macropinosomes inhibitor. Our results show that α-Syn PFF mainly internalizes into the SH-SY5Y cells and differentiated neurons via the macropinocytosis pathway. The elucidation of the molecular and cellular mechanism involved in the α-Syn PFF internalization will help improve the understanding of α-synucleinopathies including PD, and further design specific inhibitors for the same.

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM
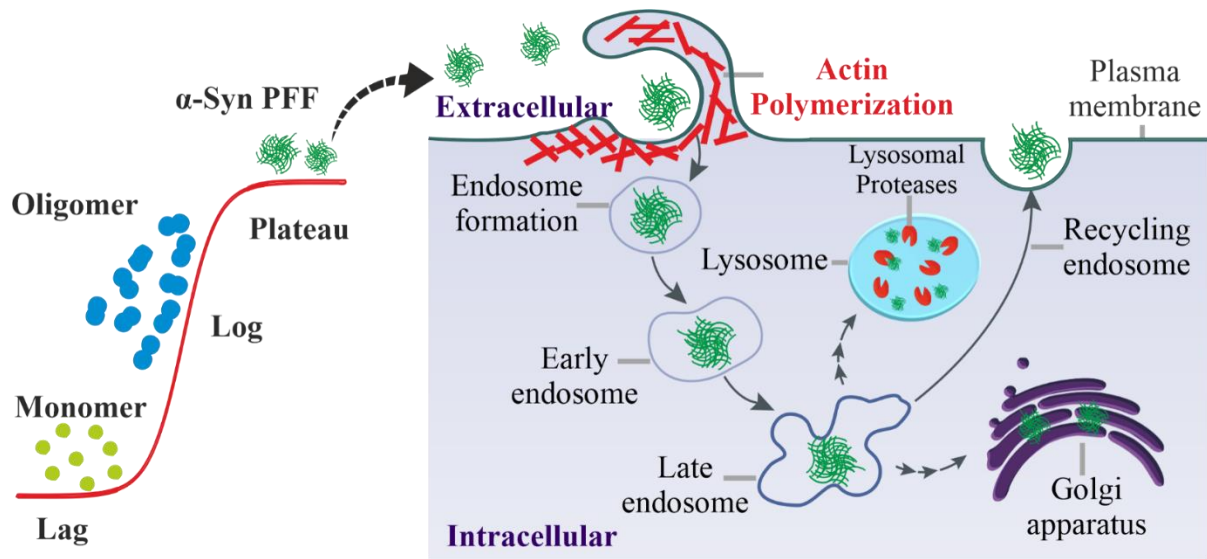
Presentation Number: 593.04

Topic: C.03. Parkinson's Disease

Title: Intracellular expression of anti-alpha-synuclein nanobodies prevents fibril-induced synucleinopathy

Authors: *A. TANDON[1], S. ARMSTRONG[2], L. RODRIGUEZ[1], D. C. BUTLER[3];
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Abstract: Synucleinopathies such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), and Multiple System Atrophy (MSA) are characterized by the progressive spread of intracellular inclusions containing phosphorylated and aggregated alpha-synuclein (asyn). Therapeutics that can reduce asyn protein levels or prevent its aggregation may be useful disease-modifying strategies. Here we investigate single-domain antibodies, also called nanobodies, as potential therapeutics to prevent asyn aggregation in a cellular model of pre-formed fibril (PFF)-induced synucleinopathy. The VH14 nanobody was previously identified in a yeast display screen of a non-immune human cDNA library by its affinity for the asyn NAC domain, which is essential for asyn self-assembly and aggregation. Earlier work showed that

fusion of VH14 to a ubiquitin-independent proteasome degradation sequence (PEST) stabilized its expression and reduced intracellular asyn levels *in vitro* and *in vivo*. Based on those studies, we hypothesized that VH14-PEST could reduce intracellular asyn and thereby prevent the propagation of asyn pathology in neurons induced by seeding with asyn PFF. To test this hypothesis, we treated primary hippocampal neurons with asyn PFF which induces aggregation of intracellular endogenous asyn in the neurons and activated expression of VH14-PEST using a Tet-ON lentiviral system. The formation of phosphorylated serine-129 asyn (p-asyn) pathology was measured over 14 days. Exposure to asyn PFF progressively increased p-asyn inclusions throughout neurons over 14 days and induced lysosome enlargement. In contrast, neurons expressing VH14-PEST had significantly reduced p-asyn levels as compared to those expressing a non-specific control. In addition, VH14-PEST expression also prevented the corresponding lysosomal enlargement, suggesting that nanobody-induced removal of intracellular asyn prevented the development of asyn pathology and the destabilization of lysosomal structure. Our results demonstrate the ability of intracellular asyn nanobody expression to reduce asyn pathology and to mitigate downstream morphological changes linked to impaired protein degradation. In conclusion, this study supports the evaluation of nanobody as a gene therapy approach in models of synucleinopathy.

Disclosures: **A. Tandon:** None. **S. Armstrong:** None. **L. Rodriguez:** None. **D.C. Butler:** None.

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 593.05

Topic: C.03. Parkinson's Disease

Title: Seeding an acute α-Synuclein pathology with defined cytopathological inclusions in wild-type mice and non-human primates: selection of the inoculated α-Synuclein fibril strains using an external probe triplex

Authors: **F. ICHAS*[1], M. KASHYRINA[2], F. DE NUCCIO[2], F. LAFERRIÈRE[1], G. PORRAS[3], D. D. LOFRUMENTO[2], E. BEZARD[1], F. DE GIORGI[1];
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Abstract: Several in vivo animal models attempt to recapitulate the cerebral α-Synuclein (α-Syn) pathology observed in human α-Synucleinopathies (Parkinson disease - PD, multiple system atrophy - MSA, dementia with Lewy bodies - DLB). Some models are based on the overexpression of wild-type (wt) or mutant α-Syn, either in transgenic animals or after acute intracerebral administration with AAV vectors in wt animals. Others are based on stereotaxic injections (i) of synthetic α-Syn fibril strains, sometimes amplified using patient brain extracts as

tentative "conformational templates", or (ii) of brain-derived extracts after various semi-purifications procedures. Finally, certain authors combine overexpression and α-Synuclein fibril injections. It is however striking that the published neuropathological iconography characterizing the models operated by the different groups shows a very large variability in what is considered and tolerated as positive signs of a "bona fide" α-Syn pathology: this can include fuzzy and global stainings, images of a diffuse intraneuronal distribution, or alternatively neuronal cytoplasmic inclusions, almost systematically associated with neuritic inclusions, and in some cases with intranuclear inclusions. Clear-cut "inclusion type" images combining somatic and neuritic inclusions that are most reminiscent of the human pathology are generally observed after the injection of synthetic α-Syn fibrils or of MSA or DLB brain extracts purified with detergents rather than with water-based Lewy Body fractions or with overexpression alone. Still, even with synthetic fibrils there is a lab-to-lab variability in the neuropathology displayed. This is probably due to an uncontrolled α-Syn fibril strain variability that develops during the production procedures, a problem which is generally unaddressed. We show here that phenotyping the fibrils with a very simple triplex endpoint readout based on 3 external fluorescent probes allows to rapidly select α-Syn fibril strains that seed an acute α-Syn pathology with well-defined cytopathological inclusions in wild-type mice and in non-human primates.

Disclosures: F. Ichas: None. **M. Kashyrina:** None. **F. De Nuccio:** None. **F. Laferrière:** None. **G. Porras:** None. **D.D. Lofrumento:** None. **E. Bezard:** None. **F. De Giorgi:** None.

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 593.06

Topic: C.03. Parkinson's Disease

Title: A mouse model to test novel therapeutics for Parkinson disease: the Thy1-aSyn (line 61) mice

Authors: F. RICHTER[1,2], C. KÄUFER[1], B. GERICKE[1,2], M. FEJA[1,2];
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Abstract: Development of neuroprotective therapy for Parkinson disease (PD) is hampered by a lack of translation from pre-clinical to clinical trials. One strategy for improvement is to increase predictive validity of pre-clinical studies by using extensively characterized animal models with a comprehensive set of validated pharmacodynamic readouts. Mice over-expressing full-length, human, wild-type alpha-synuclein under the Thy-1 promoter (Thy1-aSyn line 61) reproduce key features of sporadic PD, such as progressive loss of striatal dopamine, alpha-synuclein pathology, deficits in motor and non-motor functions and elevation of inflammatory markers. Extensive work with this model by multiple laboratories over the past decade increased knowledge on pathomechanisms of alpha-synuclein pathology and down-stream pathways.

Interestingly, while postnatal transgene expression is widespread in central and peripheral neurons, the extent and progression of downstream pathology differs between brain regions, thereby replicating the characteristic selective vulnerability of neurodegenerative diseases. In depth characterization of these readouts in conjunction with behavioral deficits has led to more informative endpoints for pre-clinical trials. Each drug tested in Thy1-aSyn line 61 enhances knowledge on how molecular targets, pathology and functional behavioral readouts are interconnected, thereby further optimizing the platform towards predictive validity for clinical trials. Here we present latest discoveries and the current state of the art using Thy1-aSyn line 61 for drug target discovery, validation and pre-clinical testing.

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 593.07

Topic: C.03. Parkinson's Disease

Title: Development & Characterization of a Human Tissue Engineered Nigrostriatal Pathway as a Testbed for Understanding Pathophysiology in Parkinson's Disease

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Abstract: Parkinson's Disease (PD) is a progressive neurodegenerative disease characterized by motor deficits leading to significant disability. PD motor deficits are caused by degeneration of the nigrostriatal pathway, which consists of dopaminergic neurons in the substantia nigra pars compacta and their long-projecting axonal tracts that innervate the striatum. Nigrostriatal pathway degeneration is believed to be due to the accumulation of alpha-synuclein (α-syn) protein fibrils within axons and neuronal cell bodies, which leads to a blockage in axonal transport and ultimately causes denervation of striatal medium spiny neurons (MSNs). To

understand the axonal pathophysiology of PD, we have developed a three-dimensional anatomically-inspired *in vitro* model referred to as a tissue engineered nigrostriatal pathway (TE-NSP) using human induced pluripotent stem cells differentiated to dopaminergic neurons and striatal neurons, with these populations connected by long-projecting encapsulated dopaminergic axonal tracts. TE-NSPs are built using a tubular hydrogel microcolumn (>1.5 cm long) comprised of an outer methacrylated hyaluronic acid (MeHA) encasement and an extracellular matrix (ECM) cocktail of collagen type I and laminin (1 mg/mL each) in the lumen. The MeHA-ECM micro-columns serve as a scaffold to seed aggregates of dopaminergic and striatal neurons at either end, which project their axons and processes, respectively, to integrate over time. We found that the TE-NSPs demonstrated long bundled axonal tracts (> 12mm) from the dopaminergic neurons projecting towards the MSNs showing axonal-dendritic integration between the two discrete cell populations. Herein, we employ these human TE-NSPs as a testbed by injecting α-syn pre-formed fibrils (PFFs; 4 µM/mL) within the dopaminergic neuronal aggregate. TE-NSPs injected with α-syn PFFs were viable to at least 8 weeks of *in vitro* culture showing 90-95% viability of both cell types. Immunocytochemistry confirmed the presence of α-syn PFFs within dopaminergic neurons and their axons, as well as within the MSNs, suggesting intra-axonal PFF transport and subsequent transmission to MSNs. Ongoing studies are focused on elucidating the mechanisms of this transmission and evaluating synucleinopathy in long-term culture conditions. Our novel approach aims at mimicking key aspects of the native nigrostriatal pathway - e.g., human neurons, segregated cell populations, and bundled long-projecting axonal tracts - and disease pathology, which will aid in understanding mechanisms of α-syn transmission and testing novel therapeutic interventions to prevent transmission and neurodegeneration.

Disclosures: D. Chouhan: None. **K.D. Browne:** None. **R. Patel:** None. **D.K. Cullen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); D.K.C. is a scientific co-founder of Innervace Inc., a University of Pennsylvania spin-out company focused on translation of advanced regenerative therapies to treat central nervous system disorders., Patents related to this work: U.S. Patent App. 15/032,677 titled "Neuronal replacement and reestablishment of axonal connections";, U.S. Patent App. 16/093,036 titled "Implantable living electrodes and methods for the use thereof";, U.S. Provisional Patent App. 62/758,203 titled "Engineered neural networks in tailored hydrogel sheaths and methods for manufacturing the same".. **J.E. Duda:** None.

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 593.08

Topic: C.03. Parkinson's Disease

Support: FRQS
                 HBHL
                 CIHR

Title: Alpha-synuclein-induced brain pathology and symptomatology patterns across disease progression in mouse models

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Abstract: Intro: Recent evidence suggests synucleinopathy-associated pathogenesis in Parkinson's disease is mediated by the prion-like spreading of alpha-synuclein (aSyn). Small animal magnetic resonance imaging (MRI) is a useful tool for longitudinal examination of the progression of pathology in the brains of mouse models of disease, and presents an ideal methodology of examining brain-behaviour correlates. We present a characterization of pathological aSyn spreading from a known locus via voxel-wise aSyn-induced changes in anatomy and symptomatology.
Methods: 11-week old wild-type (WT) and hemizygous M83 aSyn^{A53T} transgenic mice received an injection of mouse [Ms-] or human [Hu-] preformed fibrils [PFF] of aSyn, or phosphate buffered saline (PBS; control) in the right striatum (n~8 mice/group/sex/time point). T1-weighted MRI images (100 μm^3 isotropic voxels; Bruker 7T), motor performance on pole test, rotarod and wire hang test, and symptom score were acquired at -7, 30, 90 & 120 days post-injection (dpi). Partial least squares regression (PLS) was used to examine the relationship between MRI-derived atrophy (voxel-wise relative Jacobian difference between the -7 & 90 dpi symptom onset time point) and symptom profile (sex, weight, genotype, injection, symptom score & motor performance).
Results: We observed a widespread bilateral pattern of atrophy, particularly involving regions that project to or receive input from the injection site, that was strongly correlated with symptomatic (smaller weighted) M83 Ms-PFF-injected mice with behavioural deficits observed on the wire hang and rotarod test. This latent variable explained ~34% of the variance and was highly significant (p<<0.0001).
Conclusion: PLS provides additional information on the brain-behaviour relationship of the MRI-derived atrophy patterns. The results support our survival data as M83 Ms-PFF mice developed symptoms earlier and succumbed to their symptoms fastest of any group, and support a univariate analysis revealing a similar widespread pattern of volumetric decline over time for the M83 Ms-PFF mice.
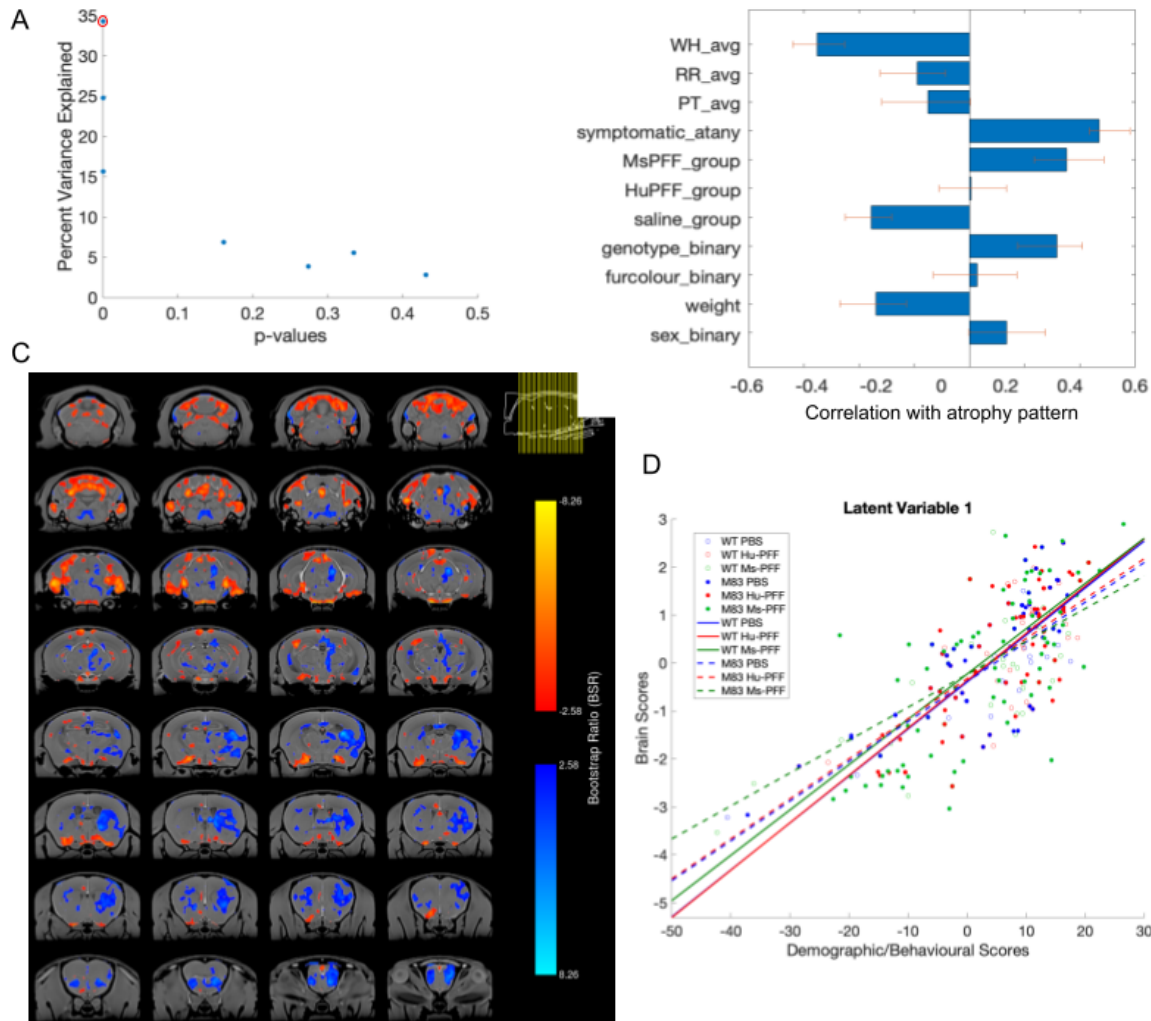
Figure. Partial least squares (PLS) analysis results for the first latent variable (LV1). (A) Covariance explained (y-axis) and permutation p-values (x-axis) for all 11 LVs in the PLS analysis. LV1 is circled in red (p<<0.0001, %covariance=34%) and was chosen for subsequent investigation based on the covariance explained and behavioural relevance of results. (B) Behaviour weight for each behavioural measure included in the analysis showing how much they contribute to the pattern of LV1. Singular value decomposition estimates the size of the bars whereas confidence intervals are estimated by bootstrapping. Bars with error bars that cross the 0 line should not be considered. (C) Brain loading bootstrap ratios for the LV1 deformation pattern overlaid on the population average, with negative bootstrap ratios in orange-yellow (indicative of larger volume), and positive in blue (indicative of smaller volume). Coloured voxels make significant contributions to LV1. (D) Correlation of individual mouse brain and behaviour score, coded by injection group (colour; blue for PBA, red for Hu-PFF and green for Ms-PFF) and genotype group (marker shape and line style; solid lines and hallow points for WT mice and dashed lines and filled points for M83 hemizygous mice) with a trend line per group (genotype-injection group). M83 Ms-PFF injected mice (green dashed line) express this pattern more strongly than any of the other genotype-injection group.

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 593.09

Topic: C.03. Parkinson's Disease

Support: ZIA AG000944, AG000928

Title: Deficiency in endocannabinoid synthase DAGLB contributes to early-onset Parkinsonism and nigral dopaminergic neuron dysfunction

Authors: J. DONG[1], Z. LIU[1], N. YANG[1], W. TIAN[1], B. SULLIVAN[1], L. WANG[1], B. TANG[2], *H. CAI[1];
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Abstract: Endocannabinoid (eCB) 2-arachidonoyl-glycerol (2-AG), the most abundant eCB in the brain, regulates diverse neural functions. Like dopamine, eCB signaling is also altered in Parkinson's disease (PD), the most common degenerative movement disorder. However, whether the observed eCB changes are a cause or compensatory response of the disease remains unclear. Using homozygosity mapping and whole-exome sequencing, we linked multiple homozygous loss-of-function mutations in diacylglycerol lipase beta (*DAGLB)* to a form of early-onset autosomal recessive PD. We then used RNA sequencing and fiber photometry with genetically encoded eCB sensors to demonstrate that DAGLB is the main synthase of 2-AG in nigral dopaminergic neurons (DANs). In mice, the nigral 2-AG levels were markedly correlated with the motor performance during motor skill acquisition. Genetic knockdown of *Daglb* in nigral DANs substantially reduced nigral 2-AG levels and impaired motor skill learning, particularly the across-session learning, whereas pharmacological inhibition of 2-AG degradation increased nigral 2-AG levels, DAN activity and dopamine release and rescued the motor skill learning deficits. Together, we demonstrate that *DAGLB*-deficiency contributes to the pathogenesis of Parkinsonism, reveal the importance of DAGLB-mediated 2-AG biosynthesis in nigral DANs in regulating neural activity and dopamine release, and provide preclinical evidence for the beneficial effects of 2-AG augmentation in alleviating Parkinsonism.

Disclosures: J. Dong: None. **Z. Liu:** None. **N. Yang:** None. **W. Tian:** None. **B. Sullivan:** None. **L. Wang:** None. **B. Tang:** None. **H. Cai:** None.

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 593.10

Topic: C.03. Parkinson's Disease

Title: Cell autonomous role of LRRK in dopaminergic neuronal survival

Authors: *J. KANG[1], **G. HUANG**[1], **Y. TONG**[1], **L. MA**[1], **L. CUI**[1], **A. SHAHAPAL**[1], **P. CHEN**[1], **J. SHEN**[1,2];
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Abstract: Mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) gene are the most common genetic cause of Parkinson's disease. Genetic studies revealed that LRRK2 regulates the autophagy-lysosomal pathway, and that germline deletion of *LRRK2* and its functional homologue *LRRK1* results in progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), along with earlier lethality and reduced body weight. In this study, we created dopaminergic neuron-specific *LRRK* conditional double knockout (cDKO) mice, in which LRRK1 and LRRK2 are selectively inactivated in dopaminergic neurons by Cre recombinase expressed under the control of the endogenous *DAT* promoter, to determine whether LRRK is required for dopaminergic neuronal survival in a cell autonomous manner. We found that dopaminergic neuron-specific *LRRK* cDKO mice of both sexes exhibit normal body weight and mortality, but they develop age-dependent loss of dopaminergic neurons in the SNpc, as evidenced by the normal number of dopaminergic neurons in *LRRK* cDKO mice at 15 months of age and decreases of dopaminergic neurons in the SNpc at the ages of 20 months (-14%) and 25 months (-23%), compared to littermate controls. However, the reduction of dopaminergic neurons in the SNpc of *DAT-Cre* driven *LRRK* cDKO mice occurs at a later age of onset and is less severe, compared with *LRRK* DKO mice lacking LRRK1 and LRRK2 in all cells (*e.g.* no reduction of dopaminergic neurons in cDKO mice at 15 months of age vs. ~20% loss of dopaminergic neurons in DKO mice at 15 months). In addition, dopaminergic neurodegeneration in *LRRK* cDKO mice is accompanied with increases in apoptosis, microgliosis, and accumulation of autophagic and autolysosomal vacuoles in surviving DA neurons. These results demonstrate an intrinsic, essential role of LRRK in the protection of dopaminergic neurons during aging, and suggest a non-cell autonomous role of LRRK in contribution to dopaminergic neuronal survival.

Disclosures: J. Kang: None. **G. Huang:** None. **Y. Tong:** None. **L. Ma:** None. **L. Cui:** None. **A. Shahapal:** None. **P. Chen:** None. **J. Shen:** None.

Nanosymposium

593. Parkinson's Disease and Mechanisms

Location: SDCC 7

Time: Wednesday, November 16, 2022, 8:00 AM - 10:45 AM

Presentation Number: 593.11

Topic: C.03. Parkinson's Disease

Title: Parkinson disease-associated mutation in miro1 leads to mitochondrial impairments and dopaminergic neuron degeneration

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Abstract: Selective loss of dopaminergic neurons in the nigrostriatal pathway is the main characteristic of Parkinson's disease (PD). Neurodegeneration in PD is believed to occur in a retrograde fashion, with mitochondrial dysfunction being one of the shared features of both monogenic and idiopathic cases. Miro1 (gene: RhoT1), an element of the motor/adaptor complex, is a key regulator of mitochondria morphology, movement and Ca2+ buffering capacity. Interestingly, recent publications reported Miro1 mutations as possible cause of PD, and showed that Miro1 function is altered in fibroblasts of PD patients (monogenic and idiopathic). These suggest a broad involvement of Miro1 in the PD pathology, supporting the hypothesis that Miro1 might represent a convergent path in the onset and/or progression of PD, namely through mitochondrial defects. We hypothesize that mitochondrial damage present in PD patients is dependent on Miro1 function and alterations in the Miro1 pathway might be tolerated until a certain tipping point after which neurodegeneration occurs. To assess our hypothesis, midbrain organoids (MO) derived from induced pluripotent stem cells of a PD patient caring the Miro1 p.R272Q mutation were used. Our MO model resembles the human midbrain, area affected in PD, and is able to reproduce PD pathological phenotypes. MO were characterized at days 20, 30, 60 and 90 of culture. First, Miro1 R272Q MO showed a lower amount of tyrosine hydroxylase (dopaminergic neuron marker) and higher fragmentation index, from day 30 onwards, comparing with healthy MO or isogenic control MO. Then, despite the similar amount of mitochondrial mass observed (TOM20 and VDAC), Miro1 R272Q MO presented a lower basal respiration and ATP production and higher mitochondrial ROS at day 35 of culture – peak of neuronal differentiation. Moreover, principal component analysis of the MO intracellular non-polar metabolites showed a clear separation between the 3 groups (healthy MO, Miro1 R272Q MO and isogenic control MO), with Miro1 R272Q MO presenting an overall reduction in the TCA metabolites. Interestingly, when exposed to different subtracts, Miro1 R272Q MO showed a significant increase in the consumption of succinic acid (complex II subtract), suggesting that complex I might be affected. At the same timepoint, no alterations in the autophagy pathway are observed (LC3 ratio or P62 levels). However, a deeper analysis in terms of the mitophagy process is needed. Altogether, our results support the hypothesis of Miro1-dependent mitochondrial damage in dopaminergic neuron degeneration and support the role of Miro1 as a possible common molecular target in PD.

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Nanosymposium

594. Mechanisms of Brain Injury and Recovery

Location: SDCC 33

Time: Wednesday, November 16, 2022, 8:00 AM - 9:30 AM

Presentation Number: 594.01

Topic: C.08. Ischemia

Title: Tet3 prevents mitochondrial dysfunction and secondary brain damage after stroke via epigenetic regulation

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Abstract: The CNS-enriched epigenetic modification known as 5-hydroxymethylcytosine (5hmC) has been shown to play an important role in neuroprotection in the diseased brain. We previously showed that the ten-eleven translocase 3 (TET3), a hydroxylase enzyme involved in producing 5hmC, is robustly induced and modulates global levels of 5hmC in the post-stroke brain. In the current study, we wanted to further determine the mechanism by which TET3 promotes neuroprotection after stroke. We subjected adult C57BL6/J mice to transient focal ischemia by middle cerebral artery occlusion following TET3 knockdown or TET3 overexpression. Hydroxymethylation DNA immunoprecipitation sequencing revealed hundreds of TET3-dependent differential hydroxymethylation regions on genomic loci associated with mitochondrial function in the peri-infarct cortex. Gene ontological analysis identified several mitochondrial processes related to the 5hmC-associated genes including mitochondrial membrane potential, mitochondrial bioenergetics, mitochondrial calcium homeostasis, mitochondrial structural components, and mitochondrial-mediated apoptosis. Immunohistochemical analysis demonstrated that TET3 maintained mitochondrial integrity and inhibited mitochondrial-mediated apoptosis following stroke. Knockdown of TET3 exacerbated secondary brain damage after stroke whereas, overexpression of TET3 decreased edema, infarct, and improved motor function recovery. Collectively this evidence indicates that TET3 may protect the brain after stroke by modulating mitochondrial gene expression and mitochondrial function.

Disclosures: S.M. Probelsky: None. **V. Arruri:** None. **A.B. Gaillard:** None. **R. Vemuganti:** None. **K.C. Morris-Blanco:** None.

Nanosymposium

594. Mechanisms of Brain Injury and Recovery

Location: SDCC 33

Time: Wednesday, November 16, 2022, 8:00 AM - 9:30 AM

Presentation Number: 594.02

Topic: C.08. Ischemia

Support: NIH 1R01NS126273-01
AHA 19CDA34660032
AHA 21CDA856826

Title: The role of serum/glucocorticoid-regulated kinase 1 in ischemic brain injury following cardiac arrest

Authors: Y. ZHANG[1], C. LI[1], C. Y.-C. WU[1], H. W. LIN[2], ***R. H.-C. LEE**[1];
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Abstract: Cardiopulmonary arrest (CA) is a major cause of death/disability in the U.S. with poor prognosis and survival rates. The current CA therapeutic challenges are physiologically complex because they involved hypoperfusion [decreased cerebral blood flow, (CBF)], neuroinflammation, and mitochondrial dysfunction. Therefore, identify these complex regulatory elements that ultimately control neuronal viability can lead to novel therapies against CA. We previously discovered that novel serum/glucocorticoid-regulated kinase 1 (SGK1, a serine/threonine kinase) is highly expressed in brain neurons that are susceptible to ischemia (e.g., hippocampus and cortex). SGK1 plays a critical role for numerous cellular processes, including regulating homeostasis, inflammation, and apoptosis in various organs. However, the role of SGK1 in the brain is understudied. To explore the potential role of SGK1 in CA-induced brain injury, we inhibited SGK1 following CA using pharmacological (specific SGK1 inhibitor) and cell type (neuron)-specific genetic approaches (e.g., shRNA) in our well-established rodent models of CA (asphyxia- and potassium chloride-induced cardiac arrest). Intra-vital two-photon laser scanning microscopy and laser speckle contrast imaging revealed that pre-treatment with GSK 650394 (1.2 µg/kg, intracerebroventricular injection) or SGK1-shRNA ($1x10^{11}$ viral particles, retro-orbital injection) attenuated cortical hypoperfusion after CA. Interestingly, neuroinflammation and mitochondrial dysfunction (via Seahorse respirometry) were reduced, while neuronal survival was enhanced in the CA1 region of the hippocampus after pre-treatment with GSK 650394 or SGK1-shRNA. Finally, rodents' neurological outcomes after CA were evaluated using Y and elevated plus mazes, ladder rung walking, hanging wire, and adhesive removal tests. Rodents pre-treated with GSK 650394 or SGK1-shRNA exhibited better neurological outcomes following CA as compared to untreated animals. In conclusion, SGK is one of the major contributors to CA-induced brain injury, while pre-treatment with GSK 650394 or SGK1-shRNA to diminish SGK1 expression provides neuroprotection against CA-induced hypoperfusion, neuroinflammation, mitochondrial dysfunction, neuronal cell death, and neurological deficits. Since the FDA has approved over 46 kinase-related drugs for the treatment of various diseases, our study will be promptly translated into human clinical trials for the patients suffering from CA.

Disclosures: Y. Zhang: None. **C. Li:** None. **C.Y. Wu:** None. **H.W. Lin:** None. **R.H. Lee:** None.

Nanosymposium

594. Mechanisms of Brain Injury and Recovery

Location: SDCC 33

Time: Wednesday, November 16, 2022, 8:00 AM - 9:30 AM

Presentation Number: 594.03

Topic: C.08. Ischemia

Title: Ischemic preconditioning maintains adaptive mitophagy while suppressing maladaptive mitophagy induced by transient global cerebral ischemia.

Authors: *T. JOVER-MENGUAL[1,2], H.-R. BYUN[3], B. L. COURT-VAZQUEZ[4], M. DELGADO-ESTEBAN[5,6], J.-Y. HWANG[7], R. S. ZUKIN[1];
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Abstract: Transient global cerebral ischemia affects millions of people each year and arises as a consequence of cardiac arrest. Global cerebral ischemia (GCI) causes selective, delayed death of hippocampal CA1 pyramidal neurons resulting in severe cognitive deficits. To date, there is no known treatment for the neurodegeneration associated with this devastating insult.
Loss of mitochondrial function is an early event during cerebral ischemia, ultimately triggering neuronal cell death. Mitochondria are involved in ATP production, ROS generation, inflammation, as well as apoptosis. Selective degradation of damaged or dysfunctional mitochondria through autophagy is known as mitophagy.
Ischemic preconditioning (IPC) is a well-known phenomenon in which a brief, sublethal ischemic insult confers robust neuroprotection to hippocampal CA1 neurons against a subsequent severe ischemic challenge. Unmasking the endogenous mechanisms triggered during IPC could help to understand how the brain protects itself. However, the molecular mechanisms underlying ischemic tolerance by preconditioning are not fully understood.
In our previous study, we showed that GCI triggers a transient increase in biochemical markers of autophagy, pS317-ULK-1, pS14-Beclin-1, and LC3-II, a decrease in the cargo adaptor p62, and an increase in autophagic flux, a functional readout of autophagy, in selectively vulnerable hippocampal CA1 neurons. However, the role of mitophagy in neuronal death after cerebral ischemia has not yet been delineated.
Here we show that mitophagy is activated in response to GCI in the hippocampal CA1 pyramidal neurons *via* PINK/PARKIN/p62/Optineurin and BNIP3 signaling pathways. Consistent with this,

recruitment of dynamin-related protein-1 (Drp1), a marker for mitochondrial fission, was detected in the mitochondrial fraction. Additionally, ischemia induces phosphorylation of NFκB and increases the expression of the proinflammatory IL-1β in the cytoplasmic fraction. However, a poor translocation of NFκB to the nucleus and no change in the mitochondrial fraction expression was observed. We further show that IPC induced a decrease of PINK, P62, Optineurin, BNIP3, and Drp1 expression in the mitochondrial fraction and IL-1β expression in the cytoplasmic fraction of the CA1 neurons. In contrast, NFκB was increased in the mitochondrial fraction suggesting a role of NFκB signaling in the mitochondria, possibly by mitochondrial dynamics regulation. These findings indicate that GCI induces maladaptive mitophagy while IPC can maintain adaptive mitophagy, most likely; through mechanisms related to mitochondrial dynamics and modulation of inflammation.

Disclosures: T. Jover-Mengual: None. **H. Byun:** None. **B.L. Court-vazquez:** None. **M. Delgado-Esteban:** None. **J. Hwang:** None. **R.S. Zukin:** None.

Nanosymposium

594. Mechanisms of Brain Injury and Recovery

Location: SDCC 33

Time: Wednesday, November 16, 2022, 8:00 AM - 9:30 AM

Presentation Number: 594.04

Topic: C.08. Ischemia

Support: 2RF1NS037459-18

Title: The role of the UCHL1 hydrolase activity in ischemic injury and recovery

Authors: *N. POVYSHEVA[1], Z. MI[2], M. ROSE[2], J. MA[2], D. ZEH[2], I. BHUIYAN[2], S. GRAHAM[2];
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Abstract: UCHL1 neuronal protein responsible for axonal and synaptic integrity carries promise as a potential therapeutic target in cerebral ischemia (Liu et al., 2019). The current study addresses the role of the UCHL1 hydrolase activity in axonal and synaptic neuronal responses to the oxygen- glucose deprivation (OGD). Brain slices obtained from the 10-12-week-old C57BI6J mice were incubated with the LDN 57444, an UCHL1 hydrolase inhibitor. Evoked axonal responses were recorded in corpus callosum (CC) and hippocampal synaptic responses in the CA1 region. OGD resulted in transient suppression of axonal and hippocampal synaptic responses that were restored after reperfusion with oxygen and glucose. LDN 57444 treatment significantly delayed recovery of axonal and synaptic responses in CC and hippocampus and decreased the amplitude of the responses after OGD and reperfusion in hippocampus. To further assess the role of the UCHL1 in neuronal responses to ischemia, OGD experiments were conducted in brain slices from the knockin mice bearing a C90A mutation devoid of UCHL1 hydrolase activity and WT controls. Recovery of axonal and hippocampal synaptic responses after OGD was delayed and their amplitude was attenuated in C90A mice as compared to control

WT mice. In addition, white matter injury detected by SMI-32 immunocytochemistry and Luxol fast blue, and motor deficits on the balance beam and cylinder test were more severe in the C90A mice subjected to transient middle artery occlusion than in WT controls. Together, these data support the involvement of UCHL1 hydrolase activity in injury and recovery of neuronal function in response to ischemia.

Nanosymposium

594. Mechanisms of Brain Injury and Recovery

Location: SDCC 33

Time: Wednesday, November 16, 2022, 8:00 AM - 9:30 AM

Presentation Number: 594.05

Topic: C.08. Ischemia

Title: CSF1R antagonism abolishes ischemic preconditioning mediated protection in white matter

Authors: *A. MCDONOUGH[1], M. A. HAMNER[1], C. NIELSON[1], D. C. GONG[1], L. J. TODD[2], S. HODECKER[1], A. GUO[1], G. ROJAS[1], C. B. RANSOM[1], T. A. REH[2], B. R. RANSOM[3], J. R. WEINSTEIN[1];
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Abstract: Background: Ischemic preconditioning (IPC) is a robust protective phenomenon whereby brief ischemic exposure confers protection against a subsequent prolonged ischemic challenge. IPC has been studied primarily in gray matter predominant models, however, stroke in human patients frequently impacts white matter (WM). We have previously reported our development of a WM IPC model in the mouse optic nerve (MON), a fully myelinated CNS WM tract. We identified innate immune signaling pathways as required for axonal protection, however the cell type(s) responsible for IPC in WM are unknown. Recent studies suggest that the length of the nodes of Ranvier (NoR) in CNS WM correlates strongly with axonal conductance and may increase due to ischemic injury. Here we characterize the effects of microglial depletion on IPC-induced protection against OGD-mediated injury to: (i) axonal compound action potential recovery, (ii) axonal structural integrity, (iii) oligodendrocyte viability and (iv) NoR length in the MON.
Methods: Following microglial depletion by pharmacologic treatment with colony stimulating factor 1 receptor (CSF1R) inhibitor PLX5622, MONs were exposed to transient ischemia in

vivo, acutely isolated, and subjected to oxygen-glucose deprivation (OGD) ex vivo to simulate a severe ischemic injury (stroke). Functional and structural axonal recovery was assessed by electrophysiology with recording of compound action potentials and by immunofluorescent/confocal microscopy followed by quantitative stereology.

Results: Microglial depletion eliminated IPC-mediated protection of axonal function but intriguingly had no effect on recovery after acute ischemic injury alone (i.e. in the absence of IPC). In addition, we observed that microglial depletion via CSF1R antagonism abrogated IPC-mediated protective effects on both axonal integrity measurements and the survival of mature (APC+) oligodendrocytes after exposure to OGD. IPC-mediated protection was determined to be independent of retinal injury. Effects on NoR length remain under investigation.

Conclusions: Based on these findings, we conclude that preconditioned, but not naïve, microglia are critical in the endogenous IPC-induced protective response against ischemic injury that occurs in WM. Thus, preconditioned microglia are a critical cellular target for future therapeutics designed to enhance WM recovery from acute ischemic injury (stroke). Furthermore, our data suggest that IPC-mediated protection in WM is anatomically intrinsic to WM and not dependent of upstream gray matter neurons.

Disclosures: A. McDonough: None. **M.A. Hamner:** None. **C. Nielson:** None. **D.C. Gong:** None. **L.J. Todd:** None. **S. Hodecker:** None. **A. Guo:** None. **G. Rojas:** None. **C.B. Ransom:** None. **T.A. Reh:** None. **B.R. Ransom:** None. **J.R. Weinstein:** None.

Nanosymposium

594. Mechanisms of Brain Injury and Recovery

Location: SDCC 33

Time: Wednesday, November 16, 2022, 8:00 AM - 9:30 AM

Presentation Number: 594.06

Topic: C.08. Ischemia

Support: NIH Grant NS112805
               AHA Grant in Aid

Title: The delta opioid receptor and ASIC-mediated cell death

Authors: *C. ASKWITH[1], L. BAUER[1], T. W. SHERWOOD[2], H. SECHRIST[3];
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Abstract: The acid sensing ion channels are essential for normal brain function, but can initiate neuronal death and contribute to brain injury. Specifically, prolonged reductions in the extracellular pH of the brain accompany cerebral injury, inflammation, and ischemia. ASIC inhibition in animal models of these conditions limits neurological damage. Yet, capitalizing on the therapeutic potential of ASIC attenuation has been hampered by the fact that ASICs play an important role in normal physiological processes and there is a lack of therapeutics which specifically target ASICs. We have discovered that the toxic effect of ASICs in central neurons

can be reduced by agonists of the delta opioid receptor (DOR). This limitation of ASIC-induced neuronal toxicity (acidotoxicity) occurs with both peptide and non-peptide DOR agonists and is dependent on G-protein activation. An important aspect of these findings is that acidotoxicity is inhibited without a reduction in ASIC1a current, showing that the toxic and physiological actions of the channel can be separated. DOR-induced neuroprotection from acidotoxicity occurs through activation of intracellular signaling cascades and involve portions of the intracellular domain of ASIC1a. Further work will determine how DOR activation affects the protein-protein interactions shown to govern ASIC1a-mediated toxicity. The outcomes of the proposed work will reveal novel regulatory mechanisms controlling ASIC1a-induced toxicity, suggest new interventions to mitigate ASIC-induced death using existing DOR agonists, and reveal strategies to separate the physiological and pathological actions of ASIC1a. These results will be significant as they are expected to have broad implications for the prevention of brain injury in disorders where neuronal acidotoxicity plays a role.

Disclosures: C. Askwith: None. **L. Bauer:** None. **T.W. Sherwood:** None. **H. Sechrist:** None.

Nanosymposium

595. Visual Memory and Categorization in the Cortex of Humans and Non-Human Primates

Location: SDCC 24

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 595.01

Topic: D.06. Vision

Title: Identifying the neural network dynamics underlying one-shot perceptual learning with intracranial EEG

Authors: *J. SHOR, T. J. BAUMGARTEN, S. DEVORE, D. FRIEDMAN, P. DUGAN, W. K. DOYLE, O. DEVINSKY, B. J. HE;
NYU Grossman Sch. of Med., New York, NY

Abstract: Object recognition under ambiguity requires matching the sensory input with one's prior knowledge to arrive at a best fit. Individuals vary in how strongly prior knowledge influences perception, and disturbances of this process may lead to hallucination. Distinct sources of prior knowledge have been identified to influence recognition, including past experiences and top-down expectation. Here, we investigate neural network dynamics underlying past experiences' influence on perception using Intracranial EEG.
Using a dramatic one-shot perceptual learning task, prior studies from our laboratory have shown that multiple large-scale cortical networks are involved in prior-knowledge-guided perceptual recognition. Notably, a recent study in brain-lesioned patients showed that the hippocampus and related medial temporal lobe structures are not needed for successful one-shot perceptual learning. This finding confirms that cortical networks implement prior-knowledge-guided

perceptual recognition; however, the involved network dynamics remain largely unknown. In this study, we leveraged the high spatial and temporal resolution of intracranial EEG (iEEG) to track the flow of information during prior-guided visual recognition to better understand the involved network mechanisms. Patients undergoing surgical evaluation with iEEG monitoring are shown degraded black-and-white images and asked if they can recognize a coherent object in the image. By presenting a degraded image both before and after the corresponding original grayscale image (which induces disambiguation of the degraded image), the distinct neural activity of prior knowledge-induced recognition can be discerned. As expected, we find that degraded images that have been disambiguated by previously viewing the matching original image are slower to generate significant ventral stream activity than the corresponding original image (as assessed by broadband gamma power), suggesting the need for additional processing due to weakened sensory evidence. Interestingly, after disambiguation, neural activity in prefrontal electrodes represents degraded images similarly to the matching original images at ~400 ms after stimulus onset. This distinction in frontal representation appears to start prior to the significant ventral stream activation driven by post-disambiguation images, which is consistent with the notion that top-down feedback from the prefrontal cortex—potentially carrying relevant prior knowledge—facilitates ventral stream processing for recognizing ambiguous visual stimuli.

Disclosures: J. Shor: None. **T.J. Baumgarten:** None. **S. Devore:** None. **D. Friedman:** None. **P. Dugan:** None. **W.K. Doyle:** None. **O. Devinsky:** None. **B.J. He:** None.

Nanosymposium

595. Visual Memory and Categorization in the Cortex of Humans and Non-Human Primates

Location: SDCC 24

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 595.02

Topic: D.06. Vision

Support: NSF 2050833

Title: Effects of motor preparation on category-specific representations in human cortex

Authors: *C. LUO, E. ESTER;
Univ. of Nevada, Reno, Reno, NV

Abstract: Categorization is the ability to classify physically similar yet conceptually different objects. The neural circuits responsible for categorization are unclear, with invasive electrophysiological studies in non-human primates pointing toward prefrontal and parietal cortex and human neuroimaging studies pointing towards early sensory areas. This discrepancy could result from signal differences (e.g., spikes vs. LFPs vs. hemodynamic response), training regimens, or a general species difference. Here, we consider an additional factor: task structure. Invasive studies in non-human primates typically rely on delayed-match-to-category (DMC)

tasks, which allow researchers to track category-selective signals independently of motor preparation; in contrast, human neuroimaging studies typically employ category discrimination (CD) tasks that require speeded responses. Thus, an important difference between non-human primate and human experiments is that the former precludes motor preparation while the latter does not. To examine whether and how motor signals influence categorization, we isolated posterior alpha-band EEG activity while participants performed a spatial delayed match to sample task. Activity measured at each electrode was modeled as a set of hypothetical spatial channels. Regression coefficients from this model were then used to track the evolution of category biases reconstructed from EEG activity while participants performed CD and DMC tasks. During the CD task, participants learned to categorize stimuli appearing at 12 positions into discrete groups, i.e., Category 1 and Category 2. Participants were free to respond following stimulus onset and instructed to prioritize accuracy and speed. During the DMC task, participants were required to determine whether two successive stimuli appearing in different spatial positions were drawn from the same category. Thus, this task requires participants to encode and remember the category membership of the first stimulus, but participants cannot plan or execute a response until the second stimulus is presented. Reconstructions of stimulus position were systematically biased towards the center of the appropriate category during the CD and DMC tasks. However, biases emerged significantly earlier during the CD relative to the DMC task. Since the primary difference between the CD and DMC tasks is that the former required an immediate response while the latter did not, we interpret this difference as evidence that decision and/or motor preparation signals influence the development of category biases in human cortex.

Disclosures: C. Luo: None. **E. Ester:** None.

Nanosymposium

595. Visual Memory and Categorization in the Cortex of Humans and Non-Human Primates

Location: SDCC 24

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 595.03

Topic: D.06. Vision

Support: NIH Grant EY017921

Title: Neurophysiological and optogenetic dissociation between feature attention and working memory

Authors: *D. MENDOZA-HALLIDAY[1], H. XU[2], R. DESIMONE[3];
[1]McGovern Institute, MIT, [2]MIT, MIT, Cambridge, MA; [3]Mit, Mit, cambridge, MA

Abstract: Visual attention and working memory (WM) are two different cognitive functions. However, because of their close relationship and interactions, it is often claimed that they share the same underlying neuronal substrates. Here we examined whether the brain areas and neurons that are modulated by selective attention to a visual feature are the same as or different from

those engaged in the maintenance of WM representations of the same feature. We trained two macaque monkeys to perform a WM-guided feature attention task that required maintaining a visual motion direction representation in WM and then selectively attending to stimuli with that motion direction. We recorded the activity of motion direction-selective neurons in multiple visual processing stages, including areas MT, MST, LIP, and LPFC. We found that the percentage of neurons exclusively showing either attentional modulation or WM coding far exceeded the percentage showing both signals. This dissociation was present in all areas and was most striking in LPFC. The posterior subregion of LPFC (LPFC-p) has been proposed as a pivotal source for feature attentional signals that, through feedback projections, selectively modulate neuronal activity in feature-selective visual cortical neurons. To examine whether LPFC-p plays a causal role in both feature attention and WM, we employed an optogenetic method for large-scale bilateral inactivation of LPFC-p during either the WM period or the sustained attention period. We replaced the native dura with a transparent artificial dura over LPFC-p bilaterally and expressed the inhibitory opsin *Jaws* across a ~100 mm^2 area via 168 viral injections. LPFC-p was bilaterally stimulated with 635 nm lasers located above the cortical surface. We confirmed that optogenetic stimulation decreased neuronal firing rates in LPFC-p; surprisingly, stimulation also decreased firing rates in MT, MST, and LIP neurons, far from the stimulated region. LPFC-p inactivation during the sustained attention period reduced the strength of feature attentional effects not only locally (in LPFC-p) but also in MST and LIP. In contrast, inactivation during the WM period reduced the strength of WM coding only in MST neurons and did not affect the strength of feature attentional effects in any area. Interestingly, LPFC-p inactivation during the sustained attention period impaired task performance, whereas inactivation during the WM period did not, suggesting that LPFC-p plays a pivotal role in feature attention but not in WM. Together, our results indicate that across multiple visual processing stages, the neuronal substrates underlying feature attention and WM are largely dissociable.

Disclosures: D. Mendoza-Halliday: None. **H. Xu:** None. **R. Desimone:** None.

Nanosymposium

595. Visual Memory and Categorization in the Cortex of Humans and Non-Human Primates

Location: SDCC 24

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 595.05

Topic: D.06. Vision

Support: NIH Grant EY019466

Title: Feature-based attention in category-learning induced transfer of visual perceptual learning

Authors: *L. ROSEDAHL, T. SERRE, T. WATANABE;
Cognitive, Linguistic, and Psychological Sci., Brown Univ., Providence, RI

Abstract: Visual Perceptual Learning (VPL; often defined as a long-term performance increase as the result of visual experience) is highly specific to trained features. Previous work found that performing category learning before VPL training causes VPL to transfer to stimuli from the same category as the trained stimulus (Category-Learning Induced Transfer of VPL or CIT-VPL, Wang et al, 2018, Current Biology). However, the mechanism of transfer is unknown, providing an obstacle to utilizing CIT-VPL. Here, we present a novel theory that CIT-VPL occurs through Feature-Based Attention (FBA) induced representational shifts. We implement this theory in a novel unified model of category learning, attention, and VPL (CAPL) and use the model to interpret the results of experiments testing two hypotheses. First, we test the hypothesis that targeted FBA is necessary for CIT-VPL by utilizing two category structures: Rule-Based (RB) and Information-Integration (II) structures. In RB structures, performance is optimized if FBA is targeted to specific feature values. For II structures, the features must be integrated at a pre-decisional stage and targeted FBA leads to suboptimal performance. Subjects (n=6) were divided evenly between the two conditions and underwent category learning followed by 5 days of VPL training. VPL for the trained stimulus and a transfer stimulus from each category was measured using pre and post-testing. The RB condition showed transfer to the stimulus from the same category as the trained stimulus but not to the opposing category stimulus, while the II condition showed no transfer. CAPL was then fit to the results by varying the strength of FBA. FBA in the RB condition was higher than the II condition. These results support the hypothesis that FBA is necessary for CIT-VPL. We then test the hypothesis that CIT-VPL occurs through representational shifts using a same-different (SD) task. If representational shift increases the overlap of stimulus representations, SD performance should decrease. Subjects (n=8) were split equally between RB and II conditions and underwent SD task pre-testing, 400 trials of category learning, and SD task post-testing. The RB condition showed decreased same-different performance after category learning while the II condition did not. CAPL was then fit to the data and the model's representational shift was calculated. The model exhibited more shift for the RB condition than the II condition. These results support the hypothesis that representational shifts are the mechanism for CIT-VPL. Overall, this work demonstrates that FBA likely plays a role in CIT-VPL by inducing representational shifts.

Disclosures: L. Rosedahl: None. **T. Serre:** None. **T. Watanabe:** None.

Nanosymposium

595. Visual Memory and Categorization in the Cortex of Humans and Non-Human Primates

Location: SDCC 24

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 595.06

Topic: D.06. Vision

Title: Convergence zones for scene perception and visuospatial memory at the anterior edge of visually-responsive cortex

Authors: C. E. ROBERTSON, B. GARCIA, A. MYNICK, K. GOYAL, A. STEEL;
Dartmouth, Hanover, NH

Abstract: The image on our retina is a limited window to our surrounding environment. What neural systems integrate the view of the scene in front of us with our memory of the immediate surrounding environment (i.e., the local visuospatial context)? Previous studies have identified high-level visual areas of the brain that selectively process visual scenes, but how memory for the wider visuospatial context surrounding a scene interfaces with these regions is not well understood. To address this knowledge gap, participants (N=17) learned a set of 20 controlled, real-world visuospatial environments using first-person head-mounted virtual reality (VR). Each environment depicted a small portion of an unfamiliar city (e.g., Padua, Seville, etc.), and the extent of the depicted visuospatial environment varied across three conditions: 1) single images (45° from a photosphere, 315° occluded), 2) panoramas (270° visible, 90° occluded), and 3) streets (three contiguous 360° photospheres). Then, we used fine-grained individual subject fMRI to test which brain areas support memory of the visuospatial context associated with a scene during recall (Exp. 1) and perception (Exp. 2). We found that, Aacross the whole brain, activity in three patches of cortex scaled with the amount of known visuospatial context during recall (vertex-wise F>6.4, p<0.0001). Interestingly, each patch was located immediately anterior to one of the three scene perception areas of the brain. Individual subject analyses revealed that these anterior patches corresponded to the three place memory areas, regions which selectively respond when visually recalling personally familiar places and show strong connectivity to the hippocampus (Steel et al., 2021). In addition to showing activity levels that scaled with the amount of visuospatial context (all p<0.01), multivariate decoding analyses showed that these anterior areas represented the identity of the specific environment being recalled (all ps < 0.01). Together, these results suggest a convergence zone for scene perception and memory of the local visuospatial context at the anterior edge of visually-responsive cortex.

Disclosures: C.E. Robertson: None. **B. Garcia:** None. **A. Mynick:** None. **K. Goyal:** None. **A. Steel:** None.

Nanosymposium

595. Visual Memory and Categorization in the Cortex of Humans and Non-Human Primates

Location: SDCC 24

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 595.07

Topic: H.03. Decision Making

Support: DFG BA 6601/2-1

Title: Similarity-based category learning in neurotypical individuals high in autistis traits and individuals with autism-spectrum disorder

Authors: R. BAUMERT[1], K. DIERMANN[1], T. FADAI[1], D. G. SCHÖTTLE[3,2], *J. BAYER[1]; [1]Dept. of Systems Neurosci., [2]Dept. of Psychiatry and Psychotherapy, Outpatients' Dept. for Autism Spectrum Disorders, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [3]Dept. of Psychiatry and Psychotherapy, Asklepios Med. Ctr. Hamburg Harburg, Hamburg, Germany

Abstract: Individuals with autism spectrum disorder (ASD) have been shown to exhibit a deficit in similarity-based categorization, a vital cognitive skill necessary for structuring and learning from the world. It has been speculated that the detail-focused cognitive style in ASD interferes with abstracting the categories' central tendency, the so-called prototype, which is an alternative to storing category knowledge through memorizing a category's exemplars. Alternatively, an ASD-specific reduction in top-down influences of prior experience on perception contents could lead to a more general deficit in similarity-based categorization. As the interpretation of previous findings is hampered by unclear influences of comorbidities and high proportions of chance-level performers, we aimed at replicating the reported deficits in neurotypical (NT) individuals high in autistic traits and investigated potential underlying neural mechanisms. Behavioural and functional magnetic resonance imaging (fMRI) data were collected from 67 NT individuals (age: $M = 25.60$, $SD = 3.70$; $n = 30$ women) characterized for autistic traits, performing a single-category prototype-distortion task with abstract patterns. Individuals had to learn which items belong to a category 'A' via visual feedback. During a transfer phase, individuals categorized previously seen as well as novel stimuli without feedback. Only individuals who performed above chance levels were included in the analyses ($N = 62$). Statistical analyses were based on median splits of autism quotient questionnaire sum scores and the 'attention to detail' subscale. Particularly individuals with a high focus on detail required more training rounds, showed worse overall training accuracy, and exhibited difficulties to endorse novel category members in the transfer phase. Model-based analyses did not indicate an association between autistic traits and behavioral strategy preferences. In contrast, analyses of parameter estimates indicate a lowered reliance on both prototype and exemplar representations. Preliminary model-free and model-based fMRI analyses also rather speak to a link between autistic traits and activity associated with the general processing of abstract patterns in striatal and prefrontal areas rather than specific differences in prototype abstraction and representation. Results confirm that deficits in similarity-based categorization can be replicated in NT individuals high in autistic traits and indicate that lowered top-down influences underlie these deficits. Existing findings will be complemented by further model-based analyses as well as data from individuals with ASD diagnoses.

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Nanosymposium

595. Visual Memory and Categorization in the Cortex of Humans and Non-Human Primates

Location: SDCC 24

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 595.08

Topic: D.06. Vision

Support: NIH R01 EY032085 (to B.J.H)
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The Irma T. Hirschl/Monique Weill-Caulier Trust (to B.J.H)

Title: Resolving the spatiotemporal neural dynamics of object recognition under uncertainty in humans

Authors: *Y.-H. WU, E. PODVALNY, B. J. HE;
NYU Grossman Sch. of Med., New York, NY

Abstract: Recent human neuroimaging work indicates that object recognition under uncertainty is orchestrated by the complex interplay between ventral visual and high-order frontoparietal regions. However, while these studies demonstrate where in the brain the recognition-related processes may take place, they provide only limited insights into how these signals evolve in each of these brain locations and how they coordinate with each other. In this study, we investigated the spatiotemporal dynamics of object recognition processing under uncertainty in the human brain by combining MEG (n=24, 15 females) and 7T fMRI data (n=25, 17 females) obtained from the same visual object recognition task. Participants were instructed to view images of objects presented at the recognition threshold and report the object category and their recognition experience after a short delay. We combined MEG and fMRI data with model-based analysis based on representational similarity analysis, allowing us to track the temporal unfolding of recognition-related signals in brain regions distributed across large-scale brain networks at millisecond resolution. We observed an early, parallel rise of recognition-related signals across ventral visual regions and frontoparietal cortices (110-120 ms following stimulus onset), with temporal dynamics and representational formats varying across regions. Recognition-related effects in ventral visual regions were relatively transient in time and best explained by a two-state representational format whereby brain activities associated with both recognized and unrecognized images were confined in two independent, relatively constrained representational subspaces, respectively. In contrast, recognition-related effects in frontoparietal regions were more stable and remained evident throughout the delay period. They were primarily driven by a representational structure whereby activities associated with the recognized images were confined in a relatively restrained subspace, whereas activities associated with the unrecognized images were more variable and distributed over a larger subspace. Together, our results reveal how neural activities underlying object recognition under uncertainty unfold across space and time and shed new insights on how the interplay between different processes in widespread cortical regions underlies object recognition under uncertainty.

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Nanosymposium

595. Visual Memory and Categorization in the Cortex of Humans and Non-Human Primates

Location: SDCC 24

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 595.09

Topic: D.06. Vision

Support: NSF Grant #2123069

Title: A connectivity-constrained computational model of the topography of human ventral temporal cortex

Authors: *N. M. BLAUCH[1], M. BEHRMANN[2], D. C. PLAUT[2];
[2]Dept. of Psychology and Neurosci. Inst., [1]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Inferior temporal (IT) cortex of primates is topographically organized, with spatial clusters of selectivity for multiple stimulus domains, including faces, bodies, and scenes, organized along a medial-lateral axis corresponding to the peripheral-foveal layout of earlier retinotopic cortex. In the homologous ventral temporal cortex (VTC) of humans, additional lateral word selectivity is seen, with a relative hemispheric left-lateralization that mirrors the relative (but weaker) right-lateralization of face selectivity. How does this topographic organization emerge, and what factors govern its consistent global layout? Recent computational modeling work using Interactive Topographic Networks has demonstrated that learning under biological constraints on the spatial cost and sign of connections within IT/VTC cortex is sufficient to produce domain-selective clusters. Here, we test whether additionally constrained connectivity with early retinotopic areas and with downstream non-visual areas, in combination with domain-biased viewing conditions and task demands, can produce the global layout of human VTC in a bi-hemispheric model. Retinotopic constraints are modeled by adding a spatial cost on feedforward connections from the polar-coordinate convolutional retinotopy of V4 into posterior VTC within each hemisphere of the model. Viewing conditions are modeled as distributions of relative image size, with scenes viewed at typically larger sizes than words, faces, and objects. Downstream language demands are modeled by an additional left-lateralized "language" system with connectivity restricted to model LH anterior VTC. Analyzing selectivity patterns and the results of model lesions, we find that learning in the model accounts for 1) the retinotopically-constrained layout of domain-selectivity for words, faces, objects, and scenes along a lateral-medial or foveal-peripheral axis, and 2) hemispheric organization in which words are relatively left lateralized and, due to competition with words, faces are relatively but more weakly right lateralized. Analysis of a group of randomly initialized models reveals a consistent group-level layout and laterality. Our work demonstrates how simple connectivity constraints combined with visual task demands can give rise to a consistent global organization of high-level visual representations in human VTC, and paves the way for the modeling of other connectivity constraints, as well as a targeted investigation of factors underlying individual variability.

Disclosures: N.M. Blauch: None. **M. Behrmann:** None. **D.C. Plaut:** None.

Nanosymposium

596. Neuro-Emotional Consequences of Aging

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 596.01

Topic: G.04. Emotion

Support: NIH Grant 501885

Title: Positive affective experience as a potential mechanism of cognitive reserve in older adults

Authors: *M. ANTHONY[1], **A. TURNBULL**[1], **D. TADIN**[1], **F. LIN**[2];
[1]Dept. of Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY; [2]Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Palo Alto, CA

Abstract: "Cognitive reserve" is used to explain the fact that some older adults show little to no cognitive decline despite evidence of brain aging. Current measures of cognitive reserve, including sociodemographic proxies (e.g., socioeconomic status, education) and residual measures of cognition accounting for brain aging, have had limited success predicting age-related longitudinal changes in cognition, and few established mechanisms exist to explain how cognitive reserve protects cognition. One potential understudied mechanism involves positive affective experience (PAE; i.e., one's capacity to maintain positive and stable arousal/valence from day to day). Aging is associated with paradoxical increases in emotional well-being and positive affect, despite declines in episodic memory (EM) and executive function (EF). Longitudinal studies have shown that better PAE is linked to a reduction or even an absence of noticeable cognitive decline in older adults. We examined whether PAE may act as a potential mechanism to explain how some older adults maintain robust cognitive function as they age. Data from a double-blinded intervention design (primarily aimed at understanding brain stimulation effects on mood) was used to examine relationships between PAE (Self-Assessment Manikin ratings of valence and arousal), neurodegeneration (cortical thickness), and cognition (change in EM or EF) in older adults with mild cognitive impairment (N = 39, mean age = 71.5, SD = 7). PAE was assessed at 14 timepoints over one month, with stability and level of PAE defined as SD and mean, respectively; cognition was assessed at baseline and after one-month intervention. Moderation analyses of PAE showed that stability of both arousal (*interaction term*: $t(34) = 3.03$, $p = .0046$) and valence (*interaction term*: $t(34) = 2.49$, $p = .018$) significantly moderated by lessening the negative influence of neurodegeneration on EF but not EM. Findings indicate that PAE may be a novel mechanism for explaining how some older adults maintain cognitive function despite brain aging and may serve as an intervention target for enhancing cognitive reserve.

Disclosures: M. Anthony: None. **A. Turnbull:** None. **D. Tadin:** None. **F. Lin:** None.

Nanosymposium

596. Neuro-Emotional Consequences of Aging

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 596.02

Topic: G.04. Emotion

Title: Effect of online tDCS to left somatomotor cortex on neuropsychiatric symptoms among older adults at risk for dementia

Authors: *A. TURNBULL[1], M. ANTHONY[1], D. TADIN[1], K. HEFFNER[1], A. PORSTEINSSON[1], F. V. LIN[2];
[1]Univ. of Rochester, Rochester, NY; [2]Stanford Univ., Palo Alto, CA

Abstract: Neuropsychiatric symptoms (NPS) are behavioral disturbances prevalent in mild cognitive impairment (MCI) and Alzheimer's disease (AD) that cause significant distress to patients and caregivers, and accelerate functional decline. tDCS is a non-invasive treatment that has shown promising effects for improving NPS in AD and MCI. In the current double-blinded randomized control trial pilot study, we assessed the behavioral and neural effects of a 4-week anodal tDCS intervention targeting left sensorimotor cortex (LSMC) during a visual attention task (compared to sham tDCS during the same task), in 40 older adults (24 females, mean age=71) with MCI. NPS was primarily measured by a composite score of participant-report depression, anxiety, and apathy, as well as using a caregiver-reported questionnaire, at baseline and immediately after intervention. Using GEE models, we found that, although there was no significant group by time interaction, intervention (Wald's X^2 = 3.80, p = .051), but not control (Wald's X^2 = 0.16, p = .69), showed significant improvement in mood immediately after intervention from baseline. Intervention (Wald's X^2 = 2.93, p = .087), but not control (Wald's X^2 = 0.20, p = .65), showed a trend towards a significant decrease in LSMC activation during a visual attention task after intervention from baseline. There was a significant relationship between decrease in LSMC activation and improvement in mood symptoms (Wald's X^2 = 9.20, p = .002). Intervention (Wald's X^2 = 3.13, p = .077), but not control (Wald's X^2 = 2.64, p = .11), had a trend towards significant enhancement in LSMC-amygdala functional connectivity (FC) after intervention from baseline. There was a significant relationship between increase in LSMC-amygdala functional connectivity (FC) and decrease in mood symptoms (Wald's X^2 = 4.72, p = .030). There were no significant findings related to caregiver-reported NPS. Follow-up analyses revealed that results were stronger for left postcentral gyrus activation and FC compared to left precentral gyrus (both regions make up LSMC). Overall, we found tentative evidence that tDCS applied to LSMC in older adults with MCI while they engaged in a visual attention task may improve emotion dysregulation and NPS via changes in LSMC activation and FC to the amygdala. Our findings suggested that patient-reported mood may be more sensitive to these changes than caregiver-reported NPS, and that effects may be stronger for left postcentral gyrus, laying the foundation for follow-up studies with more precise mechanistic investigation and efficacy testing.

Nanosymposium

596. Neuro-Emotional Consequences of Aging

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 596.03

Topic: G.04. Emotion

Support: R01MH127737

Title: Stress affect mouse mPFC activity and the performance in attentional set-shifting task

Authors: *S. MA, Y. ZUO;
Univ. of California Santa Cruz, Santa Cruz, CA

Abstract: Stress, a prevalent problem in modern society, is a well-known risk factor for psychiatric disorders. Stress adversely affects many brain regions, with the prefrontal cortex (PFC) being a major target. The rodent PFC has several subdivisions. Among them, the medial PFC (mPFC) is crucial for cognitive flexibility, the ability to change perspectives or approaches to a problem, flexibly adjust to new demands, rules, or priorities. Previous studies and my data show that stress impairs cognitive flexibility, however, little is known about how stress affect mPFC neuronal coding, and how such effects contribute to stress induced defects in cognitive functions. To test the cognitive flexibility, I use attentional set-shifting task (AST), a two-choice discrimination task consisting of several sessions. Among different sessions, the extradimensional shift (EDS) session requires the animal to shift between different sensory modalities to make decisions. I found restraint stress (RS) treatment cause impaired EDS and decreased mPFC neuronal activity. Excitatory neuron activity in mPFC is very important for EDS. Optogenetic activate mPFC excitatory neurons improve cognitive flexibility evidenced by decreased trial numbers to finish EDS. Additionally, increase mPFC inhibition by optogenetic activating Parvalbumin+ (PV+) interneuron impair EDS. Then we looked at mPFC calcium (Ca) activity in individual cell level and found mPFC excitatory neuronal activity patterns are highly informative of behavioral variables. We hypothesis stress causes decreased excitatory neuron activity and then disrupts the neuronal coding and leads to impaired EDS. We are currently working on comparing stress and non-stressed mice neuronal coding at mPFC during EDS. As stress is also involved in many pathologies and aging, with mPFC shows significant changes and cognitive flexibility declines, thus, we plan to investigate how stress affects cognitive flexibility under pathological conditions and aging in the future.

Disclosures: S. Ma: None. **Y. Zuo:** None.

Nanosymposium

596. Neuro-Emotional Consequences of Aging

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 596.04

Topic: G.04. Emotion

Title: Factors related to subjective well-being and responsible molecules interacting the brain and the locomotor system

Authors: *Y. INADA, C. TOHDA;
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Abstract: Subjective well-being (SWB) is an important research topic being addressed from a variety of perspectives, including philosophy, psychology, sociology, economics, public health, and medicine. High SWB has been shown to correlate with longevity and healthy state. Several studies suggested that factors influencing SWB were at least physical activity, cognitive function, and social resource. The fact that SWB, which is an emotional issue, is influenced by physical activity and cognitive activity, and vice versa SWB affects physical activity and cognitive activity, suggests that the locomotor system and cognitive function are closely related to mental health. However, the molecular basis of these interactions has not been clarified at all. We hypothesized that some molecules responsible for these interactions are transferred from the locomotor system to the brain or from the brain to the locomotor system. This clinical study aimed to investigate what are related activities to SWB, and find molecules responsible for controlling SWB from the blood circulation.Subjects were healthy elderly people over 65 years old who have no functional troubles in daily life. Evaluation items were (1) SWB, (2) lifestyle, (3) cognitive function (CF), (4) motor function (MF) and (5) daily activity (DA). Based on all points in those items, Structural Equation Modeling was conducted to clarify the relationship among them. The most fit model showed that CF, MF, lifestyle and DA are needed to explain SWB. Next, to elucidate features of elder people with high SWB, subjects were divided by their SWB scores and other activities patterns. As a results, scoring patterns were divided into 4 groups. High SWB was associated with high scores in CF, MF and DA. Plasma samples of typical subjects in those 4 groups were served for tandem mass tag-based quantitative proteomics. Comprehensive analysis of responsible molecules in plasma for controlling high SWB are under investigation.There has been no molecular explanation of why physical activity, cognitive activity, and social activity affect SWB. The present study has the potential to answer the question and to provide a new perspective for health and longevity research with significant impact for medical, psychological, and social sciences.

Disclosures: Y. Inada: None. **C. Tohda:** None.

Nanosymposium

596. Neuro-Emotional Consequences of Aging

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 596.05

Topic: G.04. Emotion

Title: Trait Empathy and Emotional Reactions to a Virtual Adaptation of the Trier Social Stress Test in Older Adults

Authors: *J. BEADLE[1], D. E. WARREN[2];
[1]Dept. of Gerontology, Univ. of Nebraska at Omaha, Omaha, NE; [2]Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Older adults experience aging-related brain and motivational changes that may affect how they respond to social stressors in late life, such as making medical decisions about a loved one. Individual differences in social cognition may affect how older adults react to various life stressors, with some factors relating to greater resilience. Yet, it is not known the degree to which older adults' empathy relates to their emotional reactions to a social stressor in real time in a virtual setting. The present study investigated relationships between trait empathy (i.e., empathy as a general tendency), and state emotion (i.e., in the moment emotional responses) to a social stressor using a virtual adaption of the well-validated Trier Social Stress Test (TSST). Participants included 35 older adults (Mage=69.83) who did not report a history of mental illness or neurological disease. The study entailed undergoing a virtual adaptation of the TSST through the internet at their residence. Participants' state emotion prior to, during, and after the TSST was measured using a modified version of the Positive and Negative Affect Schedule (PANAS). A trait measure of empathy (the Interpersonal Reactivity Index-IRI) was also completed. We examined the three main subscales of the IRI which included Empathic Concern (e.g., compassion), Perspective Taking (e.g., understanding others' mental states), and Personal Distress (e.g., feelings of anxiety/distress in response to others' suffering). We investigated the relationship between trait empathy and state emotion in response to the TSST. We found that older adults with higher trait personal distress reported higher levels of state stress/anxiety (p<.01) and sadness (p<.01) during the TSST. Furthermore, older adults with higher trait perspective taking reported lower feelings of state sadness (p<.05) during the TSST. Finally, we found that older adults with higher trait empathic concern reported greater feelings of state sympathy and compassion (p<.05) during the TSST. Our results suggest that in older adults, individual differences in trait empathy are related to in the moment emotional reactions to a social stressor. Future studies may examine the degree to which these responses relate to real-world emotional reactivity to social stressors in older adults.

Disclosures: J. Beadle: Other; I serve as a Section Editor on the Journal of Current Behavioral Neuroscience Reports. I have received an annual honorarium fee for serving as a Section Editor for the Journal.. **D.E. Warren:** None.

Nanosymposium

596. Neuro-Emotional Consequences of Aging

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 596.06

Topic: G.04. Emotion

Title: Maintaining factors for loneliness in later life: exploration of mechanisms in a clinical trial

Authors: *K. A. VAN ORDEN, A.-T. NGUYEN;
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Abstract: Background: Loneliness and isolation in later life are associated with cognitive impairment and dementia, while social connection is associated with healthy brain aging. Community programs to foster social connection are widely available (e.g., senior centers, friendly calling, volunteering) but are under-utilized, even when barriers to participation are removed (e.g., cost, transportation). While transient loneliness in response to life transitions is normative (e.g., bereavement, retirement, relocation) and most often motivates social behavior to reduce loneliness, when the experience of loneliness becomes chronic, it sets in motion a cycle of hypervigilance to social threat and social withdrawal that exacerbates and maintains loneliness over time, including by preventing social behaviors that would reduce loneliness. Our premise is that one mechanism whereby chronic loneliness is associated with unhealthy brain aging by creating a self-propagatory loop in which greater severity and duration of loneliness increases the likelihood that a lonely older adult will decline to engage in interventions to reduce loneliness. Our study examines this hypothesis in a clinical trial in which lonely older adults were randomly assigned to 12 months of either an AmeriCorps Seniors volunteering program or active control (self-guided life review). **Methods:** 291 older adults were randomized and followed for one year. Loneliness was assessed via the De Jong Gierveld Loneliness Scale. Cognition was assessed by the Montreal Cognitive Assessment. **Results:** Results indicated high rates of non-compliance in line with low utilization in community agencies: only half of subjects engaged in assigned interventions for the full year, with 56% for volunteering and 45% for active control. Most subjects completed study assessments at 1-year follow-up, allowing us to examine predictors of non-compliance with interventions. We found that greater loneliness (6.37, std 3.07 vs. 5.60, std 2.74, p<.05) and lower cognitive performance (MoCA; 22.21, std 3.53 vs. 23.36, std 3.44, p<.05) at baseline were both associated with reduced likelihood of engaging in study interventions. **Discussion:** Loneliness and its correlates in later life, including cognitive impairment, may serve as maintaining factors for loneliness by reducing likelihood of engaging in social approach behaviors. Future work will examine neural circuitry that may underlie a feedback loop between cognitive decline and loneliness, including the potential role of executive functioning to disrupt the loop and promote social behaviors.

Disclosures: K.A. Van Orden: None. **A. Nguyen:** None.

Nanosymposium

596. Neuro-Emotional Consequences of Aging

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 8:00 AM - 9:45 AM

Presentation Number: 596.07

Topic: G.06. Anxiety Disorders

Title: Aberrant model-based learning in Obsessive-Compulsive Disorder is normalized after laser capsulotomy

Authors: *P. M. LAURO[1], D. D. LIU[2], M. A. SHERIF[3], B. D. GREENBERG[3], S. A. RASMUSSEN[3], N. C. R. MCLAUGHLIN[3], W. F. ASAAD[4];
[1]Neurosci., [2]Alpert Med. Sch., [3]Psychiatry and Human Behavior, [4]Neurosurg., Brown Univ., Providence, RI

Abstract: Background: Obsessive-compulsive disorder (OCD) is characterised in part by aberrant model-based learning (fears persisting despite no negative external outcomes). Surgery can be effective for treatment-refreactory OCD but its mechanisms are unclear.
Methods: To understand if clinical improvement in OCD symptoms (Yale-Brown Obsessive-Compulsive Scale - YBOCS) was tied to changes in learning strategies, patients performed a virtual spatial navigation task before and after ablation of the internal capsule. At each timepoint subjects performed two blocks within the same maze. The first "unobstructed" block allowed subjects to freely explore while the second "obstructed" block removed paths based on the subject's previous preferred choices to probe whether subjects could find a new optimal path. In both conditions, subjects were instructed to navigate to a reward location in the fewest number of moves.
Task performance was quantified by the number of moves above the shortest distance, and was compared to 6 age-matched controls (3 F) using a 2x2 mixed ANOVA (OCD status, timepoint). To disentangle model-free and model-based learning from task behavior, SARSA reinforcement learning models were fit to behavioral data. Model-free (learning rate) and model-based parameters were also compared across timepoints using a 2x2 mixed ANOVA. To understand how surgical lesions influenced clinical outcomes and behavioral changes, lesion volumes were mapped to preoperative 256-direction diffusion tensor imaging.
Results: Six patients (1 F) between the ages of 21 and 53 with treatment-refractory OCD underwent capsulotomy (pre- v. post-surgical YBOCS: 31.2 ± 3.7 v. 22.5 ± 8.6, paired T-test: T = 2.77, p = 0.039). Patients with OCD took a greater number of moves to reach the target (p = 0.014). Both model-free (learning rate) and model-based parameters were decreased between patients and controls (p < 0.044). Exploratory pairwise T-tests of ANOVA models revealed a significant OCD x timepoint interaction, with post-surgical OCD subject performance and model-based learning becoming indistinguishable from controls (p < 0.022).
YBOCS improvement with surgery correlated with lesions intersecting with tracts projecting to the right hippocampi and middle temporal lobe (p < 0.033), whereas increased model-based learning parameters trended with increased tracts projecting to left lateral orbitofrontal cortex (p = 0.066).
Conclusions: Model-based parameters were modified by surgery, with an apparent improvement in task performance. Surgical intervention may alleviate OCD symptoms by disrupting pathological model-based learning mediated by fronto-temporal networks.

Disclosures: P.M. Lauro: None. **D.D. Liu:** None. **M.A. Sherif:** None. **B.D. Greenberg:** None. **S.A. Rasmussen:** None. **N.C.R. McLaughlin:** None. **W.F. Asaad:** None.

Nanosymposium

597. Perception and Behavior in Human Social Interactions

Location: SDCC 25

Time: Wednesday, November 16, 2022, 8:00 AM - 10:30 AM

Presentation Number: 597.01

Topic: H.06. Social Cognition

Support: National Natural Science Foundation of China 32022031

Title: Unfolding social interaction perception over time in the human brain

Authors: *Q. LIANG, C.-Y. TIAN, J.-Y. CHENG, S.-G. KUAI;
The Sch. of Psychology and Cognitive Sci., East China Normal Univ., Shanghai, China

Abstract: Humans are social animals, excelling in integrating clues to recognize interacting others. Previous fMRI studies mainly compared typical interacting and non-interacting situations and found a series of brain regions supporting social interaction perception. Due to the limitation of simple comparison methodology and temporal resolution of fMRI, how these regions play different roles in utilizing cues and unfolding social interaction information remains unclear. In this study, we altered spatial cues to quantitatively investigate social interaction perception and used MEG to capture neural dynamics. Participants were asked to watch images of two virtual humans standing in different spatial relationships and judge whether they were interacting or not while a 306-channel MEG device recorded their neural activities. Stimuli were screenshots in a gray-background virtual reality environment, where the participant was observing 7m away from two 1.7m height virtual humans. The interpersonal distance between them changed at 4 levels from 1 to 6m, and the heading orientation of one virtual human changed at 6 levels from 0 to 75° while the other one kept heading toward the virtual human, resulting in 24 conditions. In each trial, the stimulus was presented for 1000ms and the inter-trial interval was randomized from 500 to 700ms. Behavioral results showed that participants were more likely to judge virtual humans standing close and facing each other as interacting. But when the interpersonal distance was too close or too far, heading orientation played a little role in social interaction judgments. A sensor level RSA combined with further sourcing analysis of neural data showed that distance information was read out at 50ms after stimulus onset in V1, heading orientation was decoded at around 400ms in fusiform gyrus, lateral occipital cortex and superior temporal sulcus (STS), and the probability of judging as interacting was represented at 600ms in STS, parietal areas and prefrontal cortex. Since orientation was more important in ambiguous distance conditions, we calculated the RDM of entropy provided by orientation in each distance condition and decoded them from neural activities. Interestingly, the influence of orientation was represented at about 120ms in fusiform gyrus, lateral occipital cortex and prefrontal cortex, quickly after distance was decoded. Connectivity analysis showed a top-down influence from prefrontal cortex to ventral visual areas at 120ms. These results indicate that the human brain perceives others' social interaction by utilizing cues optimally under the top-down control of prefrontal cortex.

Disclosures: Q. Liang: None. **C. Tian:** None. **J. Cheng:** None. **S. Kuai:** None.

Nanosymposium

597. Perception and Behavior in Human Social Interactions

Location: SDCC 25

Time: Wednesday, November 16, 2022, 8:00 AM - 10:30 AM

Presentation Number: 597.02

Topic: H.06. Social Cognition

Title: Naturalistic sitcom-viewing fMRI paradigm elicits reliable and ecologically valid BOLD responses for humor comprehension and appreciation

Authors: *M. PRENGER[1,2,3], K. VAN HEDGER[2,3], K. N. SEERGOBIN[3], A. M. OWEN[2,3,4], P. A. MACDONALD[2,3,5];
[1]Neurosci., [2]BrainsCAN, [3]Western Inst. for Neurosci., [4]Departments of Physiol. & Pharmacol. and Psychology, [5]Clin. Neurolog. Sci., Univ. of Western Ontario, London, ON, Canada

Abstract: Humor is a cognitively complex phenomenon that promotes social and personal well-being. However, its scientific investigation in humans (e.g., with functional magnetic resonance imaging; fMRI) has been limited by a reliance on behavioral responses and low ecological validity. Our objective was to validate and replicate a naturalistic sitcom-viewing paradigm for humor processing introduced by Moran *et al*., (2004) using 3 Tesla fMRI. Healthy, young participants ($n = 20$; $M_{age} = 22.80$) underwent fMRI to evaluate blood-oxygen-level-dependent (BOLD) responses to full episodes of the sitcom *Seinfeld*. Humor comprehension (i.e., "getting the joke") was defined by 2-second epochs immediately preceding the onset of laughter in the sitcom's laugh track. Humor appreciation (i.e., subjective amusement) was defined by variable-length epochs lasting the full duration of laugh track laughter. These events were individually contrasted with the remainder of the episode in subject-level analyses. Following this, group-level, whole-brain analyses revealed significant BOLD activation (corrected for multiple comparisons; family-wise error [FWE] $p < .05$, $k = 10$ consecutive voxels) in the bilateral superior temporal gyrus (STG), the bilateral middle temporal gyrus (MTG), the left inferior frontal gyrus (IFG), and the right temporal pole during humor comprehension. These areas are consistent with previous studies of humor comprehension (including Moran *et al*., 2004) and are thought to be involved in language processing (IFG), problem solving and insight (STG), ambiguity resolution (MTG), and theory of mind (temporal pole). This aligns well with the theory that humor comprehension relies on resolving incongruities that contradict the brain's predictions. For humor appreciation, significant BOLD activation (FWE$p < .05$, $k = 10$) was found in the bilateral middle occipital gyrus, the bilateral precuneus, the left inferior occipital gyrus, the left inferior parietal lobule, the left cuneus, the right posterior insula, the right thalamus, the right posterior cingulate cortex, and the right amygdala. Again, these areas are consistent with those reported in previous literature and align with the notion that humor appreciation activates regions involved in emotional processing and reward (e.g., insula, amygdala). Overall, these findings suggest that this naturalistic sitcom-viewing paradigm invokes reliable and valid BOLD responses and can facilitate the investigation of humor

processing in humans, particularly in clinical populations in which eliciting behavioral responses might be difficult (e.g., cognitive impairment, locked-in syndrome).

Disclosures: M. Prenger: None. **K. Van Hedger:** None. **K.N. Seergobin:** None. **A.M. Owen:** None. **P.A. MacDonald:** None.

Nanosymposium

597. Perception and Behavior in Human Social Interactions

Location: SDCC 25

Time: Wednesday, November 16, 2022, 8:00 AM - 10:30 AM

Presentation Number: 597.03

Topic: H.06. Social Cognition

Support: The Royal Society
             The Academy of Medical Sciences
             China Scholarship Council

Title: Does laughter make things funnier? An fMRI study from a neurodiversity perspective.

Authors: *Q. CAI[1], N. LAVAN[2], S. CHEN[1], S. J. GILBERT[1], S. J. WHITE[1], S. K. SCOTT[1];
[1]UCL Inst. of Cognitive Neurosci., UCL Inst. of Cognitive Neurosci., London, United Kingdom; [2]Dept. of Biol. and Exptl. Psychology, Sch. of Biol. and Behavioural Sciences, Queen Mary Univ. of London, London, United Kingdom

Abstract: Laugher can either be genuine emotional vocalizations or serve as social signals in daily interaction. A previous neuroimaging study found that neurotypical adults automatically engages high-level cognitive skills, such as mentalizing ability, to understand and interpret the intention and meaning behind laughter. Intriguingly, the social and emotional meaning of laughter is processed implicitly by participants: genuine laughter has been found to amplify the funniness of jokes more than posed laughter amongst both neurotypical and autistic adults. However, there have been no studies researching whether autistic adults have a different neural mechanism of implicit laughter processing relative to neurotypical adults and, if so, how it relates to their mentalizing difficulties. To address the above questions, we asked autistic and neurotypical adults (comparable for age, gender and IQ) to passively listen to funny words paired with genuine laughter, social laughter or non-emotional non-contagious human vocalizations in a fMRI study. In a preliminary analysis (6 Autism and 6 NTs; sample size to increase), we found NT participants showed greater activation in the medial prefrontal cortex and several regions in sensorimotor cortex than autistic adults during words paired with social laughter versus words paired with genuine laughter. We will apply a region of interest (ROI) analysis on an implicit mentalising localizer to further explore how implicit laughter processing relates to mentalizing ability. Parametric modulation will also be conducted to explore brain-behaviour associations. Overall, our preliminary results show neural differences between autistic adults with high IQs and neurotypical controls during implicit laughter processing, and indicates the medical

prefrontal cortex and sensorimotor cortex to be crucially involved in the implicit processing of social laughter.

Nanosymposium

597. Perception and Behavior in Human Social Interactions

Location: SDCC 25

Time: Wednesday, November 16, 2022, 8:00 AM - 10:30 AM

Presentation Number: 597.04

Topic: H.06. Social Cognition

Title: Neural correlates of social information processing and gossip

Authors: *J. LEE[1,2], Y. SONG[1], J. JEONG[1,2];
[1]Bio and Brain Engin., [2]Program of Brain and Cognitive Engin., KAIST, Daejeon, Korea, Republic of

Abstract: Gossip, a conversation between two people sharing social information about an absent person (i.e., a target), is a ubiquitous form of social interaction easily found in daily life. Gossip is generally considered a malicious behavior that violates solidarity norms within a group or community; at the same time, studies have suggested that gossip serves important functions such as social control and social bonding. Although gossip is a clear example of decision-making that has reasons for it (e.g., social control and social bonding) and against it (e.g., risks of breaking solidarity norms and retaliation from the gossip's target), there is a limited number of studies attempting to find neural evidence that gossiping involves such benefit-cost frameworks. Here, we used fMRI techniques to investigate how the brain processes various types of social information and decision variables (i.e., benefits and costs) for gossip. To systematically generate input stimuli varying in traits, we identified three basic building blocks of social information: target, content, and valence. With the data obtained from 50 participants, we performed a series of contrast analyses and found specific areas showing greater activities for particular types of information: bilateral occipital pole for gossip about morality and social norm, precuneus and angular gyrus for daily social affairs, posterior cingulate gyrus and frontal pole for ingroup friends, lateral PFC and dmPFC for negative events, etc. Next, we performed conjunction analysis to find if there is any area specialized for processing costly gossip. We found dmPFC showing greater activity when the information that may cause negative consequences in the future (e.g., a possible social conflict between the sender and the target after gossiping) was given, suggesting the area's involvement in the cost processing of gossip. Finally, to find an area processing benefits of gossip, we calculated gossip spreading rates that represent

the total benefit (or estimated value) of taking the action. We found vmPFC showing correlated activities, which suggests its role in processing total benefit for gossip decisions. Our results provide neural evidence that there are dedicated areas for processing specific types of social information and that gossip involves benefit and cost processing, which is a key feature of value-based decision making. The evidence of such decision variable processing also suggests the presence of a system for integrating these signals in gossip decision-making, and research towards identifying such processes in the brain may be an interesting point for further study.

Disclosures: J. Lee: None. **Y. Song:** None. **J. Jeong:** None.

Nanosymposium

597. Perception and Behavior in Human Social Interactions

Location: SDCC 25

Time: Wednesday, November 16, 2022, 8:00 AM - 10:30 AM

Presentation Number: 597.05

Topic: H.06. Social Cognition

Title: Sensitivity to the action and attention cues of others in autistic and non-autistic observers

Authors: *B. CHOUINARD[1], A. PESQUITA[3], J. ENNS[4], C. S. CHAPMAN[2];
[2]Physical Educ. and Recreation, [1]Univ. of Alberta, Edmonton, AB, Canada; [3]Univ. of Birmingham, Birmingham, United Kingdom; [4]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Previous work indicated that Observers had a quicker response to another person's pointing if the Pointer had chosen where to point as opposed to being directed where to point (Pesquita, Enns, Chapman, 2016). Notably, the size of the effect, referred to here as the Locus of Decision effect (LoDe), was smaller for those with higher scores on a measure of autistic-like traits and tendencies (i.e., Autism Spectrum Quotient). Here, we expanded the original study by (i) evaluating the effect online; (ii) having an additional observer group of autistic individuals; and (iii) collecting mouse trajectories of Observers. All participants (Observers) watched video clips of a person pointing and were instructed to 'beat the Pointer', by moving their mouse to the same side that the Pointer was going to point to, faster than the Pointer in the video. For half of the clips the Pointer had been directed where to point, and half were self-chosen. After 8 practice trials, each observer watched 100 clips of each of 3 different Pointers. The order in which Pointers were viewed was randomized, as was the order of each Pointer's clips. Response times and mouse trajectories were collected for all 300 trials. Of the 83 online no-autism Observers (no-AUT), 31 used a trackpad, which is not amenable to mouse trajectory analysis, and three were ineligible, leaving a total of 47 no-AUT datasets for analysis. Thus far, nine online autistic Observers have completed the study, with data collection ongoing. Analysis of the entire response time (i.e., from when the video appeared until when the mouse reached the target)

indicated presence of the LoDe effect in the no-AUT as well as the AUT group. However, for mouse trajectory analysis, response time is separated into: 1) reaction time, which is the time between video appearing and Observer beginning their mouse movement; and 2) movement time (MT), which is the time from initiation of mouse movement to mouse entering target area. Both groups exhibited LoDe effect for MT, but there was an absence of the LoDe effect for reaction time unique to the AUT group. Thus, although the autistic participants are picking up the subtle differences between the points, this effect does not emerge until their mouse begins moving toward the target. To summarize: (i) we successfully replicated this simple yet elegant design in an online setting; (ii) we, somewhat surprisingly, found that autistic individuals exhibit an implicit sensitivity to the locus of control of the decision making of others; and (iii) our finer-grained mouse trajectory data suggested that in autistic participants, detection of the effect was only possible once the movement portion of their response began.

Disclosures: B. Chouinard: None. **A. Pesquita:** None. **J. Enns:** None. **C.S. Chapman:** None.

Nanosymposium

597. Perception and Behavior in Human Social Interactions

Location: SDCC 25

Time: Wednesday, November 16, 2022, 8:00 AM - 10:30 AM

Presentation Number: 597.06

Topic: H.06. Social Cognition

Title: Neural processing of social gaze cueing in typical and ASD adults

Authors: *T. PARKER[1], X. ZHANG[2,3], J. A. NOAH[2], M. KELLEY[1], J. MCPARTLAND[4], J. HIRSCH[2,5];
[1]Interdepartmental Neurosci. Program, Yale Sch. of Med., Hamden, CT; [2]Dept. of Psychiatry, Yale Sch. of Med., New Haven, CT; [3]Yale Univ., New Haven, CT; [4]Yale Child Study Ctr., New Haven, CT; [5]Dept. of Med. Physics & Biomed. Engin., Univ. Col. London, London, United Kingdom

Abstract: Gaze cueing adjustably orients the perceiver's attention to a specific external location and is a form of joint attention. In typically-developed (TD) individuals, gaze-directed attentional orientation is associated with responses in the dorsal parietal and medial prefrontal cortices.[1,2] Atypical variations in social cognitive processing of gaze, contrarily, are symptomatic features of autism spectrum disorder (ASD) that may indicate aberrant joint attention mechanisms.[3] More recently, we have begun to examine live face-to-face joint attention.[4] Here, we examine the role of live two-person joint attention in ASD and TD groups to test the hypothesis that dorsal parietal brain activity is related to gaze cueing using functional near-infrared spectroscopy (fNIRS; Shimadzu LABNIRS) and eye-tracking (Tobii Pro X3-120). Twenty-five TD and 19

ASD participants engaged in a gaze cueing task in which an initiator (robot or human) used eye gaze to direct the participant's eyes to one of two circular targets on a glass partition. During the gaze cueing task, the initiator looked at the participant's eyes for two seconds and then averted their gaze to a dot (left or right) for two seconds. The participant's task was to follow the initiator's gaze. Eye following accuracy was >99% for both diagnostic groups across both conditions. A direct neural comparison of TD and ASD findings indicated that neural activity in the human gaze cueing condition increased in the right somatosensory association cortex, right visual association cortex, and right frontal eye fields for TD > ASD (Fig 1, red). In contrast, in ASD, neural responses to human gaze cueing were observed in the left angular gyrus, left somatosensory cortex, and right superior temporal gyrus (Fig 1, blue). These findings suggest that gaze cue processing modulates right dorsal parietal and frontal neural systems related to joint attention mechanisms in TD. However, ASD participants elicited atypical left functional lateralization to human gaze, consistent with a model of alternative neural processing systems for attention to faces and eyes.[5]
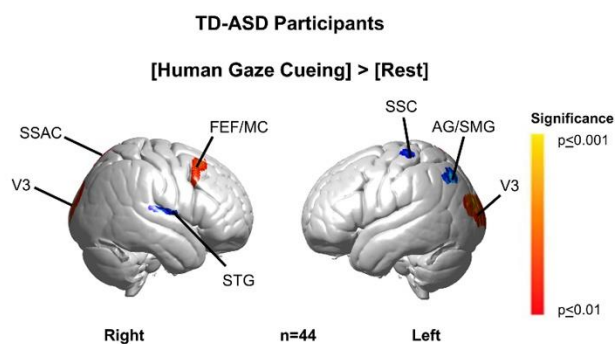

Figure 1. Contrast comparison [Human Gaze Cueing] > [Rest], typically-developed (TD) participants relative to autism spectrum disorder (ASD) participants. TD participants (red clusters) showed comparatively greater activation in right dorsal somatosensory association cortex (SSAC); right visual association cortex (V3); and right frontal eye fields and pre-motor cortex (FEF/MC), while heightened activity was observed for ASD (blue clusters) participants in right superior temporal gyrus (STG); left somatosensory cortex (SSC); left angular gyrus and supramarginal gyrus (AG/SMG).

Disclosures: T. Parker: None. **X. Zhang:** None. **J.A. Noah:** None. **M. Kelley:** None. **J. McPartland:** None. **J. Hirsch:** None.

Nanosymposium

597. Perception and Behavior in Human Social Interactions

Location: SDCC 25

Time: Wednesday, November 16, 2022, 8:00 AM - 10:30 AM

Presentation Number: 597.07

Topic: H.06. Social Cognition

Title: Neural correlates of emergent prosocial behavior during dynamic human group formation

Authors: *W. ZAJKOWSKI[1], **R. BADMAN**[1], **M. HARUNO**[2], **R. AKAISHI**[1];
[1]RIKEN Inst., RIKEN Brain Sci. Inst. - Wako, Wako-shi, Japan; [2]Natl. Inst. of Information and Communication Technol., Natl. Inst. of Information and Communication Technol., Osaka, Japan

Abstract: Probing the emergent changes in cognition as group sizes increase is vital for understanding the evolution of large cooperative societies. Larger group sizes often require complex self-versus-other trade-offs, as well as possibly greater mental capacity to process than smaller groups or dyads—issues which have been rarely studied in social neuroscience. Specifically, to date, there has been few studies exploring how dynamic cognitive changes associated with group size increases and decreases affect cooperation within economic decision making experiments, as group size is typically fixed rather than modulated within-session. We thus deploy a novel, social network-embedded-dyad version of the classic iterative prisoner's dilemma (PD) task to study how preference for cooperation changes, within-subject, as a function of group size ($N=87$ for behaviour, $N=26$ for fMRI). Each trial consisted of a two-way PD game with one randomly chosen group member. New group members were added every ~5-10 trials in 180-trial session (up to a group size of 5 partners), but both the subject and current partner could unilaterally break ties on select trials. Being in larger groups is assumed to affect both memory and social behavior, which are examined by behavioral and neural analyses. Subjects consistently followed a well-performing decision policy (*tit-for-tat, TFT*), in which players imitate the prior choice of the current partner from their previous interaction, suggesting subjects could strategically track and respond to multiple partners even in larger groups and over multi-trial timescales. However, subjects became more forgiving as group size increased. with higher cooperation rates that resulted in larger group sizes being maintained from partners breaking social ties less (despite defection being more optimal for score). Response time (RT) also increased with group size, suggesting larger group sizes required more mental capacity to process, while there was a default preference shift from faster RT for defect in dyads to faster RT for cooperate in larger groups. Larger group size was associated with deactivation in the precuneus, a complex brain region with both social and memory functions, as well as components of the dorsal attention network. Choice (e.g. TFT) and new partners each correlated with changes in possibly memory-related hippocampal activation and functional connectivity within social and salience networks. Overall, humans seem to default to more cooperative strategies partly due to intrinsic preference for larger group sizes, and this prosocial behaviour transition may be governed by the brain's social memory systems.

Disclosures: W. Zajkowski: None. **R. Badman:** None. **M. Haruno:** None. **R. Akaishi:** None.

Nanosymposium

597. Perception and Behavior in Human Social Interactions

Location: SDCC 25

Time: Wednesday, November 16, 2022, 8:00 AM - 10:30 AM

Presentation Number: 597.08

Topic: H.06. Social Cognition

Title: Cortical correlates of inhibition when observed by synchronised vs. non-synchronised peers

Authors: *R. MOFFAT[1,2], N. CARUANA[1,2], E. S. CROSS[1,2,3];
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Abstract: Response inhibition can improve under a non-judgemental watchful eye and, in the longer-term, through training. Dyads who engage in synchronising motor activities (such as mirroring each other's body movements) can reap collaborative, cognitive, and social benefits. It stands to reason that the level of synchrony between an observer and the observed may improve the observed individual's ability to inhibit motor responses, offering a potential therapeutic tool for inhibition training. To determine if a high degree of motor synchrony with an observer improved inhibition while being observed, we compare behavioural and cortical measures of inhibition among synchronised and non-synchronised dyads. In this preregistered study, 60 participants were assigned to either the synchronised or non-synchronised group (n=30 each). Both groups completed a 5-minute seated movement task facing a peer-confederate, which was video recorded for off-line quantification of synchrony. In the synchronised group, the dyad was instructed to mirror each other's hand movements as closely as possible, with each member leading one turn. In the control group, each member of the dyad took a turn moving their arms while the other observed. To assess the influence of synchrony on general cognitive inhibition while being observed, all participants completed a go/no-go task while the confederate remained in the room observing them. We recorded reaction times and error rates, as well as cortical responses from the inferior frontal and pre-frontal cortical regions with functional near-infrared spectroscopy (fNIRS). Preliminary analyses suggest that relative to the non-synchronised group, the synchronised group shows reduced haemodynamic activity in frontal cortical regions, despite little-to-no difference emerging in error rates or reaction times for the two groups. We cautiously interpret this as evidence that synchronising with a peer-confederate reduces the cognitive burden of being observed as shown by cortical activity during an inhibition task. These findings from young healthy adults have implications for rehabilitation strategies targeting deficits in inhibition and social skills, which stand to benefit clinical populations such as those with attention-deficit/hyperactive or bipolar disorders.

Disclosures: R. Moffat: None. **N. Caruana:** None. **E.S. Cross:** None.

Nanosymposium

597. Perception and Behavior in Human Social Interactions

Location: SDCC 25

Time: Wednesday, November 16, 2022, 8:00 AM - 10:30 AM

Presentation Number: 597.09

Topic: H.06. Social Cognition

Support: NIH Grant R56MH091113

Title: The posterior cerebellum is recruited when processing social relationship knowledge

Authors: *H. POPAL[1], K. JOBSON[1], Y. WANG[3], M. A. THORNTON[4], I. R. OLSON[2];
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Abstract: We asked whether the posterior cerebellum was involved in understanding the nuances of social relationships. Young adults received an fMRI scan while performing a task that required a decision about which scenario between two individuals was more likely, given their particular social relationship. The task did not include sequential information and working memory load was minimal. Results showed that regions of the "social brain", such as the ventromedial prefrontal cortex, were recruited during this task. In addition, portions of crus I/II were activated. Searchlight RSA showed that crus I/II was specifically sensitive to the "formality" dimension of social relationships (e.g. knowledge about boss-employee relationships). Last, connectivity analyses show that the activated region of the posterior cerebellum was preferentially connected to the social cerebrum. It is possible that inhibitory activity in crus I/II dampens spreading activation in cerebral social knowledge networks, helping to pinpoint appropriate social meaning.

Disclosures: H. Popal: None. K. Jobson: None. Y. Wang: None. M.A. Thornton: None. I.R. Olson: None.

Nanosymposium

597. Perception and Behavior in Human Social Interactions

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Presentation Number: 597.10

Topic: H.06. Social Cognition

Support: ERC-2015-STG-677270
ERC-2016-STG-716974
PLP-2018-152

Title: Socialness matters more than human-likeness when attributing mental states to robots

Authors: *E. S. CROSS[1,2], L. JASTRZAB[3,1], B. CHAUDHURY[1], S. ASHLEY[3], K. KOLDEWYN[3];

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Abstract: In the mid-20th century, Alan Turing formalized the philosophical debate as to whether machines think, a question that continues to captivate many today. Here, we ask what can be thought of as the opposite question: namely, regardless of whether or not robots think, do we perceive robots as having minds of their own? If so, do we primarily base this decision off how human-like a robot looks, or does its *perceived socialness* also matter? We typically and intuitively think of other people as having minds that are different from our own, a skill known as mentalizing. Imaging studies probing brain activity related to this skill identify bilateral temporoparietal junction (TPJ) and medial prefrontal cortex as core nodes of a putative mentalizing network. Prior work examining how people socially interact with different robots suggests that these mentalizing regions show increasingly robust responses the more human-like a robot appears. In the present preregistered study, we sought to replicate this finding, while also asking the extent to which perceived socialness, defined here by participant perceptions of a robot's behavior, shapes mentalizing network engagement. Forty healthy participants underwent fMRI while playing a rock-paper-scissors (RPS) task against a computer, a small mechanoid robot (Cozmo), a humanoid robot (Pepper), and a human. Importantly, while Pepper looked more humanlike, Cozmo was rated higher on fun, competitiveness and sympathy (all measures of socialness), based on its responses to winning or losing the RPS task. fMRI revealed that linear models evaluating a partner's humanness (computer < Cozmo < Pepper < human) reliably captured percent signal changes within core mentalizing regions (including bilateral TPJ, middle frontal gyrus and precuneus), but linear models evaluating socialness (computer < Pepper < Cozmo < Human) more robustly captured engagement of these same mentalizing regions. Findings suggest that while human-likeness matters when thinking about robot minds, a robot's perceived socialness might even more strongly impact mind attribution and social engagement. Incorporating knowledge-based social cues into robots designed to socially engage humans thus has potential to bolster social relationships with our robotic partners.

Disclosures: E.S. Cross: None. **L. Jastrzab:** None. **B. Chaudhury:** None. **S. Ashley:** None. **K. Koldewyn:** None.

Nanosymposium

598. Genomics and Transcriptomics in Health and Disease

Location: SDCC 23

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 598.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant HG011513-01

Title: A transcriptome-wide association study reveals human endogenous retroviruses as novel risk factors for schizophrenia

Authors: *R. R. R. DUARTE[1], M. L. BENDALL[2], M. M. ROUGVIE[2], O. PAIN[1], C. E. ORMSBY[3], G. A. BECKERLE[2], S. SELVACKADUNCO[1], C. TROAKES[1], G. REYES-TERÁN[3], K. A. CRANDALL[4], D. P. SRIVASTAVA[1], D. F. NIXON[2], T. R. POWELL[1]; [1]King's Col. London, London, United Kingdom; [2]Weill Cornell Med., New York, NY; [3]Ctr. for Res. in Infectious Dis., Mexico City, Mexico; [4]The George Washington Univ., Washington, DC

Abstract: Background: Human endogenous retroviruses (HERVs) correspond to DNA that originated from the infection of germ cells with ancient retroviruses during evolution. Most HERVs are assumed to be regulatory, but many contain remnants of retroviral genes previously used for replication (Gag, Pol, or Env), which have transcriptional and protein-coding potential. Population studies of schizophrenia highlight the substantial contribution of risk attributed to the non-coding genome, where HERVs are located. Despite previous reports suggesting associations with schizophrenia, HERVs have been ignored in genomic studies due to methodological limitations associated with their repetitive nature and incomplete annotation.
Methods: We created TWAS weights for the FUSION pipeline using RNA-sequencing data from post-mortem dorsolateral prefrontal cortex tissue (DLPFC) from the CommonMind Consortium datasets (N = 910). We quantified gene and HERV expression using kallisto (Bray et al., 2016) and Telescope (Bendall et al., 2019), and followed the GTEx guidelines for processing count data. Genotype files were imputed and processed following standard quality control steps (Marees et al., 2018). We performed the TWAS using FUSION, summary statistics from the latest schizophrenia genome-wide association study (Trubetskoy et al., 2022), and the European subset of the 1000 Genomes Project as reference panel. To infer HERV function, co-expression networks were identified using WGCNA (Langfelder et al. 2008), and gene ontology analysis was performed using Webgestalt (Liao et al., 2019).
Results: We observed 5,034 HERVs expressed in the DLPFC. A preliminary analysis suggests at least 150 expression profiles associated with schizophrenia (Bonferroni $P < 0.05$). These included 4 HERVs that were downregulated on chromosome 2q33, 6p21 and 6p22, and 6 that were upregulated on chromosomes 20q13, 8q24, 11p14, 2p13, 6q14, and 6p21. We identified 19 co-expression modules. The HERVs identified in the TWAS belonged to WGCNA modules enriched with gene ontology terms pertaining to the regulation of mitochondrial and synaptic function.
Discussion: Our findings suggest that locus-specific HERVs are subject to cis-regulatory mechanisms associated with schizophrenia risk genotype and that they are co-expressed with genes involved in specialized biological processes. Functional research is now warranted to understand the underlying mechanisms of neuropsychiatric pathology associated with HERV expression in the adult brain.

Disclosures: R.R.R. Duarte: None. **M.L. Bendall:** None. **M.M. Rougvie:** None. **O. Pain:** None. **C.E. Ormsby:** None. **G.A. Beckerle:** None. **S. Selvackadunco:** None. **C. Troakes:** None. **G. Reyes-Terán:** None. **K.A. Crandall:** None. **D.P. Srivastava:** None. **D.F. Nixon:** None. **T.R. Powell:** None.

Nanosymposium

598. Genomics and Transcriptomics in Health and Disease

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Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 598.02

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: R44HG008907
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R44GM116584

Title: A field survey of histone PTM antibodies in epigenomic mapping approaches reveals widespread liabilities: best practices and resources for reliable epigenetic studies

Authors: *A. JOHNSTONE, D. N. MARYANSKI, K. L. RODRIGUEZ, M. C. KEOGH; EpiCypher, Durham, NC

Abstract: Histone post-translational modifications (PTMs) play a critical role in chromatin regulation. It has long been suspected that the poorly understood capability of 'PTM-specific' antibodies (*i.e.* their specificity and efficiency) is a prime driver of the reproducibility crisis in biomedical research. Here we confirm the validity of this concern as it applies to epigenomic mapping studies. Extensive spike-in panels of PTM-defined DNA-barcoded nucleosome standards show that 70% of over 500 commercial antibodies (and over 80% of the most highly cited) to histone lysine methyl and acyl states have failing performance in ChIP (over 20% cross reactivity, less than 5% target recovery; www.ChromatinAntibodies.com). Variable lot behavior (of both polyclonals and monoclonals) shows the danger of focusing on catalog numbers without considering the inherent variability of biological reagents. Ultimately, these studies support the inclusion of *in situ* standards to control genomic mapping assays as an improved path out of this morass. Despite these advances, the application of ChIP-seq for neuroscience research has been hampered by its requirement for high cell inputs and low reliability. The recent development of immunotethering assays, such as Cleavage Under Targets and Release Using Nuclease (CUT&RUN), deliver high signal-to-noise mapping data using a fraction of the required cells compared to ChIP-seq. These innovations enable the application of epigenomics for neuroscience research, particularly for primary cells, brain tissue, and clinical samples where cell numbers are limited. By adapting our nucleosome spike-in control approach for CUT&RUN assays, we show that while the same antibody problems observed in ChIP-seq also pervade CUT&RUN, continuous use of spike-in controls improves assay rigor and reproducibility to realize the potential of CUT&RUN to advance neuroepigenetic research.

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Nanosymposium

598. Genomics and Transcriptomics in Health and Disease

Location: SDCC 23

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 598.03

Topic: I.02. Systems Biology and Bioinformatics

Title: Heroin abuse-associated transcriptional and epigenetic alterations in the orbitofrontal cortex concentrate in GABA interneurons and provide insight into cell type-specific regulatory response

Authors: *J. M. NOSHAY[1], K. A. SULLIVAN[1], A. KOZLENKOV[2,4], A. TOWNSEND[1], G. ROMPALA[2], R. VADUKAPURAM[2], Y. L. HURD[2,3], D. A. JACOBSON[1], S. DRACHEVA[4,2];
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Abstract: Understanding the cell type-specific effects of heroin on chromatin accessibility and gene expression in brain regions controlling decision making can elucidate the biological effects of this potent drug of abuse. Here we generated transcriptomic (RNA-seq) and epigenomic (ATAC-seq) datasets in FACS-sorted nuclei from glutamatergic and GABAergic neurons and oligodendrocytes from autopsied orbitofrontal cortex, a prefrontal cortex region implicated in impulse control, goal-direction, and addiction behaviors. Tissue was collected from subjects who died of heroin overdose and controls (n = 30/cell type/condition) to assess heroin-specific (epi)-genomic responses. H3K27ac ChIP-seq data were also acquired from a 9 heroin and 9 control donor subset of the same cohort. Differential expression (DE) analyses showed that there were more DE genes in GABAergic neurons compared with glutamatergic neurons or oligodendrocytes. Notably, the precursors of opioid peptides, *PENK* and *POMC,* were downregulated in GABAergic neurons, whereas multiple immediate early genes (e.g., *ARC* and *NPAS4*) were downregulated in both GABAergic and glutamatergic neurons. Moreover, through multi-omic integrative analyses we identified several cases of differential chromatin accessibility in or near genes demonstrating differential expression, including heroin-induced decreased accessibility in the TSS of *ADAMTS1* in oligodendrocytes that correlated with decreased gene expression. Furthermore, in GABAergic neurons we identified genic or intergenic differential ATAC-seq peaks with the closest gene being identified as DE, including *PCSK1* and *VGF*. By integrating Hi-C data from control samples, we identified putative enhancer regions from these chromatin interactions that may elucidate deeper mechanistic interpretation of opioid-induced changes to epigenetic regulation. Together, these novel tissue- and cell type-specific analyses identify important regulatory dynamics and gene expression changes in the orbitofrontal cortex resulting from heroin use.

Disclosures: J.M. Noshay: None. **K.A. Sullivan:** None. **A. Kozlenkov:** None. **A. Townsend:** None. **G. Rompala:** None. **R. Vadukapuram:** None. **Y.L. Hurd:** None. **D.A. Jacobson:** None. **S. Dracheva:** None.

Nanosymposium

598. Genomics and Transcriptomics in Health and Disease

Location: SDCC 23

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 598.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Spatial mapping of pain-associated G-protein coupled receptors and biomarker localization in mouse brain using RNAscope[TM] HiPlex v2 and RNA-protein Co-detection assay

Authors: S. BASAK[1], L. CHATELAIN[1], S. ZHOU[1], C.-W. CHANG[1], *J. V. PULLIAM[2], A. DIKSHIT[1];
[1]Bio-Techne, Advanced Cell Diagnostics, Bio-Techne, NEWARK, CA; [2]Bio-Techne, Bio-Techne, Derwood, MD

Abstract: The mammalian brain is highly complex, comprised of distinct cell populations that contribute to the function of each distinct neuroanatomical area. Spatially mapped gene expression and cellular resolution is critical for better understanding the phenotypes of various central nervous system (CNS) disorders such as Alzheimer's disease, schizophrenia, autism and epilepsy, with poorly defined etiologies. The G-protein coupled receptors (GPCR) are an important component of pain modulation. They are widely distributed in the peripheral and CNS and are one of the most important therapeutic targets in pain medicine. However, detection of GPCRs can be challenging due to difficulties in obtaining suitable antigen accessibility and their low expression levels. In this study we demonstrate detection of GPCRs and Neuropeptide Y (NYP), known to be involved in pain perception and transmission in the brain, using the RNAscope HiPlex v2 and integrated codetection workflow assays.
The RNAscope HiPlex v2 assay can detect 48 targets in fixed and fresh frozen samples and up to 12 targets in formalin fixed paraffin embedded (FFPE) tissues. We have leveraged this technology to investigate spatial expression profile of 12 GPCR targets implicated in the pain modulation in the mouse brain. Spatial expression profile of 12 GPCR family receptors included opioid receptors, dopamine receptors and GABAergic receptors along with a neuronal marker. The copy numbers of opioid receptors in different regions of the brain were quantified using HALO Image analysis platform.
The spatial expression profiles of these markers showed high concordance to the Allen Brain Atlas resources for validating spatial gene expression (ABA Mouse Atlas ISH database). The copy number analysis indicated differential expression of *Oprm1*, *Oprd1* and *Oprk1* transcripts between cortex and nucleus accumbens, implying region-specific functions. Simultaneous RNA and protein co-detection indicated colocalization of NPY and opioid receptors in the astrocytes and microglia of the nucleus accumbens.
In summary, using the highly sensitive HiPlex v2 assay and the RNA-protein Codetection assay, we established a spatial gene expression map for visualizing GPCR family receptors within the normal mouse brain. The findings revealed cell type specific gene expression in different regions of the brain to establish functional significance of different cell types in the pain pathway. The study provides deeper insights into understanding the spatial crosstalk as well as functional

significance of different cell populations within the various brain regions thereby leading to broader understanding of disease pathology.

Nanosymposium

598. Genomics and Transcriptomics in Health and Disease

Location: SDCC 23

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 598.05

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Spatially resolved single-cell transcriptomics reveals conservation and divergence of the cellular organization in human and mouse cortices

Authors: *R. FANG[1], C. XIA[1], J. L. CLOSE[2], M. ZHANG[1], J. HE[1], A. HELPERN[1], B. R. LONG[3], J. A. MILLER[3], E. LEIN[3], X. ZHUANG[1];
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Abstract: The human cerebral cortex has billions of cells, tremendous cellular diversity, and complex cellular organization. However, how different cell types are spatially organized in the human cortex and how cellular organization varies across species remains unclear. Here, we performed spatially resolved single-cell profiling of 4000 genes using multiplexed error-robust FISH (MERFISH), identified more than 100 transcriptionally distinct cell populations, and generated a molecularly defined and spatially resolved cell atlas of the human middle and superior temporal gyrus. To systematically characterize the conservation and divergence in **cell composition**, **spatial organization** and **cell-cell interactions** between human and mouse cortex, we also performed MERFISH imaging in multiple cortical areas in the mouse brain. The **cell composition** in these human cortical regions differed markedly from that observed in mouse cortical regions. We find that human has a lower proportion of excitatory neurons and a much higher proportion of glial cells. The glia-to-neuron ratio is five times higher and excitatory-to-inhibitory ratio is three times lower in humans than that in mice. The MERFISH imaging of **spatial organization** further revealed a complex map in humans in which not only excitatory but also most inhibitory neuronal and some non-neuronal clusters adopted laminar organizations.

The high-spatial resolution cell atlases by MERFISH also allowed us to characterize soma-proximity-based **cell-cell interactions** in a cell type-specific manner and revealed drastic differences in soma interaction patterns between humans and mice. The cross-species difference is particularly pronounced between neurons and non-neuronal cells. We observed substantial enhancement in soma contact or proximity between neurons and oligodendrocytes in the human cortex compared with the mouse cortex, suggesting a potential evolutionary adaptation to higher energy demands during firing of a single neuron in the human brain. In addition, we also observed preferential enrichment for contact or proximity between microglia and excitatory neurons, compared with inhibitory neurons, in the human cortex. Some ligand-receptor pairs enriched in the contacting microglia-neuron pairs in human are genetically associated with neurodegenerative diseases, suggesting a possible molecular basis underlying the observed microglia-neuron interactions and a potential connection of these cell-cell interactions to neurodegenerative diseases.

Disclosures: R. Fang: None. **C. Xia:** Other; inventors on patents applied for by Harvard University related to MERFISH. **J.L. Close:** None. **M. Zhang:** None. **J. He:** None. **A. Helpern:** None. **B.R. Long:** None. **J.A. Miller:** None. **E. Lein:** None. **X. Zhuang:** Other; inventor on patents applied for by Harvard University related to MERFISH, cofounder and consultant of Vizgen.

Nanosymposium

598. Genomics and Transcriptomics in Health and Disease

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Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 598.06

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Map of full-length isoforms in the human brain

Authors: X. LIN[1], Y. HADAS[1], E. HADJIMICHAEL[1], L. LI[2], A. WEIMER[3], E. KOORNSTRA[1], T. WANG[3], L. ERJAVC[1], X. WANG[2], PSYCHENCODE CONSORTIUM[4], M. SNYDER[3], C. LIU[5], A. E. URBAN[3], ***D. PINTO**[1];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Univ. of North Dakota, Grand Forks, ND; [3]Stanford Univ., Palo Alto, CA; [4]PEC, New York, NY; [5]Upstate Med. Univ., New York, NY

Abstract: Regulation of transcript structure generates transcript isoform and splicing diversity and plays an important role in health and disease. Differences in isoform expression account for substantial effect sizes and genetic enrichments in neuropsychiatric disorders such as ASD and schizophrenia; however, a systematic characterization of isoform expression in the human brain is lacking as most isoforms are yet to be directly profiled at the tissue or cellular level. As a

result, our understanding of the genetic control of splicing and isoform usage remains incomplete. Here, we performed deep single-molecule real-time isoform long-read sequencing of mRNA and lncRNA-capture enriched prefrontal cortex samples from >26 individual donors. Large-scale analysis of these data with RNA-Seq profiles of >1300 PsychENCODE cortical bulk-tissue samples, resulted in a comprehensive quantitative map of coding and noncoding transcript isoforms. Our map of ~440k isoforms greatly expands the transcriptional landscape of brain-expressed genes with >320k novel unannotated complete isoforms (72%), extends known coding and noncoding gene annotations, and uncovers >1000 novel or reconstructed gene loci that can show cell-type specific expression. Orthogonal data, including, ribosome sequencing and mass-spectrometry data from >280 prefrontal cortical samples, as well as 5'-end TSS profiling and nanopore long-read sequencing, were used to further characterize novel isoforms and open reading frames. We further leveraged PsychENCODE single-nuclei transcriptomic and chromatin data to annotate cell-type-specific regulatory interactions for novel isoforms, reconstructed loci, and novel genes. Comparison to long-read data from an additional 12 cerebellum (CBL), anterior cingulate cortex (ACC) and hippocampus (HIP) samples uncovered tissue-specific patterns of isoform abundance and usage, and splicing changes in genes relevant to neuropsychiatric risk. Multi-platform analysis of >80k single-nuclei full-length transcripts further revealed that isoform diversity seen in brain tissues is often due to underlying cell-type-specific patterns. Finally, we leveraged our reference map of full-length isoforms to re-assess the functional consequence of rare genetic variants from a compendium of disease studies, and to improve quantitative trait analyses and refine the prioritization of dozens of candidate risk genes. Taken together, we provide a comprehensive resource to characterize dysregulation of isoform expression in neuropsychiatric disorders.

Disclosures: X. Lin: None. **Y. Hadas:** None. **E. Hadjimichael:** None. **L. Li:** None. **A. Weimer:** None. **E. Koornstra:** None. **T. Wang:** None. **L. Erjavc:** None. **X. Wang:** None. **X. Wang PsychENCODE consortium:** None. **M. Snyder:** None. **C. Liu:** None. **A.E. Urban:** None. **D. Pinto:** None.

Nanosymposium

598. Genomics and Transcriptomics in Health and Disease

Location: SDCC 23

Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 598.08

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Subanatomical transcriptomic characterization of Rhesus Subthalamic Nucleus for therapeutic target discovery

Authors: *R. GUNARATNA[1], M. XIMERAKIS[2], D. CHANG[2], X. LU[2], X. WANG[1], V. PETERSON[2], D. LOVATT[1];
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Abstract: The therapeutic importance of the subthalamic nucleus (STN) in Parkinson's disease (PD) has been clearly demonstrated in patients receiving deep brain stimulation (DBS), where direct stimulation of the STN attenuates pathological beta-band activity and ameliorates abnormal motor function. Hence, pharmacological manipulations of STN enriched targets that mimic STN-DBS could serve as a therapeutic approach to alleviate PD-related motor symptoms.Here, we integrated single-nuclei RNA-seq, spatial transcriptomics and RNAscope to characterize rhesus STN transcriptome at a subanatomical level. Enrichment of STN gene signature in STN neurons showed concordance with orthogonal Pitx2 RNAscope findings. Cell type deconvolution showed enrichment of neurons and astrocytes in the STN. Among spatially-confined genes, *Pvalb* expression overlapped with region of the STN innervated by motor cortex. Similar to snRNA-seq findings of the mouse STN, current data point to spatially-resolved subanatomical regions in rhesus that are transcriptomically unique. The identification of putative topographical markers may reveal region specific molecular mechanisms paving the way to druggable targets mimicking STN-DBS upon pharmacological manipulation.

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Nanosymposium

598. Genomics and Transcriptomics in Health and Disease

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Time: Wednesday, November 16, 2022, 8:00 AM - 10:15 AM

Presentation Number: 598.09

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Cross-platform long-read RNA sequencing characterization reveals isoforms associated with neuronal differentiation and disease

Authors: *S. LÉPINE[1,2], G. MAUSSION[1], S. HIGGINS[3], S. HO[4], R. A. THOMAS[1], T. DURCAN[1], R. SCHUBERT[4];
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Abstract: Alternative splicing (AS), which gives rise to multiple mRNA isoforms from individual genes, contributes to the vast diversity of the eukaryotic transcriptome. Regulation of gene expression through AS has been established as a critical factor during development and acquisition of adult tissue identity. Moreover, dysregulation of AS has been implicated in many human diseases including cancer and neurological disorders, highlighting the need for methodologies that can reliably detect and quantify transcript isoforms. The recent development

of long-read RNA sequencing platforms, which can capture a full RNA transcript in a single read, now offer key advantages for isoform analysis. Here, we harnessed this new technology to identify isoform signatures characterizing the programs of differentiation into neuronal cell subtypes from human induced pluripotent stem cells (iPSCs) using two long-read RNA sequencing platforms (PacBio and ONT). To account for potential sources of biases and artifacts arising from sequencing and data processing, we conducted analysis comparing both sequencing platforms as well as several data processing approaches. After establishing a rigorous cross-platform workflow, we performed isoform profiling of iPSC-derived neuron subtypes (motor neurons and dopaminergic neurons) at the progenitor and neuronal stages. We identified a number of differentially expressed genes and alternative splicing events governing i) the transition from neuronal progenitors to differentiated neurons, and ii) neuronal specification to the motor and dopaminergic lineages. Interestingly, we found that differences in isoform stoichiometry sometimes occur without changes in total gene expression levels, highlighting the added value of long-read RNA sequencing over traditional transcriptomic approaches. Importantly, this data pointed to many transcripts whose genes have been associated with neurodevelopmental and neurological diseases. This led us to perform isoform profiling of dopaminergic neurons differentiated from iPSCs carrying triplication in the SCNA gene to investigate the importance of AS in the pathophysiology of Parkinson's disease. Overall, we established a robust framework for isoform characterization in several iPSC-derived models, allowing us to investigate AS in neurodevelopment and disease.

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Nanosymposium

676. Glia-Neuron Crosstalk in Disease

Location: SDCC 7

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.01

Topic: B.09. Glial Mechanisms

Title: Microglia modulate post-stroke corticospinal neuroplasticity in the cervical spinal cord

Authors: *K. POINSATTE[1], A. AJAY[1], A. NAWABY[1], W. XU[2], X. KONG[1], E. J. PLAUTZ[1], D. O. RAMIREZ[1], M. P. GOLDBERG[1,3];
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Abstract: Primary motor cortex stroke in mice leads to the degeneration of the corticospinal tract (CST) and denervation of the hemicord, leading to motor deficits. However, the healthy contralesional CST generates collateral axon sprouts into the denervated hemicord, promoting recovery. This synaptic and axonal remodeling may be modulated by activated microglia in the spinal cord. 8-11 week old male C57/B6 mice received a photothrombotic motor cortex stroke or sham surgery and a contralesional motor cortex injection of an adeno-associated virus driving

fluorescent protein expression for independent visualization of sprouting CST axons and terminals. Mice were sacrificed at 3 days, 1, 4, or 6 weeks post-stroke. Cervical spinal cords were imaged using serial two-photon tomography. Central nervous system neuroplasticity was visualized across the entire volume of the cervical cord with 3-D representations of sprouting axons and terminals. A custom developed pipeline incorporating machine learning-based image segmentation and registration into a newly-created reference atlas was used to quantify synaptic terminal density in an unbiased region-specific manner. Four weeks post-stroke, synaptic terminal density increased in the denervated dorsal horn, with significantly greater densities in lamina 4, including the intermediomedial column and internal basilar nucleus, and the lateral part of lamina 5, as well as a trending increase in the medial part of lamina 5. Microglia:terminal proximity analysis of high-resolution image subvolumes showed an acute post-stroke decrease in proximity, but by 1 and 4 weeks post-stroke, a greater percentage of terminals were touching or near microglia in the denervated central and ventral gray matter, respectively. At 6 weeks post-stroke, microglia engulfed synaptic terminals in the white matter near the denervated CST fiber tract. These results suggest that spinal cord microglia may mediate neuroplasticity in the denervated hemicord during stroke recovery.
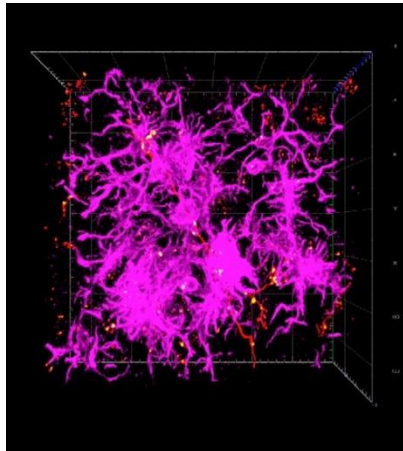
Disclosures: K. Poinsatte: None. **A. Ajay:** None. **A. Nawaby:** None. **W. Xu:** None. **X. Kong:** None. **E.J. Plautz:** None. **D.O. Ramirez:** None. **M.P. Goldberg:** None.

Nanosymposium

676. Glia-Neuron Crosstalk in Disease

Location: SDCC 7

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.02

Topic: B.09. Glial Mechanisms

Title: Microglia Actively Remove NR1 Autoantibody-Bound NMDA Receptors And Synapses In Neuron Microglia Co-cultures

Authors: *K. RAHMAN[1,2], A. ICHKOVA[3], E. ANDRZEJAK[3], H. PRÜß[4,3], C. GARNER[1,3];
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Abstract: NMDA receptor encephalitis (NMDARE) is an autoimmune disorder of central nervous system (CNS) where antibodies are produced commonly against the NR1 subunit of NMDA receptors (NMDARs). Currently, it is understood that bound antibodies lead to clustering and internalization of NMDARs from the surface of neurons. However, the effector function of these autoantibodies following antigen binding specifically the Fc region-dependent engagement of the resident immune cells, microglia, remains unexplored. Here we shed light on NMDAR autoantibody mediated microglial engagement and its downstream effects using patient derived monoclonal NR1 autoantibody (NR1-mAb). Using a co-culture model of primary mouse microglia and hippocampal neurons, we observed a decrease of NR1-mAb bound-NMDARs 6 hours after microglia addition. NMDAR puncta concomitantly appeared inside the lysosomes of CD11b positive microglia hinting at phagocytosis of these receptors by microglia. The receptor removal was specific to the NR1-mAb bound receptors as we did not observe changes in GABA$_A$ receptors (GABA$_A$Rs) after addition of microglia. Similarly, we used patient derived monoclonal autoantibody against the α1- subunit of GABA$_A$Rs and observed significant decrease in GABA$_A$R puncta 6 hours after microglia addition but without changes in the number of NMDARs. In parallel to receptor removal, we also observed significant decrease in synaptic number in particular of different post-synaptic markers like PSD-95, Homer1 however no such decrease was noted in markers of pre-synaptic boutons e.g., vGLUT1, Synapsin. Importantly, blocking the downstream engagement of microglia by introducing mutations in the Fc region of the bound NR1-mAb prevented both loss of NMDARs and synapses, indicating that antibody-microglia engagement is critical for receptor/synapse removal. Moreover, our data argue that microglia could be actively involved in mediating the loss of NMDA and other receptors in individuals with autoimmune encephalitis contributing to their associated pathophysiology.

Disclosures: K. Rahman: None. **A. Ichkova:** None. **E. Andrzejak:** None. **H. Prüß:** None. **C. Garner:** None.

Nanosymposium

676. Glia-Neuron Crosstalk in Disease

Location: SDCC 7

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.03

Topic: B.09. Glial Mechanisms

Title: Microglia-restricted Toll-like receptor-4 (TLR4) drives female-specific ethanol-induced allodynia

Authors: *S. N. ALEXANDER[1], T. A. SZABO-PARDI[1], M. D. BURTON[2];
[2]Brain and Behavioral Sci., [1]UT Dallas Cognition and Neurosci. Grad. Program, Richardson, TX

Abstract: Chronic pain affects more than 20% of Americans and there is a need to understand lifestyle factors that contribute to pain development. Data shows that Americans consume approximately 1 bottle of wine per week, on average. While chronic alcohol consumption induces alcohol-induced neuropathy, there have been little to no observations of the ability of low-to-moderate alcohol use to alter sensitivity or produce allodynia. Toll-like receptor-4 (TLR4), is an immune receptor expressed by many cells that are involved in alcohol-induced neuroinflammation and mediate sex differences in pain signaling. TLR4 on microglia is important in promoting microglial activation states and has been purported to mediate pertinent alcohol-specific behaviors, such as depression. Our hypothesis was that microglial TLR4 would be important for mediating pain in males and not females, similar to studies from us and many others. To assess whether TLR4 on central microglia only is sufficient to drive allodynia in mice, independent of TLR4 on peripheral immune cells or nociceptors, we exposed male and female mice to a liquid diet consisting of 5% ethanol or control diet for 14 days. Our study used a novel genetic model in which TLR4 expression is restricted to microglia (Cx3CR1:CreERT2: egfp: TLR4^{LoxTB}) on a TLR4-null background (TLR4^{LoxTB}). We then assessed changes in allodynia after a local intraplantar injection of subthreshold non-pain inducing stimulus. We tested our hypothesis by using battery of pain behavioral assays for spontaneous pain, mechanical hypersensitivity, and hot/cold sensitivity. Preliminary findings show that short-term alcohol exposure induced allodynia in females only, and sexual dimorphisms in microglia activity and morphology in the spinal cord via immunohistochemistry analysis. These changes led us to investigate whether TLR4 on microglia alone was sufficient to drive the allodynia seen in females. Similar to the wild-type females, reactivated TLR4 mice had higher microglial cell counts in the lumbar spinal region and adopted a more amoeboid morphology compared to their control counterparts. This study begins to uncover the cell- and sex-specific role of TLR4 in the mechanisms behind the development of short- and long-term alcohol-induced hypersensitivity.

Disclosures: S.N. Alexander: None. **T.A. Szabo-Pardi:** None. **M.D. Burton:** None.

Nanosymposium

676. Glia-Neuron Crosstalk in Disease

Location: SDCC 7

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.04

Topic: B.09. Glial Mechanisms

Title: A novel in vitro modeling system to evaluate the role of Glia and Neurons in CLN3 Batten disease

Authors: *J. A. SIERRA DELGADO[1], C. N. DENNYS[1], E. SPECTOR[1], S. SINHA RAY[1], A. HARTLAUB[1], R. RODRIGO[1], X. ZHANG[1], S. B. LIKHITE[1], K. C. MEYER[1,2];
[1]Ctr. for Gene Therapy, Res. Inst. Nationwide Childrens Hosp., Columbus, OH; [2]Col. of Med., The Ohio State Univ., Columbus, OH

Abstract: Neuronal Ceroid Lipofuscinosis 3 (CLN3) is a fatal autosomal recessive lysosomal storage disorder in the group of Batten Diseases. Disease characteristics are cognitive and motor decline, vision impairments and seizures ultimately leading to premature death in early adulthood. The cellular mechanisms by which CLN3 mutations cause Batten disease are poorly understood, with not very much known about the function and expression of CLN3 in neurons, and even less about the contributions of glial cells, especially astrocytes, to disease mechanisms and progression. To address this, we used a direct reprogramming method to generate Neural Progenitor Cells (iNPCs) from primary fibroblasts derived from CLN3 patients. We then differentiated the iNPCs into induced astrocytes (iAs) to evaluate classic stress markers such as mitochondrial and endoplasmic reticulum stress commonly observed in neurological disease. While no difference was found in levels of ER stress in either fibroblasts or iAs compared to healthy controls, CLN3 iAs showed an abnormal mitochondrial morphology, with fragmented and rounded mitochondrial network. Interestingly, the abnormal mitochondrial network and morphology was specific to astrocytes and was not observed in CLN3 fibroblasts. Based on this observation, we evaluated the mitochondrial activity profile of CLN3 iAs using the Seahorse Bioscience XF96 Extracellular Flux Analyzer platform, finding cell line specific changes in mitochondrial activity in CLN3 iAs in both basal and ATP linked respiration. While mitochondrial dysfunction was previously observed in mouse Cln3$^{\Delta ex7/8}$ astrocytes, our results suggest that patient specific variables may have a differential effect on the mitochondrial phenotype. We also developed a co-culture system using healthy mouse GFP+ neurons in contact with the patient iAs. Using this system, we found that iAs induced a high degree of neuronal death, with some lines showing a more toxic phenotype than others. Additionally, to further study the effect of mutations in neurons, we generated induced neurons (iNs) from patients fibroblasts and are currently evaluating patient specific phenotypes. These *in vitro* culture systems are also being used for testing of new therapeutic strategies in order to accelerate development of novel treatments.

Disclosures: J.A. Sierra Delgado: None. **C.N. Dennys:** None. **S. Sinha Ray:** None. **A. Hartlaub:** None. **R. Rodrigo:** None. **X. Zhang:** None. **S.B. Likhite:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amicus Therapeutics. **K.C. Meyer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alcyone Therapeutics, Amicus Therapeutics. F. Consulting Fees (e.g., advisory boards); Alcyone Therapeutics.

Nanosymposium

676. Glia-Neuron Crosstalk in Disease

Location: SDCC 7

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.05

Topic: B.09. Glial Mechanisms

Title: The role of purines and their receptors in microglial calcium signaling during epileptogenesis

Authors: *A. D. UMPIERRE, L.-J. WU;
Mayo Clin., Mayo Clin., Rochester, MN

Abstract: Microglial calcium signaling is a relatively unexplored aspect of their function, partially due to the low-to-absent levels of calcium signaling observed in naive mice. However, microglia dramatically elevate their calcium signaling in response to injury/cell death, inflammation, or neuronal hyperactivity. Using ex vivo two photon imaging combined with pharmacology and genetic approaches, we have determined the key purine receptors and pathways that influence microglial calcium signaling in the basal state and in epileptogenic mouse brain slice, as well as their overall contributions to ATP and UDP signaling. In addition, we have utilized receptor knockout approaches and in vivo two photon microscopy to explore a key purine receptor influencing microglial calcium signaling longitudinally during epileptogenesis. Our ongoing experiments, using histology, electrophysiology, and transcriptomics will determine how the attenuation of microglial calcium signaling during epileptogenesis influences disease outcomes including neuropathology, inflammatory states, and epilepsy development risk.

Disclosures: A.D. Umpierre: None. **L. Wu:** None.

Nanosymposium

676. Glia-Neuron Crosstalk in Disease

Location: SDCC 7

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.06

Topic: B.09. Glial Mechanisms

Title: Role of immature glia in modulating neuronal hyperactivity and cognition in patients with epilepsy

Authors: *A. AMMOTHUMKANDY[1], K. RAVINA[1], V. WOLSELEY[1], A. TARTT[2], P.-N. YU[1], L. CORONA[1], N. ZHANG[1], G. NUNE[1], B. LEE[1], J. SMITH[3], D. SONG[1], T. BERGER[1], C. HECK[1], R. CHOW[1], M. BOLDRINI[4], C. LIU[1], J. RUSSIN[1], M. BONAGUIDI[1];
[1]USC, Los Angeles, CA; [2]NYS Psychiatric Inst., New York, NY; [3]Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [4]Columbia Univ., New York, NY

Abstract: Abstract: The hippocampus is the most common seizure focus in people. Within the hippocampus, aberrant cell genesis plays a critical role in the initiation and progression of epilepsy in rodent models, but it is unknown whether this also holds true in humans. To deconstruct the role of cell genesis in neuronal hyperactivity and cognitive decline in human epilepsy we used a combination of histology, neural stem cell cultures and multi-electrode recordings on surgical resections from patients with mesial temporal lobe epilepsy (MTLE). We found neurogenesis declined with increasing disease duration, neuronal hyperactivity, and led to verbal learning impairment. In addition to neurogenesis, we also observed astrocytes generated in the adult human MTLE hippocampus, and immature astrocytes persist during epilepsy progression. The location and activity of immature astrocytes are dependent upon epileptiform activity, where immature astroglia activity anticorrelates with neuronal hyperactivity. Further, we identified the expression of neurotoxic (A1) and neuroprotective (A2) glial markers within the immature glial cells. Most of the immature astroglial cells expressed both A1 and A2 markers and their expression did not differ significantly based upon disease duration, age at surgery and epileptiform activity. However, higher number of immature astrocytes are associated with a decline in intelligence and language skills. We therefore suggest new born astrocytes display preserved mechanisms across patients, while their activity transitions in association with epileptiform-like activity. Immature astroglia therefore represent a new potential target to modulate neuronal hyperactivity as well as cognitive decline in adult human MTLE patients.

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Nanosymposium

676. Glia-Neuron Crosstalk in Disease

Location: SDCC 7

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.07

Topic: A.01. Neurogenesis and Gliogenesis

Title: Late-onset Alzheimer's disease risk factor BIN1 plays a novel role in oligodendrocyte health and survival

Authors: *G. SAMTANI[1], **J. LI**[2];
[1]Texas A&M Inst. For Neurosci., College Station, TX; [2]Vet. Integrative Biosci., Texas A&M Univ., College Station, TX

Abstract: White matter dystrophy in the brain occurs early in the development of late-onset Alzheimer's disease (LOAD) as well as in advanced aging. However, the contribution of white matter alterations to LOAD remains poorly understood. BIN1 (bridging integrator 1), the second-most common genetic risk factor for LOAD after ApoE, has been shown to be localized to cerebral white matter tracts and oligodendrocytes in humans and mice, yet its function in oligodendrocytes is unknown. In this study, we seek to identify the physiological functions of BIN1 in oligodendroglial development and myelination. We generated two inducible conditional knockout mouse lines where *Bin1* is selectively ablated in oligodendrocytes at different developmental stages - oligodendrocyte progenitor cells (OPCs, Pdgfrα-Cre^{ERT};Bin1$^{fl/fl}$) and mature oligodendrocytes (Plp1Cre^{ERT};Bin1$^{fl/fl}$). We found that targeted deletion of *Bin1* in OPCs and their progenies during early postnatal brain development suppressed developmental myelination, which was associated with increased apoptosis of newly formed oligodendrocytes. Consistently, in primary cultures, oligodendroglial *Bin1* deficiency resulted in process retraction and degeneration of newly matured oligodendrocytes. Moreover, our preliminary data showed that homeostatic *Bin1* deletion in adult oligodendrocytes led to neurobehavioral alterations. Together, our data reveal previously unrecognized functions of Bin1 in maintaining oligodendrocyte health and suggest that dysregulation of oligodendroglial Bin1 may compromise axon-glia interaction and sensitize axons to aging-related neurodegeneration.
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Disclosures: G. Samtani: None. **J. Li:** None.

Nanosymposium

676. Glia-Neuron Crosstalk in Disease

Location: SDCC 7

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.08

Topic: B.09. Glial Mechanisms

Title: Microglia and complement signaling contribute to neural representations of space in the hippocampus

Authors: *K. MCDERMOTT, M. FRECHOU, J. JORDAN, S. MARTIN, J. GONÇALVES;
Albert Einstein Col. of Med., Bronx, NY

Abstract: Microglia sense neuronal activity and regulate activity-dependent synaptic plasticity, a process important for learning and memory. The complement cascade is one molecular pathway involved in microglia-mediated synaptic pruning that has been implicated in memory impairment. While microglia and related factors contribute to hippocampal memory and plasticity, the corresponding effects on network activity are still being elucidated. The hippocampus is well known for its role in spatial memory and contains place cells, or neurons that fire preferentially to specific locations within an environment. We investigate the effects of microglia and complement on spatial tuning of hippocampal neuronal activity by utilizing longitudinal in vivo calcium imaging in mice. We also examine corresponding effects on hippocampal-dependent behavior. First, we study the physiological role of microglia by depleting them from the brain. Second, we knock out complement component C1q in microglia to determine the contribution of this pathway to hippocampal function. Preliminary data suggest that microglia ablation or loss of microglial complement signaling impairs spatial tuning. Furthermore, microglia-ablated animals show deficits during a spatial memory task. Ongoing experiments aim to clarify the cellular mechanisms involved in these changes. This work will further our understanding of how microglia can impact hippocampal function on a network level.

Disclosures: K. McDermott: None. **M. Frechou:** None. **J. Jordan:** None. **S. Martin:** None. **J. Gonçalves:** None.

Nanosymposium

676. Glia-Neuron Crosstalk in Disease

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Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.09

Topic: B.09. Glial Mechanisms

Title: Microglia in the presynaptic dysfunction and neurodegeneration in mice lacking the synaptic co-chaperone CSPα/DNAJC5

Authors: *N. BORJINI[1], M. VALENZUELA-VILLATORO[2], F. RUBIO-PASTOR[2], C. MESA-CRUZ[2], C. PARADELA-LEAL[2], R. FERNÁNDEZ-CHACÓN[2];
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Abstract: In the central nervous system, synapses are specialized junctions that connect neurons into circuits. To maintain the specialized structure and function of synapses, neurons have dedicated synapse-specific proteostasis, of which chaperones are a fundamental component. Imbalances in proteostasis result in neurotransmission deficits and protein aggregates that ultimately trigger synaptic loss and neurodegeneration. Co-chaperone Cysteine String Protein α(CSPα/DNAJC5), is critical for presynaptic proteostasis and synapse maintenance, especially in highly active synapses. Microglia, the CNS's resident immune cells monitor and interact with synapses to modulate neural circuit formation and function and their interaction is prevalent. In neurodegenerative diseases, studies have demonstrated that microglial activation is often detected before the first signs of neuronal cell death, leading to the hypothesis that early microglial activation may promote the degenerative disease progression. Nevertheless, the mechanisms through which microglia could mediate synaptic damage in mice lacking CSPα/DNAJC5 have not been identified. In this study, we are interested in deciphering the specific microglial contribution in mice lacking CSPα/DNAJC5. Moreover, by using newly generated mice to conditionally target *Dnajc5,* we have analyzed the involvement of microglia in the presynaptic dysfunction of highly active synapses of the GABAergic parvalbumin-positive (PV) neurons (*PVCre:Ai27D:Dnajc5^flox*), and glutamatergic neurons that operate at a low activity regime (CaMK^CreERT2:Ai27D:*Dnajc5*^flox). Our data demonstrate that microglia are activated in the absence of neuronal CSPα. Similar activation was observed in *PVCre:Ai27D:Dnajc5^flox* mice with increased microglia-PV+ neurons interaction. Interestingly, only slight involvement of microglia was detected in CaMK^CreERT2:Ai27D:*Dnajc5*^flox mice. We are investigating the molecular mechanisms by which microglia might cause dysfunction of synaptic maintenance and contribute to the pathogenesis of neurodegeneration.

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Nanosymposium

676. Glia-Neuron Crosstalk in Disease

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Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.10

Topic: B.09. Glial Mechanisms

Title: Astrocytic NKCC1 plays a pivotal role in prevention of seizures promoted by postsynaptic GABAergic excitation in epilepsy models

Authors: D. T. NGUYEN, M. ISHIBASHI, M. WATANABE, A. SINHA, R. YESMIN, *A. FUKUDA;
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Abstract: GABA is a main inhibitory neurotransmitter, however, excitatory GABA actions have been observed in brain slices of patients with temporal lobe epilepsy and in many *in-vitro* seizure models. This is caused by neuronal $GABA_A$ receptor-mediated Cl^- accumulation and consequent collapse of Cl^- gradient at the inhibitory postsynapses. Astrocytic processes tightly wrap around inhibitory synapses, enabling astrocytes to modulate synaptic cleft ion concentration. In contrast to neuron, astrocytic Cl^- concentration is high due to abundance in Na^+-K^+-$2Cl^-$ cotransporter type 1 (NKCC1), so that the astrocytic $GABA_A$ receptor activation leads to Cl^- efflux. This Cl^- efflux from the astrocyte could buffer the synaptic cleft Cl^- concentration ($[Cl^-]_o$) to sustain the postsynaptic Cl^- gradient during intense activation of GABAergic synapses (Egawa et al., J. Physiol. 2013). We therefore investigated the function of astrocytes in modulating the postsynaptic GABA action in epilepsy. We used the astrocyte-specific conditional NKCC1 knock-out (astroNKCC1 KO) mice, in which astrocytic $GABA_A$ receptor-mediated Cl^- efflux would be reduced. The decrease in astrocytic Cl^- efflux is expected to lower the $[Cl^-]_o$, hence hasten the collapse of postsynaptic Cl^- gradient. The seizure-like events (SLEs) in CA1 pyramidal neurons were triggered by tetanic stimulation of stratum radiatum in hippocampus. We found that the threshold of stimulation intensity for SLEs is significantly lower in astroNKCC1 KO, compared to wild-type littermates (WT). In addition, in astroNKCC1 KO the duration of SLEs was significantly longer and the bicuculline-sensitive excitatory GABA current was significantly larger. Consistent with these *in vitro* results, *in vivo* pilocarpine-induced seizure model indicated astroNKCC1 KO mice are significantly seizure-prone with the lower induction dose and the higher score of Racine scale. Thus, our findings suggest a protective role of astrocytic NKCC1 in excitatory GABA-mediated seizures. This might provide an answer to the question why some clinical trials of bumetanide, a cell-type nonselective NKCC1 inhibitor, failed to show appreciable results.

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Nanosymposium

676. Glia-Neuron Crosstalk in Disease

Location: SDCC 7

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Presentation Number: 676.11

Topic: B.09. Glial Mechanisms

Title: Regulation of neuronal network dynamics via glial-neural paracrine signaling in the healthy and diseased human brain

Authors: *S. KRISHNA[1], A. CHOUDHURY[1], M. KEOUGH[2], K. SEO[1], L. NI[2], S. KAKAIZADA[1], A. LEE[1], A. AABEDI[1], S. NAGARAJAN[1], D. RALEIGH[1], D. BRANG[3], M. MONJE[2], S. HERVEY-JUMPER[1];
[1]Univ. of California San Francisco, San Francisco, CA; [2]Stanford Univ., Stanford, CA; [3]Univ. of Michigan, An Arbor, MI

Abstract: Little is known about the contributions of astrocytes to behavior in the adult brain. To fully understand circuit changes underlying behavior, it is important to study both neurons and glial cells in human models. Brain tumor, namely gliomas, commonly cause cognitive deficits and represents one of the few human cognition models that offer mechanistic interrogation at both cellular and network levels. Here, we integrate both *in vivo* and *in vitro* neurophysiology spatially matched with gene and protein expression programs across 66 IDH WT adult glioblastoma (GBM) patients to identify thrombospondin-1 (TSP-1) as a molecular driver of glioma-induced network remodeling. Cognitive task-related neuronal activity was assessed in 14 adult GBM patients with cortically projecting glioma infiltration in the lateral prefrontal cortex (LPFC). Using electrocorticography, we show that speech initiation induces recruitment of not only LPFC, but also regions of tumor-infiltrated cortex not normally involved in speech processing. We also find that compared to healthy cortex, speech initiation results in task-relevant hyperexcitability (as measured by high gamma power) within tumor-infiltrated cortex. Using single cell RNA-sequencing of intratumoral regions maintaining brain functional connectivity (13,730 cells analyzed), we find elevated expression of several genes involved in neural circuit assembly, including the synaptogenic factor, TSP-1. We further show that these TSP-1 expressing astrocyte-like glioma cells have a distinct synaptogenic phenotype with increased pre- (synapsin-1) and post (PSD95)- synaptic marker expression in tumor tissues and neuron-glioma co-cultures. These findings suggest that tumor-infiltrated brain regions with increased functional connectivity include a subpopulation of synaptogenic TSP-1 expressing malignant tumor cells, and that these cells not only promote neuronal hyperexcitability but also potentially contribute to the functional circuit-level remodeling mentioned above. Therapeutic vulnerabilities to TSP-1 inhibition by gabapentin was performed *in vitro*. Multi-electrode array analysis of neuron-glioma co-cultures show increased neuronal spiking activity and network burst synchrony in the presence of TSP-1 over expressing cells. Strikingly, these increases are eliminated in the presence of gabapentin, indicating the strong link between glioma cell-derived TSP-1 and network hyperexcitability. In sum, our results identify TSP-1 mediated paracrine interactions between glioma cells and neurons as a key contributor to neuronal hyperexcitability and functional circuit remodeling in glioma patients.

Nanosymposium

676. Glia-Neuron Crosstalk in Disease

Location: SDCC 7

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 676.12

Topic: B.09. Glial Mechanisms

Title: Neuromodulator crosstalk shapes astrocyte Ca^{2+} dynamics during vigilance and is impaired in the APP^{NL-F} mouse model of Alzheimer's disease

Authors: *E. LIM[1], A. SALINAS-BIRT[1], L. YE[1], Y. YANG[2], M. PAUKERT[1];
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Abstract: Studying how vigilance is represented in the brain is essential to understanding mind as well as neurological and psychiatric diseases. Both noradrenergic and cholinergic neurons activate during periods of heightened vigilance and may contribute to behavioral state by shaping astroglia Ca^{2+} dynamics. Here we used transgenic mice with GCaMP6f expression in astrocytes or noradrenergic neurons on a linear motorized treadmill for two-photon Ca^{2+} imaging. During prolonged locomotion, visual cortex astrocytes exhibited a Ca^{2+} response characterized by multiple components: an initial transient response and a reduced sustained response that lasted until the end of locomotion. Using a combination of pharmacological tools and gene deletion, we found that these dynamics were shaped by cholinergic facilitation of activation of noradrenergic terminals and subsequent activation of α_{1A}-adrenergic receptors. Given that early degeneration of cholinergic and noradrenergic nuclei are hallmarks of Alzheimer's disease, we monitored vigilance-dependent Ca^{2+} signaling in the App^{NL-F} mouse model of Alzheimer's disease and elucidated an underlying deficit in neuromodulator signaling. Together, these data offer mechanistic insight into how astrocytes get engaged in behavioral state-dependent adjustment of neural processing in both healthy and AD brains.

Nanosymposium

677. Alzheimer's Disease and Models of Alzheimer's Disease

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 677.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PIACERI Project, University of Catania
Ricerca Corrente, Oasi Research Institute-IRCCS, Troina

Title: Inhibition of D3 receptors rescues synaptic disfunction and memory impairment in aged and Alzheimer's disease mouse models

Authors: *M. TROPEA[1], M. MELONE[3,4], V. VACANTI[1], A. CENTARO[1], W. GULISANO[1], G. LEGGIO[2], F. CONTI[3,4], D. PUZZO[1,5];
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Abstract: Alzheimer's Disease (AD) is a neurodegenerative disorder representing the most common form of dementia in the elderly. Despite decades of intensive research, AD pathophysiology remains elusive, and the therapeutic strategies proposed for its treatment have failed so far. Dopamine D3 receptors (D3-Rs) are involved in several functions such as reward, social behavior, and movement control. Indeed, D3-Rs have been proposed as a therapeutical target for addiction, schizophrenia, and Parkinson's disease. More recently, some studies have evidenced the role of D3-Rs in cognition, demonstrating that activation of D3-Rs impairs attention and working memory. Results obtained in our laboratory suggested that D3 inhibition exerts a pro-cognitive effect on long term potentiation (LTP) and recognition memory on healthy wild-type (WT) mice.Based on these findings, here, we aimed to investigate whether the positive effect shown by D3 inhibition at the synapse could be exploited to counteract the synaptic plasticity and memory impairment found in aged and AD mice. To this end, we used WT and D3 knock out (D3-KO) mice at 18-22 months of age as a physiological model of aging and the transgenic mouse model of AD 3xtg. To test whether the pharmacological inhibition of D3-Rs or their genetic deletion could rescue LTP and memory impairment, we performed electrophysiological recordings at CA3-CA1 synapses in hippocampal slices and behavioral experiments of novel object recognition, novel object location and Morris Walter Maze. We found that inhibition of D3-Rs rescued the LTP impairment in aged WT mice and 3Xtg mice. Behavioral experiments showed that D3 antagonism was able to completely restore recognition memory and spatial learning and memory in our models of aging and AD. Interestingly, the genetic deletion of D3 receptors prevented the synaptic plasticity and memory decline that occurs with aging. In fact, D3 KO mice at 18-22 months showed no alteration of LTP and memory. In conclusion, our findings suggest that D3-Rs inhibition exerts a pro-cognitive effect in synaptic plasticity and memory and could represent a viable strategy in the treatment of cognitive dysfunction occurring during aging and AD.

Disclosures: M. Tropea: None. **M. Melone:** None. **V. Vacanti:** None. **A. Centaro:** None. **W. Gulisano:** None. **G. Leggio:** None. **F. Conti:** None. **D. Puzzo:** None.

Nanosymposium

677. Alzheimer's Disease and Models of Alzheimer's Disease

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 677.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alazheimer'Association Research Grant AARG-19-614919

Title: Nucleoporin 153 deficiency in adult neural stem cells defines a pathological protein-network signature and defective neurogenesis in a mouse model of Alzheimer's disease

Authors: *C. COLUSSI[1,2], A. BERTOZZI[1], M. RINAUDO[3], L. LEONE[3,2], F. CONTE[1], G. ACETO[3,2], D. D. LI PUMA[3], C. RIPOLI[3,2], R. SOLLAZZO[3], M. GABRIELLA VITA[2], M. D'ASCENZO[3,2], C. GRASSI[3,2];
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Abstract: Reduction of adult hippocampal neurogenesis is an early critical event in Alzheimer's disease (AD), leading to progressive memory loss and cognitive decline. The nucleoporin Nup153, besides its role in nuclear transport, has been described as a key regulator of NSC plasticity through gene modulation. Here we investigated the potential role of Nup153 as target to improve neurogenesis in the 3xTg mouse model of AD in vitro and in vivo. We found that reduced Nup153 levels characterized NSCs from the 3xTg mice (AD-NSCs) and caused inefficient proliferation, migration and differentiation that were restored by Nup153 overexpression in vitro. Lentiviral-mediated Nup153 hippocampal delivery in AD mice led to an increase in the number of BrdU/DCX$^+$, BrdU/NCAM$^+$ and BrdU/NeuN$^+$ cells at 10 days and 1 month respectively. Consistently, LV-Nup153-injected AD mice showed an improvement of cognitive performance in comparison to AD control mice at 1 month after LV-Nup153 injection (MWM test). iPSC-derived brain organoids produced from control and AD patients were also used to further validate the role of Nup153 in neurogenesis and development. AD organoids produced from AD-iPSC transduced with the LV-Nup153 (AD-ORG-Nup) showed a better maturation at 1 month than control-AD-organoids as well as the presence of ventricle-like structures as in healthy control organoids. A proteomic approach was performed to identify Nup153 interactors in WT- and AD-NSCs potentially implicated in neurogenesis regulation. GO analysis showed that Nup153-bound proteins in WT-NSCs were involved in RNA metabolism (tRNA, mRNA, ncRNA, splicing and transport) and epigenetic mechanisms (DNA methylation, histone modifications). Nup153-bound proteins in AD-NSCs were involved in pathways of neurodegeneration and AD, mitochondrial dysfunction, proteasomal processing, cell cycle and RNA degradation. Our data indicate that Nup153 restoration promotes neurogenesis and cognitive performance. Molecular data suggest that the complex regulatory network orchestrated by Nup153 is based on multiple interactions that are differently regulated in WT and AD-NSCs.

Nanosymposium

677. Alzheimer's Disease and Models of Alzheimer's Disease

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 677.03

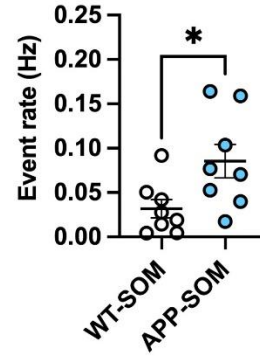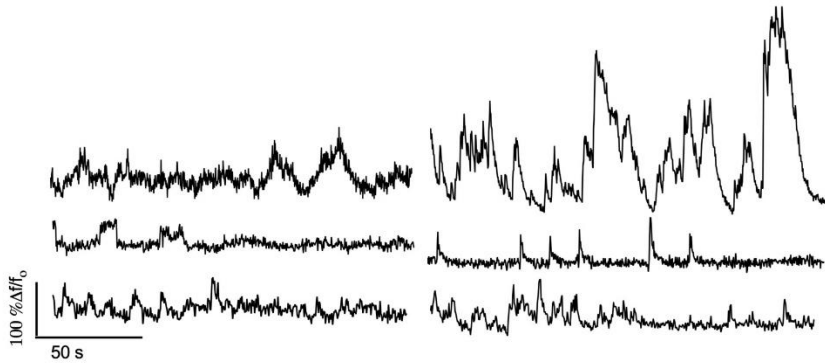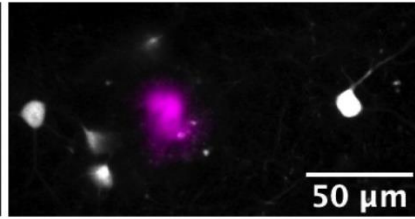**Topic:** C.02. Alzheimer's Disease and Other Dementias
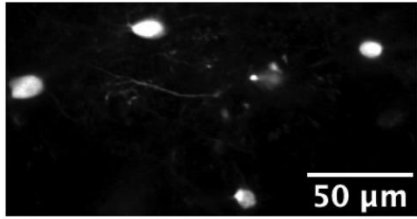
Title: Hyperactive somatostatin interneurons near amyloid plaque and cell-type-specific firing deficits in a mouse model of Alzheimer's disease

Authors: *M. ALGAMAL[1], A. N. RUSS[1], M. R. MILLER[1], S. S. HOU[1], L. MUNTING[1], M. MACI[1], Q. ZHAO[1], D. GERASHCHENKO[3], B. J. BACSKAI[2], K. V. KASTANENKA[1];
[1]Neurol., [2]Neureology, Massachusetts Gen. Hosp., Charlestown, MA; [3]Harvard Med. Sch. At VA Med. Ctr., Harvard Med. Sch. At VA Med. Ctr., West Roxbury, MA

Abstract: Alzheimer's disease (AD) is characterized by progressive memory loss and cognitive decline. These impairments correlate with early alterations in neuronal network activity in AD patients. Disruptions in the activity of individual neurons have been reported in mouse models of amyloidosis. However, the impact of amyloid pathology on distinct neuronal types remains unexplored within intact neuronal circuits. Here we use in vivo calcium imaging with multiphoton microscopy to monitor and compare the spontaneous activity of excitatory and two types of inhibitory interneurons in the cortices of APP/PS1 and control mice. We also determine the relationship between amyloid accumulation and the deficits in spontaneous activity in APP/PS1 mice. We show that somatostatin-expressing (SOM) interneurons are hyperactive, while parvalbumin-expressing interneurons are hypoactive in APP/PS1 mice. Only SOM interneuron hyperactivity correlated with proximity to amyloid plaque. These inhibitory deficits were accompanied by decreased excitatory neuron activity and decreased pairwise activity correlations in APP/PS1 mice. Our study identifies cell-specific neuronal firing deficits in APP/PS1 mice driven by amyloid pathology. These findings highlight the importance of addressing the complexity of neuron-specific deficits to ameliorate circuit dysfunction in Alzheimer's disease.
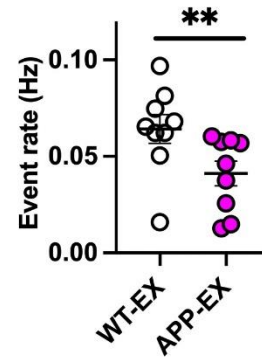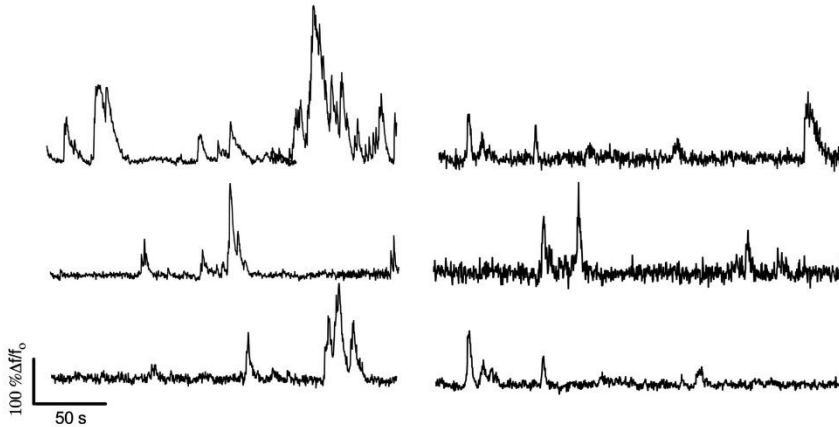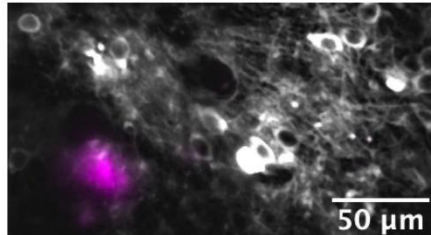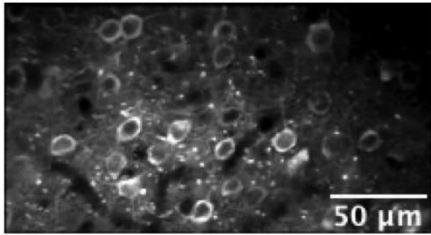
WT (SOM-jGCaMp7s)   **APP (SOM-jGCaMp7s)**

WT (CAMKII-GCaMP6s)   **APP (CAMKII-GCaMP6s)**

Event rate (Hz)

WT-SOM   APP-SOM

Event rate (Hz)

WT-EX   APP-EX

100 %Δf/f$_0$
50 s

Disclosures: M. Algamal: None. **A.N. Russ:** None. **M.R. Miller:** None. **S.S. Hou:** None. **L. Munting:** None. **M. Maci:** None. **Q. Zhao:** None. **D. Gerashchenko:** None. **B.J. Bacskai:** None. **K.V. Kastanenka:** None.

Nanosymposium

677. Alzheimer's Disease and Models of Alzheimer's Disease

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 677.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Alzheimer's disease risk gene CD2AP modulates synaptic structure and function

Authors: *M. PAVESKOVIC[1], S. A. OJELADE[1], M. KOCHUKOV[1], B. R. ARENKIEL[2], J. M. SHULMAN[1];
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Abstract: The gene *CD2-Associated protein* (*CD2AP*) has been associated with an increased risk of late-onset Alzheimer's disease in multiple genome-wide association studies (GWAS). Our published research has previously shown that loss of *CD2AP*'s *Drosophila* homolog *cindr* exacerbates tau toxicity and impairs synaptic maturation and function at the neuromuscular junction. However, little is understood about CD2AP at the central mammalian synapse, and due to the protein's important function in the kidney, knockout mice die at a young age. The present study demonstrates conservation of CD2AP function at the pre-synapse from fly to mouse, characterizes CD2AP's role in maintaining post synaptic structure, and introduces an alternative model to the full-body *CD2AP* knockout.We examine short- and long-term plasticity using electrophysiologic measurements and synaptic structure and morphology using confocal imaging *in vivo* and *in vitro*. Experiments were performed on homozygous *CD2AP* knockout mice and heterozygous *CD2AP* knockout mice to investigate potential haploinsufficient requirement. Mice constitutively expressing Cas9 endonuclease were used for brain-specific *CD2AP* knockout. We also developed a conditional knockout model, allowing studies in the mouse past 6 weeks of age, and began behavioral characterization of the model at 3 months.We find that homozygous and heterozygous *CD2AP* knockout mice exhibit increased paired-pulse facilitation - a result that matches our data from *Drosophila* and indicates altered short-term plasticity and decreased probability of presynaptic vesicle release with *CD2AP* loss. In *CD2AP* knockout neurons in vitro, we find increased dendritic branching but decreased dendritic spine density. Our characterization of dendritic spine types reveals an increase in the number and density of filopodia, potentially indicating immature synapses in the knockout neurons. Lastly, we see no difference in young *CD2AP* conditional knockout mice (3.5-4 m.o.; N=24, 10 males) compared to controls (age-matched; N=13, 7 males) across multiple behavioral assays, including Open Field, Elevated Plus Maze, Barnes maze, Y maze, and fear conditioning. Studies are currently ongoing to examine performance in these behavioral tests in older animals.Our analyses implicate *CD2AP* in modulation of synaptic plasticity and structure, suggest a conservation of *CD2AP* function from flies to mice, and support *CD2AP* haploinsufficiency. Going forward, we will investigate age-dependent effects of *CD2AP* loss using aged *CD2AP* knockout animals.

Nanosymposium

677. Alzheimer's Disease and Models of Alzheimer's Disease

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 677.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Revealing hidden sensorimotor memories in mice with AD-relevant pathology

Authors: *A. SANTI[1], S. Y. MOORE CORONA[2], A. WANG[3], J. LAWLOR BLONDEL[4], K. FOGELSON[6], K. KUCHIBHOTLA[5];
[1]Johns Hopkins University, Psychological & Brain Sci., [2]Psychological and Brain Sci. Dept., [4]Psychological and Brain Sci., [5]Psychological and Brain Sciences, Neurosci. and Biomed. Engin., [3]Johns Hopkins Univ., Baltimore, MD; [6]Biomed. Sci., UC San Diego, La Jolla, CA

Abstract: Memories must be accessible for them to be useful. Alzheimer's disease (AD) is a progressive form of dementia in which cognitive capacities slowly deteriorate due to underlying neurodegeneration. Interestingly, anecdotal observations have demonstrated that Alzheimer's patients can exhibit cognitive fluctuations during all stages of the disease. In particular, it is thought that contextual factors are critical for unlocking these hidden memories. To date, however, exploration of the neural basis of cognitive fluctuations has been hampered due to the lack of a behavioral approach in mouse models to dissociate memories from contextual performance. Our previous work demonstrated that interleaving 'reinforced' trials with trials without reinforcement ('probe' trials) in an auditory go/no-go discrimination task, allows us to distinguish between acquired sensorimotor memories and their contextual expression. Here, we used this approach, together with two-photon calcium imaging on behaving AD-relevant mice (APP/PS1+), to determine whether amyloid accumulation impacts underlying sensorimotor memories (measured using 'probe' trials) and/or contextual-performance (measured using 'reinforced' trials) in an age dependent manner. Importantly, peripheral auditory function, measured using the threshold for detecting an auditory brainstem response, was similar between WT and APP/PS1+ mice. We found that while contextual-performance is significantly impaired in young adult APP/PS1+ mice compared to age-matched controls, these animals show little to no impairments in the underlying sensorimotor memories. However, middle aged APP/PS1+ mice show deficits in both domains. The impairment found in the young adults was accompanied by a reduction in stimulus selectivity and behavioral encoding in the auditory cortex of APP/PS1+ mice that that can be partially restored in probe trials. Ongoing analyses aim to identify whether this impairment is cortex-wide or is concentrated near Aβ plaques. Finally, these effects were recapitulated by using a reinforcement learning model that accounts for

changes in contextual signals. The main network model parameters affected between the control and the APP/PS1+ mice were those governing contextual scaling and behavioral inhibition. These results suggest that Aβ deposition impacts circuits involved in contextual computations before those involved in storing memories and that neural circuit interventions, such as modulating inhibition, may hold promise to reveal hidden memories.

Disclosures: A. Santi: None. **S.Y. Moore Corona:** None. **A. Wang:** None. **J. Lawlor Blondel:** None. **K. Fogelson:** None. **K. Kuchibhotla:** None.

Nanosymposium

677. Alzheimer's Disease and Models of Alzheimer's Disease

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 677.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ministry of Education, Singapore, MOE AcRF Tier 3 Award MOE2017-T3-1-002

Title: Common hippocampal spatial coding deficits in aging and in Alzheimer's disease

Authors: *Y. FANG[1], R. LONG[3], S.-C. YEN[3], G. J. AUGUSTINE[2];
[1]Ageing Res. Inst. for Society and Education, Interdisciplinary Grad. Programme, [2]Lee Kong Chian Sch. of Med., Nanyang Technological Univ., Singapore, Singapore; [3]The N.1 Inst. for Hlth., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Hippocampal spatial memory is impaired during normal aging and in Alzheimer's disease (AD). However, the relationship between these two effects has not been defined. We characterized hippocampal spatial coding in both wild-type and AD model mice during aging. Our AD model mice had three human AD-related mutations knocked into their amyloid precursor protein gene (APP-TKI; Nature Neurosci. 17: 661). *In vivo* two-photon calcium imaging was used to measure the activity of hippocampal CA1 neurons when the mice moved on a 220-cm treadmill. The effects of aging on spatial coding were examined by comparing young (3-8 months) and old (17-24 months) wild-type (WT) mice expressing GCaMP6f (PloS ONE 9: e108697). Compared to young mice, the hippocampal neuron population of old mice exhibited a 230% larger spatial decoding error and a 19% lower spatial information content (SIC) in the responses of individual neurons. Place cell properties changed in old mice: changes included a 22% reduction in place cell fraction, 3% decrease in the fraction of multi-field place cells, a 20% increase in place field width, and a 4% reduction in the entropy of place field positions. We also characterized spatial coding in APP-TKI mice during aging. Age-related degradation of spatial coding and place cell properties were also observed in APP-TKI mice. These results indicate spatial coding declines in aging of both normal and AD mice. Next, we examined the effects of AD by comparing WT mice with APP-TKI mice. APP-TKI mice showed a mild relative impairment of spatial coding at 3-8 months, which was more severe at 17-24 months. Compared to old WT mice, old APP-TKI mice exhibited a 260% larger decoding error and a 21% lower

SIC. They also had altered place cell properties, including a 2% reduction in place cell fraction, a 5% reduction in multi-field place cell fraction, and a 10% increase in place field width. In summary, AD and aging affect spatial coding in qualitatively similar ways, differing only in severity. The common spatial coding deficits in aging and AD suggest that similar circuit-level mechanisms underlie both.

Disclosures: Y. Fang: None. **R. Long:** None. **S. Yen:** None. **G.J. Augustine:** None.

Nanosymposium

677. Alzheimer's Disease and Models of Alzheimer's Disease

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 677.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Acute and chronic ethanol alters amyloid-β and neuronal excitability/inhibitory phenotypes in APP/PS1 mice.

Authors: *S. M. DAY, S. C. GIRONDA, C. W. CLARKE, J. A. SNIPES, N. I. NICOL, W. VAUGHAN, J. L. WEINER, S. L. MACAULEY;
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Abstract: Alcohol use disorder (AUD) is a risk factor for Alzheimer's disease (AD), and preclinical studies consistently show that chronic ethanol exposure increases amyloid-β (Aβ) in male APPswe/PSENdE9 (APP/PS1) mice. Nevertheless, the underlying mechanisms by which excessive alcohol drinking drives AD-like pathology are poorly understood. In this study we used male APP/PS1 mice to explore the mechanisms by which acute and chronic ethanol increase Aβ. For the acute studies, mice were given 2.0 g/kg or 3.0 g/kg ethanol. We used in vivo microdialysis to measure changes in Aβ levels in hippocampal interstitial fluid (ISF) during ethanol exposure and withdrawal. ISF Aβ levels did not initially change in response to a 2.0 g/kg dose of ethanol but increased by ~20% during withdrawal. Conversely, ISF Aβ levels decreased by ~15% after 3.0 g/kg ethanol and increased by ~20% during withdrawal. We next used a moderate two-bottle choice drinking paradigm to measure how 10 weeks of ethanol exposure altered Aβ pathology. Ethanol-exposed APP/PS1 mice had increased brain atrophy and an increased number of amyloid plaques. Further analysis revealed that ethanol-exposed mice developed a greater number of small amyloid plaques, potentially setting the stage for greater

plaque proliferation. Aβ is released into the brain in an activity-dependent manner and ethanol bidirectionally alters brain activity during exposure and withdrawal. Therefore, we investigated how ethanol altered cortical $GABA_A$ and NDMA receptor subunit mRNA transcription. *Grin2b* mRNA levels were higher in ethanol-exposed APP/PS1 mice than in wild-type mice. There was also a trend towards decreased *Gabra5* mRNA levels in ethanol-exposed APP/PS1 mice compared to H_2O-exposed APP/PS1 mice. Together these studies demonstrate that acute ethanol directly increases ISF Aβ levels during withdrawal. Also, chronic moderate intake of ethanol may prime the brain for greater plaque proliferation later in life. We identified changes in the neuronal excitatory/inhibitory phenotypes as a potential mechanism driving these effects. Together, these data provide foundational research for a growing field investigating the mechanisms by which AUD increases the risk for AD.

Nanosymposium

677. Alzheimer's Disease and Models of Alzheimer's Disease

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 677.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Epichaperomes: an emerging target for precision medicine approach to proteostasis defects in Alzheimer's disease

Authors: *A. R. SANTHA SEELA[1], S. BAY[1], S. SHARMA[1], C. S. DIGWAL[1], A. ALAM[1], P. YAN[1], A. RODINA[1], M. J. ALLDRED[2], F. HAUT[3], O. ARANCIO[3], S. D. GINSBERG[2], G. CHIOSIS[1];
[1]Chem. Biol. Program, Sloan Kettering Inst., New York, NY; [2]Nathan Kline Inst., Orangeburg, NY; [3]Columbia Univ., New York, NY

Abstract: Alzheimer's disease (AD) is a disease of complex etiology in which genetic, epigenetic and environmental factors, alone or combined, lead to patient-specific alterations in brain circuitry and ultimately, in the cognitive decline associated with AD. Such complexity has limited our ability to identify therapeutic target(s) to account for the multiple pathological pathways that contribute to AD progression. Our recent findings that stressors and vulnerabilities

associated with AD rewire proteome-wide connectivity, and thus cellular function through epichaperomes, maladaptive disease-associated pathologic scaffolds composed of tightly bound chaperones, co-chaperone and other factors, provide such a core unifying AD mechanism. In AD, epichaperomes negatively impact assembly of proteins important for synaptic plasticity, cell-to-cell communication, protein translation, cell cycle re-entry, axon guidance, and metabolic processes and inflammation, all biological functions known to decline in AD. This disrupts and remodels brain networks ranging from intercellular to brain connectome levels. Epichaperomes therefore provides unique proteostasis-related precision medicine opportunities for detection and reversal of functional imbalances associated with AD. The discovery and clinical translation of the epichaperome agent PU-AD, currently in Phase 2 studies in AD (https://www.alzforum.org/therapeutics/pu-ad), and of a companion diagnostic, including recent developments are presented.

Disclosures: A.R. Santha seela: None. **S. Bay:** None. **S. Sharma:** None. **C.S. Digwal:** None. **A. Alam:** None. **P. Yan:** None. **A. Rodina:** None. **M.J. Alldred:** None. **F. Haut:** None. **O. Arancio:** None. **S.D. Ginsberg:** None. **G. Chiosis:** None.

Nanosymposium

677. Alzheimer's Disease and Models of Alzheimer's Disease

Location: SDCC 31ABC

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 677.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Tubulin Re-tyrosination in Synaptic Damage, Plasticity, and Neurodegenerative Disease

Authors: J. PARATO[1,2], L. PERIS[3], X. QU[1], F. LANTE[3], Y. GOLDBERG[4], A. BUISSON[5], A. SPROUL[1], M.-J. MOUTIN[6], A. ANDRIEUX[7], *F. BARTOLINI[1];
[1]Columbia Univ., New York, NY; [2]SUNY ESC, Brooklyn, NY; [3]Univ. of Grenoble Alpes, Grenoble, France; [4]yves.goldberg@univ-grenoble-alpes.fr, University of Grenoble Alpes, France; [5]Inserm U1216 - Univ. Grenoble Alpes, Grenoble Inst. Des Neurosciences, Grenoble, France; [6]Grenoble Inst. Neurosciences, Grenoble (la Tronche), France; [7]GRENOBLE INSTITUT NEUROSCIENCES, Grenoble Univ., Bron, France

Abstract: Neurons possess both stable and dynamic microtubules. Dynamic microtubules differ from stable microtubules in their ability to undergo depolymerization and polymerization and for lacking tubulin modifications. While it has long been known that stable microtubules support dendritic and axonal structure, the role of microtubules at synapses has been explored only over the past decade. Recent evidence indicates that dynamic microtubules play key regulatory functions in neurotransmission and synaptic plasticity. Stable microtubules contain de-tyrosinated tubulin while dynamic microtubules are composed almost exclusively of tyrosinated tubulin, suggesting that the tubulin detyrosination/tyrosination/(deTyr/Tyr) cycle can modulate

synaptic function. In the deTyr/Tyr tubulin cycle, the C-terminal tyrosine of newly synthesized α-tubulin is episodically cleaved on microtubules and then returned by the tubulin tyrosine-ligase (TTL) on depolymerized tubulin. We investigated whether the deTyr/Tyr cycle had an impact on synaptic plasticity and was affected in Alzheimer's disease (AD). We found that: 1) soluble oligomeric amyloid β1-42 (oAβ), the synaptotoxic peptide that accumulates in the brain of patients affected by AD, generates de-tyrosinated microtubules in primary neurons and this activity is sufficient to phosphorylate tau and induce tau-dependent neuronal injury; 2) defects in tubulin re-tyrosination are a feature of sporadic and familial AD; 3) Ttl+/- mice exhibit more de-tyrosinated microtubules and defects in synaptic activity and memory function; 4) loss of tubulin re-tyrosination inhibits microtubule dynamics, while inducing tau hyperphosphorylation and loss of dendritic spines; 5) spines invaded by dynamic microtubules are more resistant to oAβ, and normalizing tubulin re-tyrosination promotes microtubule entry into spines and prevents oAβ-induced spine pruning.Our results argue strongly for a role of tyrosinated dynamic microtubules in the regulation of synaptic function and provide evidence that induction of tubulin de-tyrosination is a feature of AD and may contribute to AD pathology by affecting synaptic function. Our data further suggest that previously unrecognized microtubule-dependent pathways regulating tau phosphorylation may lead to the identification of novel therapeutic approaches to reduce tau hyperphosphorylation in AD and related tauopathies.

Disclosures: J. Parato: None. **L. Peris:** None. **X. Qu:** None. **F. Lante:** None. **Y. Goldberg:** None. **A. Buisson:** None. **A. Sproul:** None. **M. Moutin:** None. **A. Andrieux:** None. **F. Bartolini:** None.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.01

Topic: H.08. Learning and Memory

Title: Adult neurogenesis improves spatial information encoding in the hippocampus

Authors: *M. FRECHOU, S. MARTIN, K. MCDERMOTT, S. GOKHAN, W. A. TOMÉ, R. COEN-CAGLI, J. GONCALVES;
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Abstract: The dentate gyrus (DG) is one of the few regions of the mammalian brain that continuously integrates new neurons through adulthood. Adult-born neurons (ABNs) contribute to memory discrimination tasks during a critical developmental period at 4-6 weeks post-mitosis. Exposing animals to enriched environments (EE) results in an increased number of ABNs, and improvement in context discrimination and spatial memory tasks. Conversely, decreasing the number of ABNs results in spatial memory deficits. Still, it is unknown how a small cohort of ABNs can influence the network activity and information encoding of the DG. Here, we demonstrate that exposing mice to EE results in an increase in spatial information encoding of the DG neuronal population responses compared to controls and can be largely attributed to sharper spatial tuning of individual DG neurons. Conversely, ablating DG adult neurogenesis resulted in a decrease in spatial tuning. Importantly, the increase in spatial tuning seen in EE mice is not present in mice exposed to EE where neurogenesis was ablated by focal irradiation, indicating that ABNs are necessary for the effects of EE on DG network activity. Additionally, we show that acutely silencing ABNs using a chemogenetic approach also results in a decrease in spatial tuning and in the EE-exposed animals being less able to discriminate between similar contexts during a contextual fear conditioning task. These results demonstrate, for the first time, that ABNs increase both the information content of the DG and the spatial tuning of individual DG granule cells, providing novel insight into the mechanisms behind the cognitive benefits of adult neurogenesis.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.02

Topic: H.08. Learning and Memory

Title: Ccr5 closes the temporal window for memory linking

Authors: *Y. SHEN[1], M. ZHOU[7], D. CAI[8], D. ALMEIDA FILHO[1], G. FERNANDES[1], Y. CAI[2], A. D. SOUSA[1], M. TIAN[3], N. KIM[9,10], J. LEE[10], D. NECULA[4], C. ZHOU[4], S. LI[4], S. SALINAS[7], A. LIU[4], X. KANG[4], M. KAMATA[5], A. LAVI[6], S. HUANG[4], T. SILVA[4], W. HEO[10], A. SILVA[4];
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Abstract: Real world memories are formed in a particular context and are often not acquired or recalled in isolation. Time is a key variable in the organization of memories. Previous studies in our laboratory showed that when there is substantial overlap between the neuronal ensembles of two memories encoded close in time, recall of one memory increases the likelihood of recalling the other memory, thus linking the two memories across time. Understanding the mechanisms that regulate the temporal window of memory linking is critical for understanding how the brain segregates events that are temporally distinct. Here, we show that that C-C chemokine receptor type 5 (CCR5), an immune receptor well known as a co-receptor for HIV infection, plays a key role in closing the temporal window for memory linking. Following contextual conditioning there was a delayed (12-24h) increase in the expression of the C-C chemokine receptor type 5 (CCR5). This delayed CCR5 expression in mouse dorsal CA1 (dCA1) neurons results in a decrease in neuronal excitability, which in turn negatively regulates neuronal memory allocation, thus reducing the overlap between dCA1 memory ensembles. Lowering this overlap affects the ability of one memory to trigger the recall of the other, thus closing the temporal window for memory linking. Remarkably, our findings also show that an age-related increase in neuronal CCL5/CCR5 expression leads to impairments in memory linking in aged mice, which could be reversed with a CCR5 knockout and an FDA approved drug that inhibits this receptor, a result with significant clinical implications. All together the findings reported here provide the first insights into the molecular and cellular mechanisms that shape the temporal window for memory linking.

Disclosures: Y. Shen: None. **M. Zhou:** None. **D. Cai:** None. **D. Almeida Filho:** None. **G. Fernandes:** None. **Y. Cai:** None. **A.D. Sousa:** None. **M. Tian:** None. **N. Kim:** None. **J. Lee:** None. **D. Necula:** None. **C. Zhou:** None. **S. Li:** None. **S. Salinas:** None. **A. Liu:** None. **X. Kang:** None. **M. Kamata:** None. **A. Lavi:** None. **S. Huang:** None. **T. Silva:** None. **W. Heo:** None. **A. Silva:** None.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Enhanced neurogenesis ameliorates memory impairments in Alzheimer's disease by restoring engram cell function.

Authors: *T. PHAN[1], P. KUMAR[3], Z. MORRISSEY[4], R. MISHRA[2], M. GUPTA[5], C. L. HOLLANDS[6], A. SHETTI[5], K. LOPEZ[2], R. HEN[7], M. MAIENSCHEIN-CLINE[2], H. SUH[8], O. LAZAROV[2];

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Abstract: Adult hippocampal neurogenesis is diminished with age and compromised in diseases such as Alzheimer's disease (AD). However, a direct mechanism linking the enhancement of hippocampal neurogenesis in familial AD (FAD) models to the rescue of memories is still lacking. Here, we showed that enhancing neurogenesis in an FAD model rescued associative and spatial memories by restoring memory engram. Specifically, the newly mature neurons were preferentially incorporated in the neuronal ensemble active during both the acquisition and retrieval stages of memory formation, the *bona fide* engram cells. FAD engrams cells possessed altered transcriptomic profiles with far-less developed dendrites in length, branching, spine density, and morphological changes. Remarkably, these defects were reversed in the genetically enhanced neurogenesis model in FAD. Chemogenetic silencing the newly mature neurons in the FAD with genetically enhanced neurogenesis model reversed the aforementioned rescued memory. Interestingly, AD- linked App, ApoE and Adam10 were some of the top differentially expressed genes in the engram. Taken together, this study was the first to implicate direct mechanistic evidence that targeted augmented neurogenesis ameliorates memory impairments by increasing the number of recruited engram cells, leading to memory restoration. This study implies that augmenting hippocampal neurogenesis in AD may be therapeutic.

Disclosures: T. Phan: None. **P. Kumar:** None. **Z. Morrissey:** None. **R. Mishra:** None. **M. Gupta:** None. **C.L. Hollands:** None. **A. Shetti:** None. **K. Lopez:** None. **R. Hen:** None. **M. Maienschein-Cline:** None. **H. Suh:** None. **O. Lazarov:** None.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.04

Topic: H.08. Learning and Memory

Title: Non-invasive gamma sensory stimulation impacts neural activity important for learning and memory in the hippocampus of a mouse model of Alzheimer's disease

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Abstract: Recent work reveals an important role for network dysfunction in Alzheimer's disease (AD), a devastating neurodegenerative disorder that is characterized by the accumulation of toxic proteins, aberrant neural activity, and deficits in spatial learning and memory. Alterations in neural activity have been shown to influence disease pathology and impair cognition, suggesting that neural activity may be a promising target for therapeutics to treat AD. Neural activity deficits emerge early in AD in the hippocampus, an area of the brain crucial for spatial learning and memory. AD pathology disrupts gamma oscillations (30-50 Hz, also known as slow gamma) and sharp-wave ripples (SWRs, 150-250 Hz), patterns of activity known to be important for learning and memory. Prior work has shown that exposure to 40 Hz auditory and light flicker stimulation drives gamma frequency neural activity in the hippocampus, reduces levels of toxic proteins that accumulate in AD, recruits microglia, the immune cells of the brain, and improves performance in spatial memory tasks in a mouse model of AD. However, it is unknown how prolonged gamma sensory flicker impacts endogenous neural activity crucial for learning and memory. To answer this question, we trained 5XFAD mice to navigate through a virtual reality (VR) environment to receive rewards. We recorded local field potentials and spiking activity from large populations of single neurons in hippocampal areas CA1 and CA3 as mice navigated in a VR task before and after several days of exposure to 40 Hz audio-visual flicker stimulation. We investigated the effects of prolonged exposure to 40 Hz audio-visual stimulation on aspects of hippocampal activity that have previously been found to be deficient in Alzheimer's disease or correlated with cognitive function. We found impacts on SWR properties and SWR-associated place cell firing activity following 40 Hz sensory flicker stimulation as compared to random stimulation. These findings elucidate the effects of 40 Hz sensory flicker on endogenous neural activity in a mouse model of Alzheimer's disease and suggest mechanisms through which exposure to flicker stimulation acts to improve cognitive function. This non-invasive method to impact neural activity essential for cognitive function carries promising translational applications to Alzheimer's disease, as well as other neurological diseases with altered rhythmic neural activity.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Gene replacement of mutant PRESENILIN1 normalizes gamma-secretase function in models of Autosomal Dominant Alzheimer's Disease

Authors: *B. MOORE, A. SHARMA, T. SUTER, E. SCHAEFFER;
Paros Bio, Boston, MA

Abstract: Paros Bio is developing an AAV gene therapy for the treatment of Autosomal Dominant Alzheimer's Disease (ADAD), an early onset form of AD (onset at <65 years of age). ADAD is mainly caused by mutations in the PRESENILIN1 (PSEN1) gene, which encodes the catalytic subunit of the γ-secretase complex and is responsible for the cleavage of amyloid precursor protein (APP). This cleavage produces Aβ of varying lengths, with longer peptides like Aβ42 being more prone to aggregation than shorter peptides such as Aβ40. PSEN1 mutations result in a significant loss of γ-secretase function, resulting in decreased production of Aβ40 and an increase in the ratio of Aβ42:Aβ40. Aggregation of Aβ peptides into amyloid plaques in the brain is a hallmark of ADAD. Paros Bio is employing a gene-replacement approach to normalize γ-secretase function. Using an AAV vector, Paros has successfully expressed a functional copy of *PSEN1* in mouse and patient-derived-cellular models of PS1 dysfunction. In several efficacy studies, we have demonstrated that treatment with our therapeutic in both ADAD mouse models and patient-derived cell lines effectively restores g-secretase function to normal levels. A single injection of our therapeutic both prevents pathological increases in the ratio of Aβ42:Aβ40 and normalizes functional neurodegenerative phenotypes in mouse models of ADAD. Non-human primate studies show our AAV vector can be broadly distributed and expressed in brain tissue and provides attractive expression of PS1. Paros Bio has developed a vector that enables broad expression of functional PSEN1 in brain tissue and is capable of normalizing the g-secretase dysfunction present in in vitro and in vivo models of ADAD.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Exploring flavonoid clearance of toxic amyloid-β using a genetically tractable and versatile in vivo model of Alzheimer's disease

Authors: *R. HERON[1], R. WILLIAMS[2], W. WOOD[1];
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Abstract: Flavonoids, which have numerous health benefits, are of growing interest to neuroscientists thanks to their potential to lower the risk of developing Alzheimer's disease (AD) and dementia. In animal models, some flavonoids are able to reduce the levels of toxic amyloid associated with AD and, although ongoing clinical trials remain in the early stages, they show similar promise. To help understand the ability of flavonoids to clear toxic amyloid-*beta* and to aid in the development of suitable interventions for AD, we utilise a genetically versatile *Drosophila melanogaster* model of AD that replicates the overproduction and secretion of toxic amyloid-*beta* (hAβ42) from neurons. This AD model presents distinctive behavioural phenotypes such as locomotor defects and early mortality. Immunofluorescent staining shows that these phenotypes are associated with characteristic hAβ42 accumulation in subsets of neurons. By feeding different flavonoids to these animals in a blinded and statistically powerful study, we have undertaken structure-activity relationships and observed varying abilities of flavonoids to reduce the levels of hAβ42 around the neurons and prevent locomotor defects. All animals were age-matched and sex differences were assessed in this study. Notably, when neuronal clearance of hAβ42 was partial, the locomotor rescue was correspondingly weaker. In conclusion, we have developed a genetically tractable and versatile *in vivo* model of AD that allows us to quickly explore the mechanisms through which ingested flavonoids can exert anti-amyloid activity in AD and support further development of flavonoid-based therapeutics.

Disclosures: R. Heron: None. **R. Williams:** None. **W. Wood:** None.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A CRISPR-based therapeutic for Alzheimer's disease

Authors: *B. AULSTON, K. BRANES, N. CHECKA, S. ROY;
Univ. of California San Diego, La jolla, CA

Abstract: Our lab has developed a novel CRISPR-based, APP-editing approach that effectively deletes the YENPTY endocytic domain at the extreme C-terminus of APP and favorably modulates APP processing by attenuating pathologic ß-cleavage and upregulating neuroprotective a-cleavage. Importantly, the vast majority of the APP molecule - including the

elaborate N-terminus and membrane-spanning domain - remains intact in this setting ensuring that physiological processes continue unabated. In order to assess the safety and efficacy of our approach in vivo, we used CRISPRs to generate germline and somatic edits in Wt mice and in an APP knockin (APP[NL/G/F]) mouse model of Alzheimer's disease (AD). In vivo editing of the APP C-terminus in embryos efficiently eliminated the YENPTY domain, while preserving the N-terminus. No behavioral or gross histological defects were detected in edited Wt mice and edited APP KI mice displayed a dramatic reduction of amyloid plaques and associated neuroinflammation compared to controls. Similar changes were seen with somatic AAV-mediated editing, along with a marked increase in neuroprotective sAPPα. In total, these data demonstrate the in vivo feasibility of APP C-terminus CRISPR editing as a treatment strategy for AD.

Disclosures: B. Aulston: None. **K. Branes:** None. **N. Checka:** None. **S. Roy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CRISPRAlz.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Deletion of microRNA-21 exacerbates multiple phenotypes associated with Alzheimer's disease through the RECK and ADAM10 pathway in a mouse model of Aβ amyloidosis

Authors: *B. KIM[1,2], M. D. TATE[2,3], H. KARAHAN[1,2], S. PHILTJENS[1,2], H. R. S. WIJERATNE[1,4], M. M. AL-AMIN[1,2], A. D. SHARIFY[1], J. KIM[1,2,3];
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Abstract: microRNAs have emerged as a new class of therapeutic targets that regulate the expression of target genes by binding their 3'-untranslated regions. Growing evidence suggests that dysregulation of microRNAs may contribute to the onset and/or progression of various diseases. However, the functional and therapeutic implication of microRNAs dysregulation in Alzheimer's disease (AD) remains largely unclear. To gain insight into the roles of microRNAs in AD pathogenesis, we recently performed unbiased microRNA profiling in entorhinal cortices of AD and age-matched non-demented subjects. We found that microRNA-21 (miR-21) expression was markedly increased in AD patients. Therefore, we investigated the pathogenic/therapeutic potential of miR-21 in AD. miR-21 levels were increased in neurons treated with oligomeric amyloid-beta (Aβ), suggesting that miR-21 induction is directly associated with Aβ pathology. In addition, we demonstrated that miR-21 deficiency decreases

spatial learning and contextual memory in Morris Water Maze and Contextual fear conditioning tests, respectively, in APP/PS1ΔE9 transgenic mice. Moreover, genetic deletion of miR-21 increases the levels of insoluble Aβ40 and Aβ42 accumulation, amyloid plaque, and X-34-positive fibrillar plaque load in the cortex and hippocampus. CD45-positive, IBA1-positive microgliosis, and GFAP-positive astrogliosis were also increased in mir-21 KO;APP/PS1ΔE9 transgenic mice. Mechanistic studies identified RECK as a potential target gene of miR-21. miR-21 deficiency upregulated the RECK protein level, leading to inhibition of ADAM10 activity. This finding was also corroborated by the decrease in the soluble APPα level. Taken together, our results demonstrated an important role for miR-21 in AD pathogenesis, suggesting that miR-21 may regulate multiple AD-associated pathologies. Therefore, modulation of miR-21 may represent a novel potential therapeutic strategy for AD.

Disclosures: B. Kim: None. **M.D. Tate:** None. **H. Karahan:** None. **S. Philtjens:** None. **H.R.S. Wijeratne:** None. **M.M. Al-Amin:** None. **A.D. Sharify:** None. **J. Kim:** None.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.09

Topic: H.08. Learning and Memory

Title: An aging-induced neuronally secreted factor that promotes cognitive resilience

Authors: *C. VICIDOMINI[1,2,3,4], K. MCAVOY[1,2,3,4], T. D. GOODE[1,2,3,4], G. HAYWARD-LARA[4], K. YAMAMOTO[5,6], M. MURAKAMI[6,5], S. HO SUI[7], A. SAHAY[1,2,3,4];
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Abstract: Age-associated cognitive decline is characterized by numerous physiological changes in the hippocampus including synapse loss, increased neural stem cell quiescence, and emergence of inflammatory microglia and reactive astrocytes. In a genetic screen for secreted factors induced by dentate granule neuronal synapse loss, we identified a Group IIF secreted phospholipase A2 (hereafter referred to as sPlaA2) of unknown function in the brain. We found

that sPlaA2 expression is negligible in the adult hippocampus but is gradually upregulated exclusively in dentate granule cells during aging. Genetic or viral deletion of sPlaA2 in the dentate gyrus of middle-aged animals prematurely induced age-related phenotypes in microglia, astrocytes, neurogenesis, synapse loss, and impaired hippocampal-dependent memory. In contrast, viral overexpression of sPlaA2 in the dentate gyrus of aged animals restored memory precision. Taken together, these results indicate that Group IIF secreted phospholipase A2 exerts a neuroprotective and anti-inflammatory role in the hippocampus during aging to confer cognitive resilience. Group IIF secreted phospholipase A2 represents a novel therapeutic target to slow down age-related cognitive decline and moderate the effect of aging as a risk factor for Mild Cognitive Impairment and Alzheimer's disease.

Disclosures: C. Vicidomini: None. **K. McAvoy:** None. **T.D. Goode:** None. **G. Hayward-Lara:** None. **K. Yamamoto:** None. **M. Murakami:** None. **S. Ho Sui:** None. **A. Sahay:** None.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.10

Topic: H.08. Learning and Memory

Title: REM sleep augmentation by an atypical hypnotic compound targeting G-protein inward rectifying K+ channel promotes sleep-dependent hippocampal memory consolidation

Authors: *W. P. BRANCALEONE, J. D. MARTINEZ, K. G. PETERSON, L. G. WILSON, S. J. ATON;
Molecular, Cellular, and Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: Sleep plays a critical role in consolidating many forms of hippocampus-dependent memory. While various classes of hypnotic drugs have been developed in recent years, it remains unknown whether, or how, some of them affect sleep-dependent memory consolidation mechanisms. We find that administration of ML297, a novel candidate hypnotic agent that selectively activates the G-protein coupled inwardly rectifying potassium channel subunit GIRK1, alters sleep architecture in C57BL/6 male mice over the first 6 hours following a single-trial learning event. Following contextual fear conditioning (CFC), ML297 reversed post-CFC reductions in NREM sleep spindle power and REM sleep amounts and architecture, renormalizing sleep features to what was observed at baseline, prior to CFC. Renormalization of post-CFC REM sleep latency, REM sleep amounts, and NREM spindles at the transition to REM were all associated with improved contextual fear memory (CFM) consolidation. We find that improvements in CFM consolidation due to ML297 are sleep-dependent, and can be disrupted if mice are sleep deprived following ML297 administration. Improved CFM recall is associated

with increased numbers of highly-activated dentate gyrus (DG), CA1, and CA3 neurons expressing immediate early genes after recall. Together our findings suggest that GIRK1 activation restores normal sleep architecture - including REM sleep, which is normally suppressed following CFC - and increases the number of hippocampal neurons incorporated into the CFM engram during memory recall.

Disclosures: W.P. Brancaleone: None. **J.D. Martinez:** None. **K.G. Peterson:** None. **L.G. Wilson:** None. **S.J. Aton:** None.

Nanosymposium

678. Strategies to Improve Cognition

Location: SDCC 5

Time: Wednesday, November 16, 2022, 1:00 PM - 3:45 PM

Presentation Number: 678.11

Topic: H.08. Learning and Memory

Title: Demonstration of Error-based Intracranial EEG Real-time Neurofeedback for Human Memory enhancement

Authors: *I. SONG[1], S. JUN[2], K. MENG[3], J. KIM[4], C. CHUNG[1,5];
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Abstract: **Introduction:** Being able to predict memory encoding outcomes from intracranial EEG (iEEG) during real-time encoding has significant value from a neuropsychological perspective. Previous study showed increased hippocampal theta activity (4-10 Hz) is linked to better verbal associative memory (Jun et al., 2020). Delivering real-time neurofeedback based on previous findings would allow subjects to self-regulate their memory ability and optimize their overall performance outcome. However, no study using iEEG has yet investigated neurofeedback approaches to enhance memory in verbal memory tasks. **Methods:** In this study, we aim to investigate the effect of theta (4-10 Hz) neurofeedback on memory encoding performance. We recorded iEEG from the hippocampus, while one epilepsy patient performed a word pair associative memory task. Memory task included three encoding blocks and one retrieval block. Each encoding block corresponded with the following three conditions: no sound, real-time random sound, and real-time sound neurofeedback based on theta band. During the encoding block, the subject had to memorize the given word pairs. After encoding, the subject went through some simple arithmetic questions as a distractor. Then, at the retrieval block, the subject faced a word pair memory test that was asked to answer among the three options: intact, rearrange, and new. The decoding model used the inputs of the theta band power values in the hippocampus. The number of inputs depended on subject-specific electrode placement within the hippocampus. The real-time sound neurofeedback was given during the stimuli presentation and when the subject's threshold level dropped below the criteria. **Result:** In

our behavioral results, control and testing blocks were compared to determine whether or not neurofeedback enhanced memory encoding performance. Compared to no sound and sound given, whether or not it was random or theta based, the memory performance increased when sound was given on a trial base. For the brain wave analysis, the hippocampal time-frequency map showed a high power in the 20-100 Hz frequency. A high power appeared before the word pair onset for no sound condition, and it appeared after the onset timing for theta-based sound neurofeedback condition. **Conclusion:** We discovered that giving theta-based sound neurofeedback enhances subject's memory performance. The results also suggest that giving theta-based neurofeedback may provide additional effects on other ranges on brain waves.

Disclosures: I. Song: None. **S. Jun:** None. **K. Meng:** None. **J. Kim:** None. **C. Chung:** None.

Nanosymposium

679. Microglial Activity and Dysfunction

Location: SDCC 33

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 679.01

Topic: B.09. Glial Mechanisms

Title: Heterogeneous Microglia Activation Mediates Chronic Stress-Induced Synapse Loss

Authors: *B. SOTEROS[1], H. TILLMON[2], H. CHIN[3], G. SIA[2];
[1]UT Hlth. San Antonio, [2]UT Hlth. San Antonio, San Antonio, TX; [3]Univ. of Rochester, Rochester, NY

Abstract: Stress-induced synapse loss is the biological substrate underlying many of the cognitive deficits associated with chronic stress. However, the molecular and cellular substrates of stress-induced synapse loss are not well understood. Here, we show that multiple chronic stress protocols cause complement deposition and microglia activation in the upper layers of the mouse prefrontal cortex. This heterogeneous microglia activation results in layer-specific microglial phagocytosis of synapses and synapse loss, along with behavioral deficits such as anhedonia and working memory impairment. This heterogeneous activation is also reflected in scRNA-seq data revealing the existence of multiple microglia subpopulations in the stressed mouse cortex. Importantly, mice lacking complement component C3 were protected against chronic stress-induced microglial synapse phagocytosis and synapse loss, and did not develop anhedonia or impairment in working memory. Our data indicate that stress-induced complement activation drives synapse loss and behavioral deficits through heterogeneous microglia activation. This work may provide insight into why specific neural circuits are particularly

vulnerable to stress, and how stressful life events can precipitate or exacerbate psychiatric diseases such as schizophrenia and multiple sclerosis.

Disclosures: B. Soteros: None. **H. Tillmon:** None. **H. Chin:** None. **G. Sia:** None.

Nanosymposium

679. Microglial Activity and Dysfunction

Location: SDCC 33

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 679.02

Topic: B.09. Glial Mechanisms

Title: Regulation of microglial state in aged hippocampus by blood-borne proteins

Authors: *B. M. HEMMER, A. C. FERREIRA, J. D. ZHU, A. PHAN, J. M. CASTELLANO;
Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The brain exhibits diminishing function with age that manifests as cellular, molecular, and cognitive changes. Aging renders the brain susceptible to neurological disorders, such as Alzheimer's Disease (AD), for which aging is the strongest risk factor. Given the universally unmet need to lessen the impact of AD, novel approaches are needed to curtail the influence of risk factors for the disorder. Emerging evidence has revealed rejuvenation of the aged CNS by exposure to young blood-borne factors, raising the possibility that CNS function can be improved by targeting systemic pathways. Studies have yet to evaluate how microglia sense and respond to youth-associated plasma factors to mediate plasma-induced changes in aging and AD. To begin to determine how individual factors present in young plasma alter microglia in the context of aging and AD, aged mice were systemically treated with two youth-associated blood-borne factors, tissue inhibitor of metalloproteinases-2 (TIMP2) or colony-stimulating factor 2 (CSF2), resulting in reduced microglial activation in hippocampus compared to vehicle-treated aged mice. Upon exposure to environmental challenges present in aging and AD, a subset of microglia acquire a disease-associated microglia (DAM) phenotype thought to be protective in response to debris. In addition to its role as a secreted protein acting on cells, we find that TIMP2 is expressed by microglia and is a marker of the DAM transcriptional profile, indicating that TIMP2 may play a cell-intrinsic role regulating microglial state. Mice lacking TIMP2 display hippocampal microgliosis. Transcriptomic differences were observed between primary microglia isolated from TIMP2 KO and WT mice by RNA-seq that corresponded to changes in homeostasis, activation, and immune response, based on pathway analyses. Our results argue that microglia are responsive to youth-associated plasma proteins and may regulate aging and AD phenotypes. Characterization of blood-CNS communication may facilitate development of therapies that target detrimental aging processes to limit onset of neurodegenerative diseases.

Nanosymposium

679. Microglial Activity and Dysfunction

Location: SDCC 33

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 679.03

Topic: B.09. Glial Mechanisms

Title: Microglia derived from AD and age-matched controls iPSCs have disease related molecular differences

Authors: *J. REVANNA[1], M. CUOCO[1], S. FERNANDES[1], J. JONES[1], T. SABEDOT[1], R. GOODMAN[1], T. NEWMEYER[1], E. WEST[2], S. SCHAFER[1], F. GAGE[1];
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Abstract: Neuroinflammation is a common feature of Alzheimer's disease (AD), implicating the importance of microglia's role in AD progression. Microglia are specialized resident macrophages of the central nervous system that help to clear debris, pathogens, and neuronal synapses. Under physiological conditions, microglia maintain plasticity by providing trophic support to neurons. Under pathological conditions, microglia often prune synapses excessively, increase pro-inflammatory cytokine release, and reduce trophic factor release. Currently, it is unclear how microglia impact AD. Although microglia activation is associated with amyloid deposits, a causative relationship to disease progression has not been shown. Using a recently established microglia differentiation protocol, we have converted AD and age-matched control patient derived induced pluripotent stem cells (iPSCs) from 16 individuals (8 AD/8 CTLR) into human microglia in order to investigate microglia specific changes inherent to AD. The goal of this project is to analyze the transcriptome and functionality of AD microglia cells in comparison to age-matched controls. By employing single cell RNA sequencing, we are able to analyze differences in gene expression between control and AD microglia cells, revealing AD risk factors specific to microglia that may contribute to pathogenesis. This finding suggests that microglia in AD are contributing to the disease, rather than solely reacting to AD pathogenesis. Using phagocytosis and activation assays, such as adding synaptosomes or bacteria-like lipopolysaccharides (LPS) with the microglia, respectively, allows us to assess if microglia

functionality is impaired/affected in AD. We hypothesize that AD microglia will exhibit differences in activation potential and duration compared to age matched controls. The findings from this study will elucidate aspects of microglial contribution to AD, potentially offering new therapeutic avenues for treatment.

Nanosymposium

679. Microglial Activity and Dysfunction

Location: SDCC 33

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 679.04

Topic: B.09. Glial Mechanisms

Title: Role of neuronal activity and immune signaling in regulating microglial engulfment of oligodendrocyte progenitor cells

Authors: *M. IRFAN, T. DESILVA;
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Abstract: Oligodendrocyte progenitor cells (OPCs) differentiate into mature myelinating oligodendrocytes and insulate axons with layers of myelin to increase their conduction velocity and provide metabolic support. Recent work published from our laboratory (*Nemes-Baran A. et al., 2020 Cell Reports*) showed that amoeboid microglia phagocytose OPCs in the corpus callosum during a restricted window of development before myelination. We further showed that blocking this process in a fractalkine receptor-dependent manner resulted in reduced engulfment of OPCs, increased oligodendrocytes, and thinner myelin suggesting that microglia phagocytose OPCs as a homeostatic mechanism to regulate myelin sheath formation. Changes in neuronal activity are associated with OPC proliferation and consequently changes in axonal myelination. It is, however, not clear if activity-dependent processes regulate microglial phagocytosis of OPCs. Therefore, the goal of this study is to explore the role of activity-dependent mechanisms in microglial engulfment of OPCs. Our results indicate that pharmacological inhibition of action potentials during early postnatal development caused significant changes in microglial engulfment of OPCs, suggesting that this phenomenon is sensitive to changes in neuronal activity. Furthermore, activity-dependent microglial phagocytosis of OPCs occurred during a specific time period when activity-dependent processes are also regulating microglia pruning of synapses. We propose microglial engulfment of OPCs to be a novel mechanism regulating precise axonal myelination, which ultimately has consequences for neural circuit development.

Nanosymposium

679. Microglial Activity and Dysfunction

Location: SDCC 33

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 679.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Global C3 depletion protects against aging-related hippocampal dysfunction in mice

Authors: *A. BATISTA[1,2], M. SCHROEDER[1,2], K. KHAN[1,2], S. LI[1,2], J. PRESUMEY[4,3], E. YALCIN[4,3], M. CARROLL[4,3], C. LEMERE[1,2];
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Abstract: The complement cascade modulates the innate immune system, which leads to the elimination of pathogens and dead or dying cells, as well as contributing to the extent and limit of the inflammatory immune response. Complement C3 is part of the innate immune system and participates in synapse elimination during normal brain development to refine neuronal networks. Further, this molecule may be detrimental in several neurodegenerative diseases, including Alzheimer's disease (AD). C3 levels in the brain are elevated during aging and AD. Our group previously showed that lifelong C3-deficiency protected against hippocampal synapse loss in aged WT and APP/PS1dE9 mice, correlating with improved performance on cognitive tests. Recently, we developed an inducible conditional C3 knockout mouse model (C3iKO) by crossing C3 floxed (C3$^{fl/fl}$) mice with Rosa26-Cre-ERT2+/- mice on a C57BL/6J background. Our goal was to investigate whether C3 lowering in adulthood, after normal brain development, would still protect hippocampal function in these mice. First, 4-5-mo-old male and female C3iKO mice were i.p. injected with tamoxifen (TAM) or corn oil (CO; control) daily for 5 days. Tamoxifen treatment of C3iKO mice led to a sustained ~92% lowering of serum C3 levels. Behavioral testing for hippocampal-dependent spatial memory, object memory, and object location was performed when TAM-treated and CO-treated C3iKO mice reached 16-17 mo of age. Mice that received TAM treatment performed significantly better than COtreated mice in these behavioral tasks, indicating that C3 lowering after brain development protected mice from age-related cognitive decline. In another study, mice were treated with TAM or CO at 3-4 mo of age and electrophysiological recording of long-term potentiation (LTP) was conducted 3 mo later in hippocampal slices incubated with neurotoxic Aβ S26 dimers. Remarkably, C3 lowering protected hippocampal synapses from Aβ S26 dimer-mediated LTP impairment. Further, we also analyzed the number of dendritic spines in the hippocampus when these mice reached 14-17 mo of age. C3 depletion significant alleviated the dendritic spines loss in aged mice. In conclusion,

global C3 lowering in C3iKO young adult mice protected against hippocampal dysfunction many months later, suggesting that targeting C3 may be an effective therapeutic strategy for AD.

Disclosures: A. Batista: None. **M. Schroeder:** None. **K. Khan:** None. **S. Li:** None. **J. Presumey:** None. **E. Yalcin:** None. **M. Carroll:** None. **C. Lemere:** None.

Nanosymposium

679. Microglial Activity and Dysfunction

Location: SDCC 33

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 679.06

Topic: B.09. Glial Mechanisms

Title: Amyloid β induces lipid droplets in microglia leading to their phagocytic dysfunction

Authors: *P. PRAKASH[1], P. MANCHANDA[1], E. PAOURI[2], K. BISHT[1], K. SHARMA[1], P. WIJEWARDHANE[1], C. E. RANDOLPH[1], M. G. CLARK[1], J. FINE[1], E. A. THAYER[1], C. ZHANG[1], D. DAVALOS[2], G. CHOPRA[1];
[1]Purdue Univ., West Lafayette, IN; [2]Cleveland Clin., Cleveland, OH

Abstract: Glial cell phagocytosis is essential for regulating brain function during health and disease. Alzheimer's disease (AD) is linked to amyloid β (Aβ) accumulation; yet, the Aβ-induced changes to the glial phagocytic function are poorly understood. Recent studies indicated that microglia in aged brains accumulate lipid droplets (LDs) and exhibit a proinflammatory and defective phagocytic phenotype (Nat Neurosci. 23(2), 194-208, 2020). However, the effect of Aβ on microglial lipidome and function in AD is uncharacterized to date. Here, we show that Aβ alone is sufficient to stimulate LDs in microglia and that LD-laden microglia from AD mouse brains exhibit impaired Aβ phagocytosis, which may be one of the ways they contribute to AD pathology. Specifically, unbiased lipidomics showed that microglia from wild-type (WT) mice exposed to Aβ in culture counteract the initial fatty acid accumulation by upregulating triacylglycerols (TAGs)—a major LD component. The acutely isolated LD-laden microglia from 5xFAD mouse brains are dependent on age, sex, and brain region compared to the WT microglia. Additionally, these LD-laden 5xFAD microglia showed reduced phagocytosis of AβpH—a human Aβ1-42 analog that exhibits green fluorescence upon lysosomal internalization (Chem Sci. 12, 10901-10918, 2021). Interestingly, we found that most LD-laden microglia were associated with the plaques and had a unique amoeboid reactive morphology compared to the plaque-distant microglia. Human AD brains also contained higher LD density and a higher total LD volume inside the plaque-proximal microglia. Finally, we identified and found the Diacylglycerol O-Acyltransferase 2 (DGAT2) protein to be increased in Aβ-induced LD-laden

microglia in 5xFAD and human AD brains. Pharmacological inhibition of DGAT2 reduced LDs and improved AβpH phagocytosis in 5xFAD microglia. In summary, we present a model of Aβ-induced microglial LD accumulation and phagocytic dysfunction in chronic inflammation. We propose that microglia in AD become "fat and lazy" induced by Aβ and an inflammatory environment that includes neurotoxic fatty acids secreted by reactive astrocytes (Nature. 599, 102-107, 2021) that may also be converted to TAGs and LDs leading to their dysfunction. This work shows the ever-growing dynamic role of LDs, once considered inert fatty organelles.

Disclosures: P. Prakash: None. **P. Manchanda:** None. **E. Paouri:** None. **K. Bisht:** None. **K. Sharma:** None. **P. Wijewardhane:** None. **C.E. Randolph:** None. **M.G. Clark:** None. **J. Fine:** None. **E.A. Thayer:** None. **C. Zhang:** None. **D. Davalos:** None. **G. Chopra:** None.

Nanosymposium

679. Microglial Activity and Dysfunction

Location: SDCC 33

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 679.07

Topic: B.09. Glial Mechanisms

Title: Non-binary activation, retraction of ramifications, lysosomal leakage and NLRP3 inflammasome activation following stimulation of the P2X7 receptor in microglial cells

Authors: *C. MITCHELL[1], K. CAMPAGNO[2], W. LU[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Pennsylvania, Philagelphia, PA

Abstract: Neuroinflammation is implicated in the response accompanying mechanical damage to neural tissue, but the pathways linking strain to neuroinflammation are unclear. The release of ATP through pannexin hemichannels is frequently observed following mechanical strain in neural tissues, suggesting stimulation of receptors for extracellular ATP is likely. This study examines the consequence of stimulating the P2X7 receptor for ATP on activation and cytokine release from microglial cells and asks whether this contributes to the neuroinflammation accompanying mechanical strain. Injection of the P2X7 receptor agonist BzATP increased expression of M1-like *Nos2* and *TNFa*, and M2-like *Arg1* and *Chil3 (YM1)* transcriptions in the retina. Increased gene expression was accompanied by morphological changes in microglial cells; Sholl analysis identified a shift in projections from ramified to a more retracted phenotype, with increased soma size. Similar morphological changes were observed when isolated tissue was exposed to BzATP, implying the changes did not require recruitment of extracellulr monocytes. Isolated microglia recapitulated these changes, with BzATP increasing expression of *Nos2* and *Arg1*, and retraction of ramifications detected within minutes of BzATP application. While ATP increased the migration of microglia through a Boyden chamber, the P2Y12 receptor was the primary driver of the chemotactic response. Transient elevation of intraocular pressure

induced ATP release and parallel changes in microglial morphology and gene expression in C57Bl/6J, but not P2X7 KO mice, implicating a role for the receptor in the microglial activation accompanying mechanical damage. ATP induced a release of IL-1β from isolated brain microglia. Interestingly, this IL-1β release was inhibited by CA-074, a blocker of lysosomal enzyme cathepsin B. Given that the P2X7 receptor can target lysosomes and raise luminal pH, this suggests a model where release of cathepsin B follows lysosomal leakage, with cathepsin B activating the NLRP3 inflammasome, leading to caspase-1 cleavage then IL-1β maturation and release. Overall, this study demonstrates stimulation of the P2X7 receptor mediates a non-binary activation of microglial cells, with IL-1β release at least partially linked to lysosomal leakage. As such, age-dependent lysosomal accumulations may accelerate the inflammatory response induced by receptor activation.

Disclosures: C. Mitchell: None. **K. Campagno:** None. **W. Lu:** None.

Nanosymposium

679. Microglial Activity and Dysfunction

Location: SDCC 33

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 679.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: F30AG069373
         Bluefield Project to Cure Frontotemporal Dementia

Title: Neuronal transduction of AAV-Progranulin rescues pro-inflammatory microglial morphology in a mouse model of progranulin deficiency

Authors: *S. KASHYAP[1], K. I. WILSON[2], A. E. ARRANT[3], E. D. ROBERSON[3];
[1]Univ. of Alabama, Birmingham, AL; [2]Univ. of Alabama Birmingham, BIRMINGHAM, AL; [3]Neurol., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Neuronal transduction of AAV-Progranulin rescues pro-inflammatory microglial morphology in a mouse model of progranulin deficiency Shreya N. Kashyap, Katherine I. Wilson, Andrew E. Arrant, Erik D. Roberson*Disclosures: EDR has served as consultant for AGTC, serves on the DSMB for Lilly, and is the owner of an IP related to Tau.***State of the Art:** Of the more than 70 *GRN* mutations associated with FTD, nearly all cause progranulin haploinsufficiency. Therefore, progranulin replacement is a straightforward therapeutic approach for the FTD-GRN patient population. There are two AAV-Progranulin gene therapeutics currently in clinical trials. PR006, manufactured by Prevail Therapeutics, is a human progranulin transgene product packaged in an AAV9 capsid which transduces both neurons and astroglia. PBFT02, manufactured by Passage Bio, is human progranulin packaged in an AAV1 capsid, which selectively transduces neurons. Our lab has demonstrated that mouse progranulin packaged in AAV1 rescues microglial lysosomal dysfunction in progranulin homozygous knockout ($Grn^{-/-}$) mice as measured by CD68 immunostaining, and reduces microglial soma

size, as measured by IBA1-positive particle analysis of DAB micrographs. Here, we further characterized microglial morphological phenotypes of $Grn^{-/-}$ mice and determined if neuronal restoration of progranulin influences microglial morphology. **Methodology:** A blinded experimenter used 3D Slicer to manually segment and perform 3D reconstruction of microglia from 40X Z-stacks of IBA1-stained sections of $Grn^{-/-}$ and $Grn^{+/+}$ brains. We also used a MATLAB-based script which segments and skeletonizes microglia based on a threshold set by the user. We repeated these analyses with $Grn^{-/-}$ mice that received AAV1mGrn or AAV1GFP. **Results:** $Grn^{-/-}$ mice had age-dependent increases in microglial cell volume, territorial volume, and average branch length and AAV1mGrn treatment rescued these morphologic phenotypes. Exogenous progranulin was detected only in neurons, not microglia.**Conclusions:** Neuronal transduction of AAV-Progranulin rescues pro-inflammatory microglial morphology in $Grn^{-/-}$ mice.

Disclosures: S. Kashyap: None. **K.I. Wilson:** None. **A.E. Arrant:** None. **E.D. Roberson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner of IP related to Tau. F. Consulting Fees (e.g., advisory boards); Lilly, AGTC.

Nanosymposium

679. Microglial Activity and Dysfunction

Location: SDCC 33

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 679.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Disrupted neuron-astrocyte mitochondrial communication in Alzheimer´s disease

Authors: *L. NAIA, M. PEREIRA, S. GUO, M. SHIMOZAWA, N. S. LEAL, P. NILSSON, M. ANKARCRONA;
Karolinska Institutet, Stockholm, Sweden

Abstract: Neurons have a complex morphology and consequently face a greater challenge in distributing and maintaining mitochondria throughout their arborizations. Data from our lab combining transcriptomic, imaging, and functional studies in App^{NL-G-F} mice, a knock-in mouse model of Alzheimer´s disease (AD), revealed early mitochondrial dysfunctions very localized at pre-synaptic terminals, leading to synaptic ATP loss before overall delay of energy metabolism.

Here we report an exchange of mitochondria between astrocytes and neurons, which is disrupted in the App^{NL-G-F} model and potentially contribute to the loss of heathy mitochondrial pool in axonal terminals. Using an *in vitro* coculture system, we observed that neurons release functional mitochondria via extracellular microvesicles (mitoEVs), which can be further captured by astrocytes through actin-dependent endocytosis. While WT mitoEVs mainly integrate in the host astrocytic mitochondrial network, App^{NL-G-F}-derived mitoEVs show reduced engulfment and integration. Instead, they were found to colocalize with lysosomes. An upregulation of mitophagy-related genes in App^{NL-G-F} astrocytes cocultured with neurons further support mitoEVs degradation hypothesis. Astrocytes were also observed to transfer mitochondria through tunneling nanotubes (TNTs). Remarkably, App^{NL-G-F} astrocytes fail to increase the percent of transferred mitochondria in TNTs to neighboring cells under a mitochondrial stress stimulus. Moreover, App^{NL-G-F} neurons do not benefit of mitochondrial transfer from App^{NL-G-F} astrocytes. On the contrary, WT astrocytes-derived mitoEVs mitigate mitochondrial respiratory deficits in App^{NL-G-F} neurons, an effect dependent on mitochondria since mitochondria-depleted EVs showed no ability to upregulate oxygen consumption. Overall, these data indicate that AD astrocytes are poor mitochondrial donors, and this disrupted neuron-astrocyte mitochondrial communication likely contributes to localized energy deficits in AD neurons.

Disclosures: L. Naia: None. **M. Pereira:** None. **S. Guo:** None. **M. Shimozawa:** None. **N.S. Leal:** None. **P. Nilsson:** None. **M. Ankarcrona:** None.

Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.01

Topic: A.04. Transplantation and Regeneration

Support: CIHR

Title: Unlike a conditioning crush lesion, conditioning electrical stimulation promotes nerve regeneration in a non-injurious and non-inflammatory manner

Authors: *C. A. WEBBER[1], L. ACTON[1], P. HARDY[2], K. CHAN[3], J. R. PLEMEL[1], C. BAAKLINI[2], K. LEE[2], J.-L. SENGER[1];
[1]Univ. of Alberta, [2]Univ. of Alberta, Edmonton, AB, Canada; [3]Univ. Alberta, Edmonton, AB, Canada

Abstract: Due to the slow rate of regeneration (1-3mm/day), nerve repair surgeries following proximal peripheral nerve injury are often incomplete. Despite the advancements in surgical techniques, over half of patients with peripheral nerve injury experience lifelong deficits that can severely impact quality of life. The conditioning crush lesion (CCL), a technique in which a crush injury is performed one week prior to a nerve transection and repair surgery, increases the rate of nerve growth 2-4-fold. However, CCL cannot be translated to clinical use as it requires an

intentional nerve injury which evokes an invasive inflammatory response. Conditioning electrical stimulation (CES) upregulates the same regeneration-associated genes (RAGs) as CCL to accelerate nerve regeneration and promote sensorimotor functional recovery. We hypothesized that unlike CLL, CES is non-injurious and non-inflammatory. One day post conditioning, there was an upregulation of activation transcription factor-3 expression (ATF3) in the CCL, but not the CES dorsal root ganglion (DRG) neurons. Lineage tracing studies of CX3CR1-expressing tissue-resident macrophages (TdTom+; Iba-1+) demonstrated an infiltration of blood-born macrophages at the DRG and nerve (TdTom-; Iba-1+) 3-7 days following CCL. There was no increase in tissue resident macrophage expression (TdTom+; Iba-1+) at the DRG or nerve following CCL. In contrast, CES did not increase blood born or tissue resident macrophages density within the nerve or DRG. Further, innate macrophages expression at the DRG and nerve did not increase their activation (as depicted by dectin-1 and IBA-1 expression) following CES. Ablation of the CX3CR1 population of macrophages (blood-born and tissue resident macrophages) demonstrated that the effects of CCL, but not CES, were reduced suggesting different mechanism of action from immune cells in these two conditioning events. In summary, unlike CCL which requires nerve injury that is associated with monocyte-derived macrophage entry into the DRG to evoke a pro-regenerative effect, CES activates RAG expression and accelerates nerve regeneration in a manner independent of nerve injury macrophage accumulation.

Disclosures: C.A. Webber: None. **L. Acton:** None. **P. Hardy:** None. **K. Chan:** None. **J.R. Plemel:** None. **C. Baaklini:** None. **K. Lee:** None. **J. Senger:** None.

Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.02

Topic: A.04. Transplantation and Regeneration

Title: Wilddisco reveals integrated neuronal, vascular, and lymphatic networks

Authors: *H. MAI[1], J. LUO[2], A. ERTURK[3];
[2]ITERM, [1]Helmholtz-munich, Munich, Germany; [3]ITERM, Helmholtz-Munich center, Munich, Germany

Abstract: Whole-body imaging could capture cellular insights and provide integrated biological knowledge in healthy rodents. However, while the mouse is the mouse commonly used animal

model, we still lack basic information about its body, namely how diverse organ and tissue systems are organized in the whole mouse. Here we used a new DISCO technique, which allowed high-resolution 3D imaging of the peripheral nervous system (PNS), lymphatic system, and vascular system in the whole mouse body:

1.   We could observe the PNS innervation in most organs, including the heart, lung, liver, kidney, stomach, and intestine. Moreover, we visualized the vagal nerves innervate the gastrointestinal tract.

2.   We presented lymphatic capillaries heterogeneously penetrated in the center of intestine villi and regional specific 3D villi lymphatics network. Interestingly, we discovered lymph nodes were innervated by a population of PN with immunomodulatory potential.

3.   We imaged organ-specific vascular patterns and a network of transcortical capillaries as the main support for multiple bones.

Thus, mapping whole mouse body systems can provide a roadmap for diverse studies, including the neural circuits, immunomodulation, and angiogenesis in the entire mammalian body.

Disclosures: H. Mai: None. **J. Luo:** None. **A. Erturk:** None.

Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.03

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Unraveling the Functional Architecture of the Human Spinal Cord using Neuromodulation and Functional Ultrasound Imaging

Authors: *K. A. AGYEMAN[1], D. J. LEE[2], V. EDGERTON[4], C. LIU[3], V. N. CHRISTOPOULOS[1];
[1]Bioengineering, Univ. of California, Riverside, Riverside, CA; [2]USC, Los Angeles, CA; [3]USC, California, CA; [4]Dept Integrative Biol. & Physiol., UCLA, Los Angeles, CA

Abstract: The functional architecture of the human spinal cord (SC) is not well understood, despite its vital role in sensory, motor, and autonomic functions. Disruptions stemming from injury or disease at any level of severity, can lead to functional limitations, chronic pain, and permanent paralysis. Research on the functional anatomy of the human spinal cord is limited due to susceptibility to artifacts arising from its physiological location, and technical challenges associated with existing neuroimaging modalities.
Herein, we leverage the superior spatiotemporal properties of functional ultrasound imaging (fUSI), a recent revolutionary neuroimaging technique, to illuminate the spinal cord functional organization by investigating the effects of epidural electrical spinal cord stimulation (ESCS).

We characterize and decode spinal cord hemodynamic responses to modulation at a single trial-level, using fUSI spinal cord images acquired from 4 patients who underwent ESCS stimulator implantation surgery for chronic back pain therapy. An ESCS protocol consisting of 10 ON-OFF stimulation cycles - 30 s each for a total of 10 minutes was used. The single trial decoding of ESCS induced spinal cord states was performed utilizing classwise principal component analysis integrated with linear discriminant analysis.

Our findings demonstrate that responses to ESCS reflects a spatiotemporal modulation of the human spinal cord circuitry not previously recognized - with stimulation induced spinal cord states that can be decoded from fUSI images (accuracy: 84.0 ± 11.4 % Mean ± Std, and 98.3 % optimized accuracy across patients. From statistical parametric maps and event-related-average curves, we observe region-specific increases or decreases of spinal cord hemodynamic responses during stimulation. Additionally, our analysis identifies spatial regions affected significantly by the stimulation protocol ($p < 0.05$) and show that the most informative content for decoding spinal cord induced states is contained in small arterioles and capillaries.

This first in-human study is significant as it provides analytical capabilities to assess neural activity modulations indirectly coupled to blood-flow changes with a new level of precision *in-vivo*. Our ability to decode spinal cord states in a single trial is novel and opens avenues to understand the functional anatomy, dysfunction, and effects of neuromodulation. Ultimately this is vital for translational development of real-time closed-loop neurorehabilitation systems.

Disclosures: K.A. Agyeman: None. **D.J. Lee:** None. **V. Edgerton:** None. **C. Liu:** None. **V.N. Christopoulos:** None.

Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig Neilsen Foundation Grant 648861

Title: Single cell Patch-seq in understanding the regenerative abilities of CST neurons after SCI

Authors: *H. KIM[1,2], J. SAIKIA[1], A. MOORE[1], E. HA[1], C. L. CHAVEZ-MARTINEZ[1], K. CERVANTES[1], D. LUSK[1], K. MONTE[1], B. ZHENG[1,2];
[1]Neurosci., UNIVERSITY OF CALIFORNIA, SAN DIEGO, La Jolla, CA; [2]VA, San Diego, CA

Abstract: Spinal cord injury (SCI) is a severe condition that results in loss of function in mobility. The corticospinal tract (CST) is a clinically important target for functional recovery after SCI. Multiple molecular pathways including the Pten/mTOR signaling pathway have been revealed to regulate axon regeneration and sprouting from the CST. However, among diverse populations of CST neurons, only a subset regenerates axons following molecular intervention and the number of regenerating neurons declines with age. Here, we have performed single cells

RNA-seq using Patch-seq after retrograde tracer injection in animals with Pten/mTOR pathway modification. Through differential gene expression and pathway / network analyses, we identified known and new potential regulators of CST regeneration and found that regenerating transcriptomes differentially map to previously defined neuronal clusters based on single cell seq data.
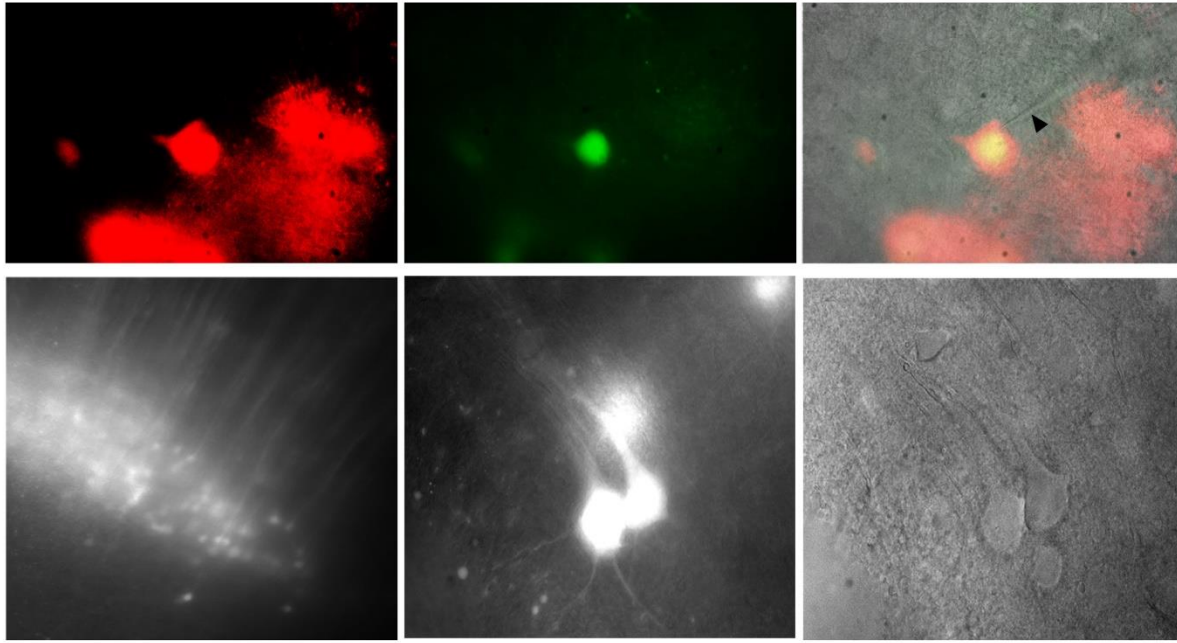
Disclosures: H. Kim: None. **J. Saikia:** None. **A. Moore:** None. **E. Ha:** None. **C.L. Chavez-Martinez:** None. **K. Cervantes:** None. **D. Lusk:** None. **K. Monte:** None. **B. Zheng:** None.

Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CSRS 21st CENTURY GRANTS

Title: Aav9-mediated kcc2 upregulation restores injury-induced synaptic alterations following traumatic spinal cord injury

Authors: *M.-M. ZAVVARIAN[1], Z. LUO[3], A. MODI[1], M. HE[4], Z. HE[5], M. G. FEHLINGS[2];
[2]Toronto Western Hosp., [1]Univ. of Toronto, Toronto, ON, Canada; [3]Univ. Hlth. Network, Toronto, ON, Canada; [4]Boston Children's Hosp., Boston Children's Hosp., Boston, MA; [5]Children's Hosp Boston, Children's Hosp Boston, Boston, MA

Abstract: Traumatic spinal cord injury (SCI) impairs local neuronal conductance and induces a subsequent synaptic remodeling cascade in the rostro-caudal perilesional zone. K^+/Cl^- cotransporter 2 (KCC2) is a differentially expressed synaptic ligand-gated channel, which is pivotal for signal propagation in inhibitory spinal interneurons. Reduced KCC2 expression post-SCI disrupts the excitatory/inhibitory (E/I) ratio in the preserved spinal interneurons and blocks the relay of signals in the injured spinal cord. The recent advances in AAV9 gene therapy present a promising approach to therapeutically upregulate KCC2 upregulation and restore the functional neurotransmission in the injured spinal cord. The aim of the study is to examine the ability of KCC2 gene therapy to improve functional recovery by altering SCI-induced synaptic neuroplasticity. We demonstrate for the first time the ability of intrathecal AAV9 administration to induce KCC2 expression in the preserved neural tissue without any deleterious off-target effects. This induced KCC2 expression alters the transcriptional profile of the targeted neurons, which improves the long-term forelimb and hindlimb motor recovery. This is also accompanied by restored electrophysiological and immunohistochemical alterations in the injured spinal cord. Overall, this study can have a significant impact on the neuromodulation regimen for SCI patients to improve their recovery and well-being.

Disclosures: M. Zavvarian: None. **Z. Luo:** None. **A. Modi:** None. **M. He:** None. **Z. He:** None. **M.G. Fehlings:** None.

Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01 NS119297

Title: Development of dorsal spinal neural progenitor cells for application in spinal cord injury therapeutics

Authors: *A. HUNTEMER-SILVEIRA, D. MALONE, P. WALSH, A. PARR;
Univ. of Minnesota, Minneapolis, MN

Abstract: In the United States, there are approximately 18,000 new cases of spinal cord injury (SCI) each year, with individual economic costs in the millions. SCI can diminish or abrogate both motor and sensory systems, though most research to date has emphasized motor impairments and recovery. With the application of human induced pluripotent stem cells (hiPSCs), the potential for targeted neural regeneration and repair in sensorimotor systems after SCI has greatly improved. In the past decade, the use of hiPSC-derived motor neurons has increased our understanding of degenerative spinal motor diseases, as well as shown successful transplantation in rat models. However, comparatively little has been published applying the same strategies to the dorsal sensory neurons and promotion of sensory recovery, despite the

prevalence of sensory dysfunction and chronic pain in SCI patients. An imperative next step is to develop protocols to generate the necessary sensory and interneuron populations. We report here the results of our work generating a rapid differentiation protocol for region specific dorsal spinal neural progenitor cells (dsNPCs) from hiPSCs. This novel protocol allows us to generate both dorsal and ventral spinal populations (ventral protocol previously published) from a shared lineage. These cell populations are being used to study injury and sensorimotor recovery in vitro and in vivo. In the future we will use our dorsal and ventral cells in tandem in a rodent transplantation model to evaluate their capacity to attain correct repair and reconnection at the site of injury, restoring both motor and sensory systems. These discoveries will push the field of spinal regeneration forward and advance the translation of cell transplantation therapies.

Disclosures: A. Huntemer-Silveira: None. **D. Malone:** None. **P. Walsh:** None. **A. Parr:** None.

Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.08

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Synaptic connectivity of neural progenitor cells with spinal locomotor circuits after spinal cord injury

Authors: *A. TUCKER[1,2], A. BALTAZAR[2], J. JANG[2], B. SINGLETARY[2], K. VO[2], J. MOSES[2], S. LETCHUMAN[2], M. PITONAK[2], M. ACEVES[2], M. TOTTY[1,3], S. MAREN[1,3], D. MCCREEDY[1,2], J. DULIN[1,2];
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Abstract: Spinal cord injury (SCI) is a traumatic and life altering event that frequently results in the loss of voluntary motor function after injury. Currently, there are no clinically effective treatments that can improve locomotor function after SCI. Neural progenitor cells (NPCs) are a promising potential therapy for SCI, due to their ability to mature into spinal cord neuronal subtypes and act as a neuronal relay between surviving host neurons rostral and caudal to the site of the injury. In order to repair locomotor circuitry following traumatic spinal cord injury using

NPCs, it is first critical to determine the number and phenotypes of transplanted NPC-derived neurons that establish direct and indirect synaptic connections onto spinal cord motor neurons (MNs) after spinal cord injury and their function in modulating locomotor recovery. We first used the monosynaptic rabies virus to characterize direct graft inputs onto spinal cord motor neurons in adult ChAT[Cre] mice following SCI and NPC transplantation. We found that less than 1% of graft neurons established monosynaptic inputs onto host lumbar MNs; of these cells, they were identified to be of cholinergic and glutamatergic fate. We next used the pseudorabies virus to characterize the direct and indirect synaptic connections between NPC graft and host locomotor circuitry. Due to our identification of different phenotypes of graft neurons synaptically connected to locomotor circuitry using the monosynaptic rabies and pseudorabies virus, we next visualized cell type-specific graft projections using AAV-SynTAG to label anatomical projections from all graft neurons, cholinergic graft neurons, or V2a graft neurons. We found that each of these subtypes exhibited a distinct termination pattern in the lumbar spinal cord, suggesting that graft neuron phenotype influences patterns of synaptic connectivity. Finally, we examined the effects of modulating graft activity on locomotor behaviour. To do this, we performed SCI on adult C57BL/6 mice and transplanted NPCs expressing excitatory DREADDs (hM3Dq) in all graft neurons. Ten weeks later, we observed that DREADD-mediated activation of graft neurons significantly altered locomotor behaviour. This is the first study to show direct and indirect synaptic connections of NPCs onto spinal locomotor circuitry and demonstrate that graft activity directly modulates locomotor function. Ultimately, these findings provide important mechanism that will inform future work on development of cell-based therapies to restore locomotor function after spinal cord injury.

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Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.09

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Transplanted human stem cell-derived interneurons functionally integrate to promote recovery after spinal cord injury

Authors: *L. ZHOLUDEVA[1], T. FORTINO[2], M. LANE[2], T. C. MCDEVITT[1], D. SRIVASTAVA[1];

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Abstract: Advances in cell-based strategies offer new promise for some of the most devastating neural injuries like spinal cord injury (SCI). However, to harness the full therapeutic potential of stem cells, it will be necessary to understand how to direct their differentiation to appropriate cell phenotypes and ensure that their phenotype and function persist after transplantation into a pathologic environment. We used a pre-clinical cervical contusion SCI in adult rats to transplant human induced pluripotent stem cell (hiPSC)-derived pre-motor spinal interneurons (SpINs). We hypothesized that donor spinal interneurons, known to contribute to plasticity post-injury, will promote novel neuronal relay formation and improve functional outcome. Quantitative PCR, immunocytochemistry, multielectrode array (MEA) analysis and single cell and nuclei RNA sequencing were used to characterize engineered human SpINs prior to transplantation to confirm identity and neuronal function. Neuroanatomical tracing (transynaptic pseudorabies virus) and immunohistochemistry were used to assess transplant integration and connectivity with injured host tissue. Optogenetic activation of hiPSC-SpINs was used to assess development of synaptic connectivity to injured host circuits with time (1- and 2-months post-transplantation). Bilateral terminal diaphragm electromyography was used to quantitatively assess functional contribution of transplanted human SpINs to the recovery of phrenic function after injury and transplantation. These studies demonstrated that transplanted human SpINs survived and integrated with injured cervical spinal cord circuits, displayed anatomical (e.g., positive for transsynaptic tracing) and functional (e.g., modulate respiratory activity when activated) evidence of connectivity. Having rigorously established improvement in diaphragm muscle activity with objective metrics, this strategy holds great promise to establish motor recovery post-SCI.

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Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life
UC Irvine ICTS

Title: Mitochondrial fitness regulates stem cell efficacy in spinal cord injury

Authors: *A. ZAHEDI[1], I. TOTOUNJI[1], J. MARTINEZ[1], A. WU[1], N. NADIMZADEH[1], N. KHAMNEI[1], R. HABIB[1], K. M. PILTTI[1], A. LAKATOS[2], A. J. ANDERSON[1];

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Abstract: Mitochondrial dysfunction is a confounding factor found in traumatic injuries such as spinal cord injury (SCI). Transplantation of human neural stem cells (hNSC) to repair damaged tissues has shown promise in pre-clinical studies and early SCI clinical trials. However, neural regeneration after SCI is hindered due to the harsh ischemic oxidative microenvironment, loss of mitochondria bioenergetics in the damaged tissues, and the presence of pro-inflammatory signals such as complement component C1q. We have previously derived and characterized several human neural stem cells (UCI-hNSCs) lines and their potential for restoring locomotor function (efficacy) after SCI in mice. We hypothesized that the survival and efficacy of donor stem cells in the SCI niche may depend on their mitochondria function. Transcriptomic analysis comparing efficacious (UCI161) and non-efficacious (UCI152) hNSC lines identified differences in key categories termed as "mitochondria fitness traits" (MFTs): bioenergetics, redox and membrane permeability, mitophagy, and biogenesis. We have previously reported that C1q, which is present in high levels at SCI site, modulates hNSC fate, bioenergetics level, and capacity for in vivo repair. Here we show that C1q exposure leads to increased mitochondria protein oxidation and mitochondria fission in the UCI hNSC cultures. Consistent with RNA-seq data for these lines, C1q treatment for 2 days also lead to loss of TFAM (a biogenesis transcription factor) and activation of the inflammasome pathway. To test whether MFTs could be modulated pharmacologically, we screened and tested several drugs that are known to modulate mitochondrial function. Mitochondrial functional assays (MTT, TMRM, ATP, MitoTracker) revealed that UCI161 were better responders to bioenergetic drugs, whereas UCI152 responded optimally to biogenesis enhancement. Our future aim is to combine stem cell transplantation and mitochondria biogenesis drug Bezafibrate to improve the resilience and therapeutic potential of hNSC lines for transplantation in SCI.

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Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Neurosurgery Research & Education Foundation Research Fellowship Grant
Physicians' Services Incorporated Foundation Research Grant
Vanier Canada Graduate Scholarship

Title: Harnessing the endogenous stem cell response after spinal cord injury

Authors: *L. D. HACHEM[1,2], J. HONG[2], A. A. VELUMIAN[2], A. J. MOTHE[2], C. H. TATOR[1,2], M. G. FEHLINGS[1,2];
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Abstract: The adult spinal cord contains a population of multi-potent ependymal-derived neural stem/progenitor cells (epNSPCs) with the capacity to regenerate and promote repair after injury. epNSPCs are normally quiescent but are acutely activated after spinal cord injury (SCI). Despite this, the activation of these cells is insufficient to promote robust neural regeneration and recovery. Thus, it is of therapeutic interest to harness the endogenous regenerative potential of epNSPCs. However, little is known about the underlying mechanisms that govern the biology of these cells and their response to injury. We thus aim to characterize the mechanisms of epNSPC activation after SCI and examine a therapeutic strategy to harness their regenerative capacity. epNSPCs isolated from the central canal region of the adult spinal cord were used for *in vitro* mechanistic analysis. *In vivo*, cervical SCI was induced in adult rodents using a clinically relevant compression-contusion model of SCI. Lineage tracing of epNSPCs was done using FoxJ1-Cre-tdT reporter mice. We found that excitotoxic levels of glutamate, a hallmark in the pathogenies of SCI, leads to calcium influx in spinal cord epNSPCs via AMPA receptors (AMPARs) and this change in calcium in concert with Notch signaling increases the proliferation of epNSPCs via pCREB, and induces astrocytic cell fate specification through Hes1 upregulation. AMPAR blockade after SCI with NBQX inhibits epNSPC proliferation, migration and differentiation. Conversely, positive allosteric modulation of AMPARs subacutely after SCI with the agent CX546 enhances epNSPC proliferation, astrogliogenesis, increases neurotrophic factor production and promotes neuronal survival and functional recovery. Our study uncovers an important mechanism by which glutamatergic signaling via AMPARs alters the proliferation and phenotype of spinal cord epNSPCs. Pharmacological modulation of AMPAR signaling offers a novel and highly translational therapeutic strategy to regulate the fate of epNSPCs and harness their regenerative potential after SCI.

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Nanosymposium

680. Spinal Cord Repair: Genes, Cells, Neuromodulation, and Imaging

Location: SDCC 11

Time: Wednesday, November 16, 2022, 1:00 PM - 4:00 PM

Presentation Number: 680.12

Topic: A.04. Transplantation and Regeneration

Support: The Methuselah Foundation

Title: Transplanted neural precursors form vascularized neocortical-like tissue

Authors: *A. QUEZADA[1], E. BADER[2], C. WARD[1], N. J. KILLIAN[3], C. XIE[4], R. BATISTA-BRITO[1], J. M. HEBERT[1];
[1]Dominick P. Purpura Dept. of Neurosci., [2]Dept. of Neurolog. Surgery, [3]Neurosurg., Albert Einstein Col. of Med., Bronx, NY; [4]Rice Univ., Houston, TX

Abstract: Recent progress in cortical stem cell therapy has demonstrated its potential to repair the brain. However, current stem cell transplant models have yet to demonstrate that the circuitry of transplant-derived neurons can encode useful function to the host after neurodegeneration or trauma. This may be due to missing cell types, their proportions, and/or their cytoarchitecture. In addition, there is a need for vasculature to increase viability and function of transplanted cells. Here, as a platform for further improvements in neocortical cell grafts, we devised a reproducible aspiration lesion and transplant paradigm. In this paradigm, donor cells differentiate into upper and deeper layer neurons, glial cells are present, and grafts are vascularized. The grafted neurons project outside of the graft to appropriate brain areas. We also find that the donor neurons fire action potentials. The graft becomes fully vascularized by 2 weeks post-transplant and perfused with blood. Finally, with this paradigm, we can organize cells into layers. Overall, we have developed a model that should allow us to build complex neocortical-like tissue, containing many of the precursor cell types and cytoarchitecture necessary for a properly developing cortex. Importantly, this *in vivo* model could also be used for investigating development and diseases.

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Nanosymposium

681. Neural Computations of Visual Cortex

Location: SDCC 25

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.01

Topic: D.06. Vision

Support: NWO Vici 016.Vici.185.050

Title: Size of the spotlight of attention influences population receptive field properties across the visual hierarchy in human cortex

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Abstract: Perception is enhanced at spatially attended locations. Previous research has demonstrated that population receptive fields (pRFs) are attracted to the position of the attentional locus, yet how the precision of attention impacts pRF properties remains to be elucidated. The attention field model is often used to summarize the locus and precision of attention by a Gaussian field. This model predicts that attentional precision, i.e. the size

(Gaussian standard deviation) of the attention field, should influence pRF properties. Here, we investigate the effect of attentional precision on pRF properties while keeping the attended location constant.

We measured pRFs using ultra-high field 7T MRI while participants performed a color discrimination task. Two attention conditions were compared: attention focused at fixation (0.1 deg) and attention maximally distributed across the entire screen (>5 deg). In both conditions the same visual stimulus was presented and only the spatial extent of attention varied. The stimulus included a standard pRF mapping contrast-defined bar.

Behavioral results showed that participants were able to modulate their spatial distribution of attention and that the difficulty of the two attention conditions was matched. The fMRI time courses revealed structured task-dependent differences. These time-series differences translated into eccentricity-dependent task responses in early visual areas. In particular, higher foveal BOLD responses to the focused attention task and higher peripheral BOLD responses to the distributed attention task were observed. Furthermore, pRF positions were also altered as a function of the attentional precision of the task with larger pRF position changes observed in higher visual areas. pRF position changes followed predictions from the attention field model. These results indicate that the precision of the spotlight of attention influences visual representations of space in line with the predictions of the attention field model.

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Nanosymposium

681. Neural Computations of Visual Cortex

Location: SDCC 25

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.02

Topic: D.06. Vision

Title: The involvement of serotonin and GABA receptors in visuospatial divisive normalization

Authors: *M. AQIL[1], T. KNAPEN[2], S. O. DUMOULIN[3];
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Abstract: We recently introduced a new population Receptive Field (pRF) model based on Divisive Normalization (DN), a mathematical operation considered a prime candidate for a canonical neural computation. Using 7T functional MRI, we showed that 1) the DN pRF model unifies and outperforms existing models throughout the human visual cortex, 2) specific model parameters, dubbed activation and normalization constants, modulate distinct response properties. We next investigated the biological correlates of the DN pRF model. Activation of specific receptors is thought to differentially modulate properties of visual responses; hence, we

reasoned that neuromodulatory mechanisms may also underlie DN computations. In particular, we hypothesised that GABA and serotonin (5-HT) 1A receptors, by modulating inhibition and baseline activity, might underlie the activation constant; and that 5-HT1B and 5-HT2A receptors, thought to exert a bidirectional gain modulation, might underlie the normalization constant.

To test these hypotheses, we compared maps of DN pRF model parameters obtained from a large-scale 7T fMRI dataset (n=180), with receptor densities obtained from PET neuroimaging. Consistent with our hypotheses, we found highly significant (both $p<10^{-6}$) opposite correlations between GABA, 5-HT1A, and the activation constant; a significant positive correlation of 5-HT1B ($p<10^{-2}$), and a n.s. negative correlation of 5-HT2A, with the model normalization constant. Next, we used a crossvalidated-GLM approach to show that the pairs of hypothesised receptors provide a significantly better prediction for model constants than individual receptors in the pair. Finally, a data-driven principal component analysis also confirmed that most of the variance is shared between the two datasets.

Our findings 1) extend the role of DN as a canonical visuospatial pRF computation and 2) provide novel evidence of the involvement of serotonin and GABA systems in DN operations. We propose that these findings provide new insights into the computational principles of information encoding in the cortex, as well as their biological correlates.

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Nanosymposium

681. Neural Computations of Visual Cortex

Location: SDCC 25

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.03

Topic: D.06. Vision

Title: Stimulus dependent variations of spike count correlation improve decoding of stimulus directions from neuron populations in visual cortex

Authors: *H. ITO[1], Y. TABATA[1], R. KOHNO[1], Y. MARUYAMA[2], Y. MORI[3];
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Abstract: Even under repeated presentations of the same stimulus, the spike counts of a single sensory cortical neuron show a considerable amount of trial-to-trial variability. One of the challenges in the BMI is to decode the stimulus information from the single trial activities of simultaneously recorded multiple neurons. We recorded multiple single neurons in the visual cortex of anesthetized cats under the moving bar of 16 different directions. We have shown that the variabilities were correlated between the neuron pairs (spike count correlation) and some neuron pairs showed stimulus dependent spike count correlations (Maruyama & Ito, 2013). Our

main interest is how the spike count correlations and their stimulus dependence contribute to the decoding performance of the stimulus directions. We applied the non-linear Support Vector Machine (SVM) model with the RBF kernel and carefully selected the hyper parameters to optimize the performance of the cross-validation. Non-linearity was essential since the non-linear SVM showed higher discrimination accuracy than the linear SVM (Wilcoxon, $P<0.001$, $N=35$). At first, we found that the original data with finite spike count correlations showed higher discrimination accuracy than the data without correlation (independent variabilities) obtained by the trial shuffling (Wilcoxon, $P=0.029$, $N=35$). Examining the contribution of stimulus dependent spike correlation, we introduced a novel stimulus shuffling method by which unit pairs in all the stimulus clusters had the identical but finite spike count correlation averaged over all the stimuli. The original data with stimulus dependent spike count correlations showed higher discrimination accuracy than the stimulus shuffled data (Wilcoxon, $P=0.042$, $N=35$). For each neuron population, the degree of stimulus dependent variations of the spike count correlations was quantified by averaging the test statistic H-value of the Kruskal-Wallis test over all the unit pairs. We also estimated the ratio of the increase in the discrimination accuracy of the original data compared with the stimulus shuffled data. We found that the ratio of the increase in the discrimination accuracy showed a significant positive correlation to the degree of stimulus dependent variations of the spike count correlations ($r=0.36$, $P=0.033$). Neuron populations with larger stimulus dependent variations of the spike count correlations tended to have larger increase ratios in the discrimination accuracy. Our findings suggested that both the spike count correlations and their stimulus dependence add extra decoding information to the population coding based solely on the spike counts of individual neurons.

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Nanosymposium

681. Neural Computations of Visual Cortex

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Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.04

Topic: D.06. Vision

Title: Visual timing-tuned responses in human association cortices and response dynamics in early visual cortex

Authors: *E. HENDRIKX[1], J. M. PAUL[2], M. VAN ACKOOIJ[1], N. VAN DER STOEP[1], B. M. HARVEY[1];
[1]Dept. of Exptl. Psychology, Utrecht Univ., Utrecht, Netherlands; [2]Melbourne Sch. of Psychological Sci., Univ. of Melbourne, Melbourne, Australia

Abstract: Quantifying the timing (duration and frequency) of brief visual events is vital to human perception, multisensory integration and action planning. For example, this allows us to follow and interact with the precise timing of speech and sports. Tuned neural responses to visual event timing have been found in areas of human association cortices implicated in visual perception, multisensory integration and action planning. Predominant models predict that such event timing representations are derived from specialized central pacemakers or processes. In contrast, we hypothesized that such event timing representations may be derived from neural response dynamics to events in sensory processing areas. Therefore, we asked whether and how timing-tuned responses are related to early visual responses, which monotonically increase with event duration and frequency. Participants were presented with repetitive visual events which gradually varied in event duration and/ or period during 7-Tesla functional magnetic resonance imaging. We characterized both monotonic and tuned responses to visual event timing using neural model-based analyses. We found increasingly clear monotonic responses to visual event duration and frequency from primary visual cortex to lateral occipital cortex. From here, we found a gradual transition from monotonic to tuned responses beginning in area MT/V5. Therefore, across successive stages of visual processing, timing-tuned response components gradually become dominant over the inherent modulation of sensory responses by event timing. This additional timing-tuned response component was independent of retinotopic location. We propose that this hierarchical emergence of timing-tuned responses from sensory processing areas quantifies sensory event timing while abstracting temporal representations from the spatial properties of their inputs.

Nanosymposium

681. Neural Computations of Visual Cortex

Location: SDCC 25

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.05

Topic: D.06. Vision

Title: Coordination of sparse intrinsic traveling waves (iTWs) in large-scale, multi-region networks of spiking neurons through feedforward and feedback connectivity

Authors: *G. B. BENIGNO[1], Z. W. DAVIS[3], M. MOFRAD[1], R. C. BUDZINSKI[1], C. C. STEWARD[2], J. H. REYNOLDS[3], L. E. MULLER[1];
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Abstract: The cortex is never silent: ongoing, spontaneous activity occurs continuously, providing cortical neurons with constantly fluctuating input (Arieli et al., Science 273, 1996). Recently, we found that spontaneous fluctuations of neural activity in Area MT of the awake, behaving marmoset are organized into intrinsic traveling waves (iTWs) that traverse MT several times per second (Davis*, Muller*, et al., Nature 587, 2020). The probability that the monkey detects a target at perceptual threshold varies as a function of iTW phase. The range of measured iTW propagation speeds is similar to the speeds of action potentials as they traverse unmyelinated horizontal fibers (0.1-0.6 m/s). We find that a model that incorporates these action potential speeds and has distance-dependent falloff in synaptic connectivity and reasonable synaptic conductance yields iTWs that are similar to those we observe in vivo. The model organizes asynchronous-irregular activity into sparse iTWs, where the spiking activity of only a small fraction of the local neural population contributes to any given iTW (Davis*, Benigno*, et al., Nature Communications, 2021). We call this unique operating mode, where the benefits of the asynchronous-irregular state can coexist with sparse iTWs across the cortex, the "sparse wave regime". We now extend this spiking model to include multiple regions. We find that diffuse feedforward and feedback projections can transiently coordinate spatiotemporal activity patterns across multiple regions. These results have several important implications for the structure of ongoing activity in the visual system. First, retinotopic coordination of spontaneous iTWs may serve to create transient windows of interareal communication. Importantly, because we have previously shown spontaneous iTWs occur during the complex, non-narrowband fluctuations of ongoing activity, the coordination observed in our models provides a novel potential mechanism of inter-area information transfer in the absence of narrowband oscillations. [GBB and ZWD contributed equally to this work.]

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Nanosymposium

681. Neural Computations of Visual Cortex

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Presentation Number: 681.06

Topic: D.06. Vision

Title: A perceptual glitch in serial perception generating time distortions

Authors: *F. SIERRA[1], R. MURALIKRISHNAN[1], D. POEPPEL[2,3], A. TAVANO[1];
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Neural Sci., New York Univ., New York, NY; [3]Ernst Strüngmann Inst. for Neurosci., Frankfurt am Main, Germany

Abstract: Precisely estimating event timing is essential for survival, yet temporal distortions are ubiquitous in our daily sensory experience. Here, we tested whether the relative position, duration, and distance in time of two sequentially-organized events —standard S, with constant duration, and comparison C, varying trial-by-trial— are causal factors in generating temporal distortions.We found that temporal distortions emerge when the first event is shorter than the second event. Importantly, a significant interaction suggests that a longer inter-stimulus interval (ISI) helps counteracting such serial distortion effect only when the constant S is in first position, but not if the unpredictable C is in first position. These results suggest the existence of a predictability-dependent perceptual glitch in perceiving ordered event durations, mechanistically contributing to distortion in time perception. Our results clarify the mechanics generating time distortions by identifying a hitherto unknown duration-dependent encoding inefficiency in human serial temporal perception, akin to a strong prior that can be overridden for highly predictable sensory events but unfolds for unpredictable ones.

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Nanosymposium

681. Neural Computations of Visual Cortex

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Presentation Number: 681.07

Topic: D.06. Vision

Title: Neural Feature Dimension Maps are Modulated by Stimulus Salience and Task Goals

Authors: *D. D. THAYER, T. C. SPRAGUE;
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Abstract: Computational theories posit that attention is guided by a combination of spatial maps that index image-computable salience for individual feature dimensions (e.g., color or motion), and which are re-weighted and combined based on task goals to form a feature-agnostic 'priority map' (Itti & Koch, 2001; Serences & Yantis, 2006). While several regions in parietal and frontal cortex have been reported to index the overall 'priority' of stimulus locations based on a combination of top-down task demands and bottom-up stimulus salience, the neural bases of individual feature dimension maps remain unknown. Here, we tested the hypothesis that retinotopic maps in visual cortex selective for specific feature dimensions (color: hV4/VO1/VO2; motion: TO1/TO2) act as neural dimension maps across two experiments. In E1, we scanned participants (n = 8) while they performed a demanding fixation task as they viewed full-field dot stimuli containing a salient location defined by color (static dots; salient location presented in a different hue from the background) or motion (black & white dots; salient location presented in a different motion direction from the background). We used a spatial inverted encoding model (IEM; Sprague & Serences, 2013) to reconstruct spatial maps of stimulus representations from activation patterns in each region. Reconstructed maps showed stronger salient stimulus representations in motion-responsive ROIs when salience was defined by motion

than when defined by color, and color-responsive ROIs showed the opposite result, consistent with a key role for these regions in computing feature-specific salience. In E2, we scanned participants (n = 8) while they viewed an identical stimulus containing colored moving dots and, between trials, were cued to report either the color or motion direction of the dots. Stimulus representations in reconstructed spatial maps were strongest when participants attended the feature dimension preferred by each region, consistent with the notion that dimension maps computed within these regions can be dynamically reweighted in service of visual cognition. Altogether, our results support a model whereby retinotopic regions responsive to specific feature dimensions index image-computable salience based on feature contrast within their preferred dimension, and the resulting activation profiles are sculpted to reflect top-down task demands prior to aggregation into a feature-agnostic priority map.

Disclosures: D.D. Thayer: None. **T.C. Sprague:** None.

Nanosymposium

681. Neural Computations of Visual Cortex

Location: SDCC 25

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.08

Topic: D.06. Vision

Title: The neural development of attentional gain mechanisms in human early visual cortex

Authors: *P. WIWATPHONTHANA[1], K. LERTLADALUCK[2], K. BENJASUPAWAN[3,1], C. PAMARAPA[4], C. PARASOMPONG[4], R. KEERATIVITTAYAYUT[4], C. CHUNHARAS[5,3], S. ITTHIPURIPAT[1];
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Abstract: Selective attention is an essential cognitive function that helps prioritize the processing of behaviorally relevant sensory information. The disruption of selective attention is thought to be the underlying cause of a wide variety of neurological and neurodevelopmental disorders. A dominant view in the field of attention posits that selective attention operates via

modulating gain of neural responses in early visual cortex. Consistent with this idea, multiple past studies in human and non-human adult primates have found that when attention is directed to a certain space on the visual field, it increases the activity of neurons that are tuned to visual stimuli spatially overlapped with the attended location. That said, there is still a paucity of data in younger organisms. Thus, it is poorly understood how selective attention operates throughout human development. Here, we tested this question by measuring spatially selective hemodynamic responses to the attended and unattended visual stimuli from retinotopically organized areas in the early visual cortex of male and female human subjects with ages ranging from 12-34 years old. We used a model-based approach to reconstruct and quantify changes in spatial extent of selective visual attention, and compared them across different age groups. We found that both groups exhibited comparable attentional gain modulations in the spatial representations reconstructed based on neural activity in early visual areas. These results suggest that attentional gain mechanisms in human early visual cortex are developed as early as 12 years of age. Ultimately, this study helps establish the experimental and modeling methods as well as produce baseline data in typically developing individuals that could be compared to future clinical data to study attention deficits in neurodevelopmental disorders.

Nanosymposium

681. Neural Computations of Visual Cortex

Location: SDCC 25

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.09

Topic: D.06. Vision

Title: Dissociable neural mechanisms in selective attention across different mild cognitive impairment subtypes

Authors: *P. SOOKPRAO[1,2], K. BENJASUPAWAN[2,1], T. CHOTIBUT[3], I. CHATNUNTAWECH[4], C. CHUNHARAS[2,5], S. ITTHIPURIPAT[1];
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Bangkok, Thailand; [5]Chula Neurosci. Ctr., King Chulalongkorn Mem. Hospital, Thai Red Cross Society, Bangkok, Thailand

Abstract: Mild cognitive impairment (MCI) is a neurodegenerative disorder found in more than 30% of the elderly population. Patients with MCI exhibit a variety of cognitive deficits, including working memory and attention. Classifying MCI into meaningful subtypes based on these functional deficits will thus help determine selective brain targets for effective interventions for Alzheimer's disease and other dementias. Recently, we have found that early selective visuospatial attention is generally impaired in MCI. We used a model-based approach (i.e., inverted encoding model; IEM) to reconstruct spatially selective representations of the attentional focus based on the activity of slow-going EEG oscillations at ~9-12Hz (i.e., alpha band activity) recorded while subjects performed a variant of the attention-cueing Eriksen Flanker tasks and compared the fidelity of the alpha-based spatial representations across the MCI and healthy aging populations. In general, we found the reduced fidelity of alpha-based spatial reconstructions in the MCI compared to the healthy aging groups. In the present study, we examined further how the neural deficit in early selective visuospatial attention is related to working memory function. Here, we divided MCI patients into different subtypes based on their behavioral performance on subsets of the Montreal Cognitive Assessment (MoCA), which require WM. We found that MCI patients with intact WM functions were slower than those with WM deficits. Moreover, these working memory-intact MCI patients showed the reduced fidelity of alpha-based reconstructions compared to the patients who exhibit substantial WM impairment. On the other hand, the MCI patients with WM deficits exhibited the increase in the activity of the late frontal negative-going wave in response to the incongruency between targets and distractors. Together, these results suggest dissociable neural deficits underling dysfunctions in selective attention in different MCI subtypes categorized based on their WM function, which could be used as potential brain targets for the future development of personalized treatments for MCI.

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Nanosymposium

681. Neural Computations of Visual Cortex

Location: SDCC 25

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.10

Topic: D.06. Vision

Support: US Army Research Office W911NF-19-2-0026
            Alfred P Sloan Foundation Research Fellowship

Title: Disentangling the impact of top-down spatial attention and bottom-up stimulus drive on voxel receptive fields in human cortex

Authors: *T. C. SPRAGUE, A. HARRISON, D. THAYER;
UC Santa Barbara, Santa Barbara, CA

Abstract: Visual attention enables the selection of relevant information in the environment for further processing. Spatial attention results in changes in spatial receptive fields (RFs) of single neurons and fMRI voxels (vRFs), including warping, shifting, and rescaling (e.g., Womelsdorf et al, 2006; Klein et al, 2014; Vo et al, 2017). In many of these studies, attention was directed either to the RF mapping stimulus or the fixation point, which typically results in an increase in measured vRF size in extrastriate cortex when attending the mapping stimulus, or attention was directed to a fixed location while vRFs are measured with an ignored mapping stimulus, which typically results in vRF position shifts. However, in all these studies, the entire mapping stimulus was either attended or ignored. Thus, it remains unknown how attentional selection of a subpart of the stimulus impacts vRFs. Here, we acquired fMRI data while participants viewed a traversing bar stimulus broken into 3 segments. Each segment contained moving black and white dots. Across scanning runs, we manipulated which segment(s) of the bar participants attended using a central cue. Importantly, the stimulus sequence was fixed across scanning runs, so changes in observed signals necessarily result from a change in the locus of attention. We compared vRF models in which the stimulus drive was modeled based on the stimulated region, ignoring attended location ('stim' model), or based on the attended location, ignoring unattended but stimulated locations ('attn' model). First, direct comparison of activation timeseries revealed similar spatial selectivity, but an attentional reweighting of activation levels when the attended bar segment passed each voxel's preferred position. Next, we compared vRF model fits between the stim and attn models: in visual cortex, the stim model performed well at predicting timeseries when only a sub-segment of the bar was attended, while in parietal cortex, this model performed worse, suggesting that it is necessary to incorporate attentional state to accurately model responses in this region. Conversely, for the attn model, fits were worse in visual cortex, because this model disregards the ignored (but still salient) segments of the bar. In parietal cortex, however, the attn model outperforms the stim model even though it does not consider ignored but salient locations on the screen, consistent with a role of these regions in directing attention. Altogether, these results suggest that it is necessary to consider both stimulus and task properties when formulating and testing vRF models, and confirm a key role for parietal cortex in the direction of top-down spatial attention.

Disclosures: T.C. Sprague: None. **A. Harrison:** None. **D. Thayer:** None.

Nanosymposium

681. Neural Computations of Visual Cortex

Location: SDCC 25

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.11

Topic: D.06. Vision

Title: Spatially distributed computation in cortical circuits

Authors: *S. GEPSHTEIN[1], A. S. PAWAR[2], S. KWON[3], S. SAVEL'EV[4], T. D. ALBRIGHT[1];
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Abstract: The traditional view of neural computation in the cerebral cortex holds that sensory neurons are specialized; they are selective for certain dimensions of sensory stimuli. This view was challenged by evidence of nonlinear contextual interactions between stimulus dimensions such that a neuron's response to one dimension strongly depends on other dimensions. Here we use methods of mathematical modeling, psychophysics, and electrophysiology to address shortcomings of the traditional view. Using a model of a generic cortical circuit, we begin with the simple demonstration that cortical responses are always distributed among neurons, forming characteristic waveforms, which we call *neural waves*. When stimulated by patterned stimuli, circuit responses arise by interference of neural waves. Results of this process depend on interaction between stimulus dimensions. Comparison of modeled responses with responses of biological vision makes it clear that the framework of neural wave interference provides a useful alternative to the standard concept of neural computation. We show in particular how selectivity of individual neurons and their lateral interaction—previously studied in the framework of separate mechanisms of classical and nonclassical receptive fields—can arise from a *single mechanism*. This work contributes to the larger body of theoretical and empirical investigation of wave phenomena in neural systems. Prior <u>theoretical</u> studies explored such phenomena as propagation of activity in spatially structured networks and formation of patterned activity in neural fields, by means of traveling waves. These investigations suggested that traveling waves can contribute to formation of stimulus selectivity in cortical mechanisms (such as directional selectivity) and that interaction between patterns of activity propagating across cortex can perform computations, even though these predictions have not yet received empirical confirmation. Prior <u>empirical</u> studies concentrated primarily on how wave phenomena coordinate cortical operations—in terms of creating synchronous patterns of activity between neural circuits that underlie various behavioral states, and in terms of transfer of information between circuits—rather than on *computational properties* of wave phenomena. Here we combine theoretical and empirical methods to investigate computational properties of wave phenomena. We find that this approach helps to understand visual phenomena that appear to be puzzling from the traditional perspective of neuronal specialization.

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Nanosymposium

681. Neural Computations of Visual Cortex

Location: SDCC 25

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.12

Topic: D.06. Vision

Title: Receptive field mapping of body selective regions supports an experiential account of visual cortex organization

Authors: *E. DANIEL HERTZ, S. GREGOREK, J. YAO, P. HOYOS, J. GOMEZ;
Princeton Univ., Princeton Neurosci. Inst., Princeton, NJ

Abstract: Using fMRI, the receptive field of a single voxel can be modeled as the collection of receptive fields of all neurons within that voxel, which is referred to as the population receptive field (pRF) (Dumoulin and Wandell, 2008; Victor et al., 1994). Previous research with pRF mapping has shown that category selective subregions of the visual cortex (such as face, place, and word selective regions) contain pRFs of unique sizes and biases across the visual field. For example, face and word selective regions tend to be biased towards coverage of the center of the visual field (Kay et al., 2015; Gomez et al., 2018, Le et al., 2017), whereas the PPA is much more peripherally biased (Levy et al., 2001; Malach et al., 2002). To date, no work has directly examined the receptive field properties of body- or limb-selective regions in visual cortex. In this study, we use a visual category fMRI localizer to identify four body-selective regions in each participant's (n=28) occipitotemporal cortex-three surrounding the hMT+ complex (e.g. one of each of the lateral occipital sulcus, the inferior temporal gyrus, and the middle temporal gyrus) and one ventrally in the occipitotemporal sulcus. The same participants underwent a sweeping-bar retinotopic mapping experiment which importantly employs new stimuli and motion parameters to more readily drive activity in extrastriate cortex and improve modeling of pRFs near the fovea. We ask the following: do different limb-selective regions display distinct pRF properties, and how do those compare to other visual regions? We demonstrate that body-selective regions contain uniquely peripheral and large receptive fields (F's>21.7, p's<0.001) when compared to face-selective regions, and that pRF properties vary significantly across body-selective regions (F=6.7, p<0.001) and across hemispheres (F=6.1, p<0.01). These findings hold implications for theories on visual cortex development, as body-selective regions contain pRFs that mirror viewing behavior well but some contain pRF's much more peripheral than would be predicted by their cortical location.

Disclosures: E. Daniel Hertz: None. **S. Gregorek:** None. **J. Yao:** None. **P. Hoyos:** None. **J. Gomez:** None.

Nanosymposium

681. Neural Computations of Visual Cortex

Location: SDCC 25

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 681.13

Topic: D.06. Vision

Title: A topographic network showing tuned responses to visual short-term memory load

Authors: *M. VAN ACKOOIJ[1], J. M. PAUL[2], N. VAN DER STOEP[1], B. M. HARVEY[1];
[1]Exptl. Psychology, Utrecht Univ., Utrecht, Netherlands; [2]Melbourne Sch. of Psychological Sci., The Univ. of Melbourne, Melbourne, Australia

Abstract: Representing image content in visual short-term memory is essential to performing many visual tasks. But how does the brain respond to the load on visual short-term memory? In an exploratory study, we measured responses to varying numbers of items in visual short-term memory in six human subjects (two female) using 7T fMRI. Our displays had a constant numerosity of six items but required remembering a variable number of items. The task was designed to elicit responses to changing visual short-term memory load independent of changes in color contrast and orientation contrast. We analyzed the responses with neural population response models tuned to the remembered number (i.e. visual short-term memory load). We found eleven bilateral areas showing tuned responses to visual short-term memory load in the dorsal stream and fronto-parietal attention network. This tuning was better captured by logarithmic Gaussian functions of remembered item number than by linear Gaussian or monotonic functions. These responses were invariant to task difficulty and trial order. Furthermore, in those participants for which we also had access to visual field mapping data, and numerosity data, observed visual short-term memory load preferences were not correlated to eccentricity preferences or numerosity preferences. We found gradual changes in the preferred visual short-term memory load of these neural populations across the cortical surface within these areas, such that they formed topographic maps. Superior parietal maps were the largest, showed the clearest responses and responded to the broadest range of short-term memory loads (i.e. had broader tuning widths). Left hemisphere maps showed clearer responses, but all maps were similarly sized in both hemispheres. We found hierarchical transformations of visual short-term memory load representations from posterior to anterior maps, focusing increasingly on higher visual short-term memory loads. These representations of visual short-term memory load mirror properties of other quantity representations and sensory cortices and demonstrate that tuned neural responses can encode task properties as well as stimulus properties.

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Nanosymposium

682. Neuronal Circuits Driving Reward and Motivated Behavior

Location: SDCC 23

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 682.01

Topic: G.03. Motivation

Title: Blunted loss sensitivity in anterior insula predicts relapse to stimulant use

Authors: *L. MORTAZAVI, K. H. MACNIVEN, B. KNUTSON;
Stanford Univ., Stanford, CA

Abstract: Psychostimulant drugs are among the most addictive substances, and patients with Stimulant Use Disorder (SUD) in particular show high rates of relapse one year after leaving treatment. While most research has focused on neural predictors of developing a SUD diagnosis, processes that promote abstinence or relapse have received less study. Since relapse is difficult to predict with conventional measures (e.g., self-report, behavior), we sought to test whether neural responses to incentives could predict relapse. We recruited 42 healthy volunteers and 60 SUD patients who were abstinent and in treatment at the time of testing, following up over 6 months after treatment discharge. Participants were scanned with Functional MRI (FMRI) as they performed the Monetary Incentive Delay (MID) task, which is designed to probe neural responses during the anticipation and receipt of monetary gains and losses. Between patients and controls, there were no differences in neural activity in any pre-registered regions of interest. Among patients, however, patients who relapsed (n=33) had lower functional activity specifically during anticipation of high losses in the Anterior Insula (AIns) than patients who abstained (n=27). This difference remained robust after controlling for several confounds (i.e., craving, negative mood, years of use, age, and gender. Blunted AIns loss anticipation activity also predicted shorter time to relapse. Behaviorally, slower reaction times for loss avoidance correlated with AIns anticipatory activity and also predicted relapse. Further, in previous research on the same sample, we reported that lower Fractional Anisotropy (FA) in the right AIns to Nucleus Accumbens (NAcc) tract predicted shorter time to relapse (Tisdall & MacNiven et al., 2022). Here, we found that AIns-NAcc FA was positively associated with loss anticipatory activity in the right AIns as well as with the correlated activity between right AIns and NAcc specifically during loss trials. Together, these findings provide convergent multi-modal evidence implicating avoidance motivation and its neural correlates in the AIns as risk factors for relapse in SUD. This neural signature may serve as a markers capable of prospectively predicting relapse above and beyond self-reported and demographic variables. These findings also more broadly implicate loss anticipation in maintenance of abstinence, consistent with distinct neurobehavioral circuits promoting "getting into" versus "getting out of" addiction.

Disclosures: L. Mortazavi: None. **K.H. MacNiven:** None. **B. Knutson:** None.

Nanosymposium

682. Neuronal Circuits Driving Reward and Motivated Behavior

Location: SDCC 23

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 682.02

Topic: G.03. Motivation

Title: Childhood Deprivation Predicts Increasing Activity of the Anterior Insula in Adolescent Girls: A Longitudinal Study

Authors: *L. R. BORCHERS[1], J. K. LEONG[2], J. P. YUAN[1], I. H. GOTLIB[1];
[1]Psychology, Stanford Univ., Stanford, CA; [2]Psychological Sci., Univ. of Arkansas, Fayetteville, AR

Abstract: Several cross-sectional studies indicate that early life stress (ELS) alters brain activity in the context of reward tasks. Importantly, childhood deprivation and sex are risk factors for a range of behavioral problems in adolescence. Despite well-documented associations, few studies have assessed these relations longitudinally. In this study, we examined the development of the anterior insula (AIns) following childhood deprivation and the moderating role of sex in order to advance our understanding of the sequelae of ELS.
We administered a child-friendly version of the Monetary Incentive Delay Task across 3 timepoints to examine neural activity during the anticipation and receipt of loss. Participants underwent functional Magnetic Resonance Imaging (fMRI) at Time 1 (n=144; Mage=11.59, SD=1.05), Time 2 (n=122; Mage=13.47, SD=1.16), and Time 3 (n=101; Mage=15.53, SD=1.08). We used fMRIPrep to preprocess the fMRI data and extracted raw percent signal change in the bilateral AIns. We computed deprivation based on the average of z-scores from the Multidimensional Neglectful Behavior Scale (Time 1), family socioeconomic status (SES), and neighborhood disadvantage (based on Census tract information). Participants completed the Sensitivity to Punishment and Reward Questionnaire (SPSRQ) at Time 3.
There was a significant interaction of sex and deprivation in predicting change in AIns activity from Time 1 to Time 3 (anticipation of loss: B=0.59, p=.024; receipt of loss B=0.73, p=.005). Females, but not males, who experienced more deprivation in early life exhibited increases in AIns activity during the anticipation (B=0.39, p=.030) and the receipt of loss (B=0.47, p=.010) across adolescence. Notably, increasing activity in the AIns during the anticipation (but not during the receipt) of loss was associated with higher levels of fear, assessed by the SPSRQ (r=0.27, p=.022).
Increasing activity in the AIns during the anticipation of loss may be a mechanism by which ELS leads to a higher prevalence of anxiety disorders in females than in males. Girls are more than twice as likely as boys to develop clinically significant internalizing symptoms. Importantly, we found a significant association between increasing AIns activity and fear during adolescence. Future work should examine the unfolding of AIns activity and the development of anxiety following adversity, which could inform the generation and implementation of effective prevention and treatment strategies.

Disclosures: L.R. Borchers: None. **J.K. Leong:** None. **J.P. Yuan:** None. **I.H. Gotlib:** None.

Nanosymposium

682. Neuronal Circuits Driving Reward and Motivated Behavior

Location: SDCC 23

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 682.03

Topic: G.03. Motivation

Title: Structural connections from midbrain to Nucleus Accumbens (NAcc) correlate with greater NAcc functional activity during reward anticipation

Authors: *E. H. ELLIS, B. M. WALKER, J. K. LEONG;
Psychological Sci., Univ. of Arkansas, Fayetteville, AR

Abstract: Do structural connections carrying dopamine to the nucleus accumbens (NAcc) correlate with NAcc functional activity during reward anticipation? Previous research could characterize the white-matter tract projecting from the Ventral Tegmental Area (VTA) to the NAcc, and separate research has found greater NAcc activity during the anticipation of monetary rewards. However, no studies have linked the structural coherence of the VTA-NAcc tract to NAcc activity. To test this link from structure to function, we analyzed raw data of 4,944 subjects from the Adolescent Brain Cognitive Development Study (ABCD). Diffusion-weighted and Functional Magnetic Resonance Imaging (FMRI) data were screened for quality (<2 mm movement in 95% of acquired volumes; discovery sample n=87, mean age=10 years, 53% female). We identified regions to seed for tractography with the FreeSurfer subcortical segmentation of the NAcc and the Pauli atlas of the VTA. We then performed constrained spherical deconvolution-based probabilistic tractography with MRtrix to visualize the VTA-NAcc tract in each hemisphere of every subject, and measured Fractional Anisotropy (FA) of the tract along its trajectory. Functionally, we extracted raw activity timecourses (percent signal change) from the NAcc, aligned the timecourse with task trial phases, and targeted NAcc activity during reward anticipation. Replicating the functional literature, NAcc activity was greatest during the anticipation of large gains ($5>$0: left hemisphere: t(86)=2.97, p<0.01; right: t(86)=2.98, p<0.01). Structural coherence at the beginning of the VTA-NAcc tract was associated with greater NAcc activity during the anticipation of large gains in both hemispheres (left: r=0.25, p<0.05; right: r=0.34, p<0.01). The tract was not associated with NAcc activity during the anticipation of smaller gains and no reward. We then reproduce the analysis in randomly-sampled subsets of the full dataset based on a power analysis corrected for the observable correlation given test-retest reliability of the structural and functional measurements (n=461; observable r=0.13 with 80% power at alpha=0.05). These findings suggest that structural tracts are associated with functional activity at tract endpoints, and further identify targets to study the reciprocal development of deep brain structure and function.

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Nanosymposium

682. Neuronal Circuits Driving Reward and Motivated Behavior

Location: SDCC 23

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 682.04

Topic: G.09. Drugs of Abuse and Addiction

Title: Pain associated fentanyl intake in males is driven by dynamic activity of ventral tegmental area dopamine neurons

Authors: *J. A. HIGGINBOTHAM[1], J. ABT[2], J. MORON-CONCEPCION[3];
[1]Anesthesiol., Washington Univ. Sch. of Med., St. louis, MO; [2]Anesthesiol., Washington Univ. Sch. of Med., St. Louis, MO; [3]Anesthesiol., Washington Univ., Saint Louis, MO

Abstract: Pain is experienced by over half of US adults. Opioids are potent analgesics used to treat pain symptoms but are highly prone to abuse - creating a major dilemma for public health. Evidence suggests that the proclivity for opioid abuse under pain conditions varies between sexes. Though women are more sensitive to pain, men are more likely to escalate opioid doses, meet diagnostic criteria for opioid use disorder, and die from overdose. Previous reports from our lab indicate that pain disrupts opioid receptor-dependent dopamine (DA) release from the ventral tegmental area (VTA). However, it remains unclear whether pain impacts the ability of VTA DA neurons to respond to opioid reinforcement in a sex-dependent manner. To investigate this possibility, GCaMP was expressed in a Cre-dependent manner and an optic fiber was implanted in the VTA of male and female TH-Cre rats (2-3 m.o.). After 14 days, rats were implanted with jugular catheters and received right hind-paw injections of saline (No Pain) or Complete Freund's Adjuvant (CFA; Pain). After 3 days, rats underwent self-administration during 15 daily 2-h sessions where correct lever presses were reinforced with an infusion of fentanyl (5 ug/kg or 2 ug/kg, i.v.) and illumination of a light cue (5 s). Effects of CFA on fentanyl motivation and dose-response were assessed during subsequent sessions (days 16-20). Simultaneous intravenous self-administration and fiber photometry recordings were achieved using wireless *in vivo* fiber photometry. Every 1-5 days, a wireless, battery-powered transmitter head stage was secured to the optic fiber and GCaMP fluorescence was measured throughout the duration of the session. Baseline fluorescence and within-session signal decay were stable over time across groups. Pain increased consumption of, and motivation for, fentanyl, selectively in males. These behavioral effects developed over time and were paralleled by sex- and pain-specific effects on tonic VTA DA $\Delta F/F$ signals throughout the sessions and phasic VTA DA $\Delta F/F$ signals time-locked to fentanyl-reinforced lever presses. Males with pain showed deficits in fentanyl-evoked VTA DA $\Delta F/F$ activity at early time points which were amplified over time in a manner that corresponded with increased fentanyl intake. Finally, we found that this activity was necessary for increased fentanyl intake in males with pain as chemogenetic inhibition of VTA DA neurons during late stages of self-administration was sufficient to normalize fentanyl intake in males with pain and associated VTA DA neuron activity. These findings reveal sex-specific pain-induced adaptations to VTA DA neuron function that underlie maladaptive patterns of opioid use.

Disclosures: J.A. Higginbotham: None. **J. Abt:** None. **J. Moron-Concepcion:** None.

Nanosymposium

682. Neuronal Circuits Driving Reward and Motivated Behavior

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Presentation Number: 682.05

Topic: G.03. Motivation

Support: Neurochoice Initiative funded by the Stanford Neurosciences Institute

Title: An analytic strategy for enhancing the reliability of FMRI responses to incentives

Authors: K. H. MACNIVEN, ***D. P. CHRISTIANO**, B. KNUTSON;
Psychology, Stanford, Stanford, CA

Abstract: Although task-based Functional Magnetic Resonance Imaging (FMRI) can replicably elicit subcortical responses to incentives in small samples, recent work in large samples has not yielded similarly robust effects (Elliott et al., 2020; Kennedy et al., 2022). Beyond suboptimal data acquisition protocols (Mortazavi et al., 2021), reduced psychometric integrity of the measures (including reliability, validity, and generalizability) may help account for these discrepancies. To explore how to increase reliability, we combined FMRI with the Monetary Incentive Delay (MID) task (Knutson et al., 2000) as a probe of Nucleus Accumbens (NAcc) and Anterior Insula (AIns) activity during anticipation of gains and losses, respectively. Healthy subjects from two cohorts (cohort 1: n=40, 18-64 years old, 19 females; cohort 2: n=37, 18-85 years old, 17 female) completed the MID task during FMRI scanning. Task trials (n=90) included an incentive cue (2 s; ±0, ±1, ±5), a delay with fixation (2-2.5 s), a briefly-presented target (~250 ms), and an outcome (2 s), separated by a variable intertrial interval (2-6 s). FMRI data were preprocessed with a pipeline optimized for identifying subcortical activity using AFNI software (Cox et al., 1996). Raw activity timecourses were averaged by trial type and extracted from NAcc and AIns volumes of interest. Analyses compared the reliability (split-half within session; ICC(3,1)) of both raw activity timecourses and modeled fits, focusing on anticipation of gains in NAcc and losses in AIns, based on previous research (Knutson and Greer, 2008). Consistent with predictions, findings in both NAcc and AIns showed higher reliability for raw peak activity in the strongest conditions than for fit activity in the strongest conditions or for fit activity contrasted between the strong versus weak conditions. This predicted a pattern of results replicated across cohorts. These findings suggest a tractable and actionable analytic strategy for immediately improving the reliability of task-related FMRI data in small and large datasets alike, potentially translating to savings of time and money.

Disclosures: K.H. MacNiven: None. **D.P. Christiano:** None. **B. Knutson:** None.

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 682.06

Topic: G.03. Motivation

Title: Stimulant medications increase nucleus accumbens activity during reward anticipation in early adolescents with Attention Deficit Hyperactivity Disorder

Authors: *A. VAVRINOVA, E. ELLIS, J. K. LEONG;
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Abstract: How is brain activity during reward anticipation different in adolescents with Attention Deficit Hyperactivity Disorder (ADHD) and can treatment with stimulant medications recover the activity? Previous findings are mixed, with some studies finding reduced activity in mesolimbic brain areas and others reporting greater activity. Furthermore, less research has tested whether treating ADHD with stimulant medication changes the activity. Here we tested whether adolescents diagnosed with ADHD might differ from healthy controls in functional brain activity during reward anticipation, and further if those treated with stimulant medications differed from unmedicated individuals. We analyzed raw data from the Adolescent Brain Cognitive Development (ABCD) study. Brain activity was measured using Functional Magnetic Resonance Imaging (FMRI) while adolescents ages 9-11 years old completed the Monetary Incentive Delay task. FMRI analyses were performed on 631 individuals with ADHD, 70 of which had ongoing treatment with stimulant medication (i.e., Ritalin or Adderall), along with 4,313 age-matched healthy controls. Raw FMRI activity was extracted from targeted regions of interest in the Nucleus Accumbens (NAcc) and Ventral Tegmental Area (VTA). The NAcc region was an 8 mm diameter sphere centered at coordinates +/-10,12,-1 in Talairach space, and the VTA region was derived from the Pauli atlas. Both regions were transformed from template space to native space with registration on the participant's T1 anatomical scan, and raw percent signal change was extracted from the FMRI timecourse. Results indicate that adolescents with ADHD had lower NAcc activity during anticipation of large gains (+$5: t=2.36, p=0.019), but not during anticipation of large losses (-$5: t=1.40, p=0.163). VTA activity was lower in adolescents with ADHD during anticipation of both large gains and large losses (+$5: t=2.01, p=0.044; -$5: t=2.45, p=0.014). Adolescents with ADHD who were treated with stimulant medication had higher NAcc activity when anticipating large losses than un-medicated individuals (-$5: t=- 2.22, p=0.0299), but not during anticipation of large gains (+$5: t=0.386, p=0.701). Stimulant medication treatment was not associated with any differences in VTA activity. These findings suggest that ADHD is associated with less mesolimbic brain activity during gain and loss anticipation, and further that stimulant medication might recover NAcc activity during loss anticipation.

Disclosures: A. Vavrinova: None. **E. Ellis:** None. **J.K. Leong:** None.

Nanosymposium

682. Neuronal Circuits Driving Reward and Motivated Behavior

Location: SDCC 23

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 682.07

Topic: G.03. Motivation

Title: Role of astrocytic exocytosis of ATP in regulating dopamine signaling and dopamine-related behavior

Authors: H. LEE[1], Q. HUANG[1], B. VOLPE[1], J. YANG[2], Y. ABUHASAN[2], S. AGRAWAL[2], L. LI[2], S. MOON[1], Y. ZHAO[1], E. N. POTHOS[2], *****W. CAI[1];
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Abstract: Astrocytes play key roles in regulating brain metabolism and injury repair. They also release neurotransmitters like glutamate, D-serine, and ATP. These "gliotransmitters" can dynamically drive synaptic plasticity, contributing to cognition, mood, food intake, and sleep. Our previous studies have shown that insulin induces ATP exocytosis in astrocytes to potentiate dopamine release in the nucleus accumbens. Loss of insulin-mediated astrocytic ATP release leads to increased depressive-like behavior in mice. To further investigate the role of astrocytic exocytosis of ATP on dopamine signaling and behavior, we have developed a new mouse model to target ATP exocytosis in astrocytes. Cytosolic ATP is loaded into secretory lysosomes through vesicular nucleotide transporters (VNUT). We have crossed VNUT-flox mice with astrocyte-specific Aldh1l1-CreERT2 mice to delete VNUT in astrocytes in a tamoxifen-dependent manner (iA-VNUTKO), thus inhibiting ATP exocytosis. In primary astrocytes, loss of VNUT reduces basal ATP release by ~60%. RNA sequencing analysis from these astrocytes reveals that loss of ATP exocytosis leads to robust transcriptional remodeling, with more than 2500 genes significantly up- or down-regulated (FDR < 0.05). Pathway analysis shows that these differentially regulated genes in VNUTKO astrocytes are enriched in many biological pathways, including cholesterol biosynthesis, axon guidance, and extracellular organization. This indicates a cell-autonomous metabolic effect of VNUT deletion in astrocytes, and potential mechanisms to affect nearby neuronal function. Given the important role of purinergic signaling on dopaminergic neural circuit suggested by previous studies, we are analyzing the modulation of dopamine release in the nucleus accumbens and dorsal striatum in the iA-VNUTKO mice using carbon fiber amperometry. In addition, we have assessed dopamine-dependent behavior in these mice. The female, but not male, iA-VNUTKO mice show significantly increased time of immobility during the forced swimming test and tail suspension test, indicating increased "despair" in female iA-VNUTKO mice in response to an inescapable stressor. In contrast, the male, but not female, iA-VNUTKO mice show a dramatic reduction in motivation in an effort-based operant task. Thus, male iA-VNUTKO mice quit on the task much sooner than the control VNUT-flox mice as the effort required to obtain a reinforcing sucrose pellet increases progressively. Together, our data show that exocytosis of ATP from astrocytes regulates multiple

metabolic and neuronal pathways that could contribute to the modulation of dopamine signaling and related behavior.

Disclosures: H. Lee: None. **Q. Huang:** None. **B. Volpe:** None. **J. Yang:** None. **Y. Abuhasan:** None. **S. Agrawal:** None. **L. Li:** None. **S. Moon:** None. **Y. Zhao:** None. **E.N. Pothos:** None. **W. Cai:** None.

Nanosymposium

682. Neuronal Circuits Driving Reward and Motivated Behavior

Location: SDCC 23

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 682.08

Topic: G.03. Motivation

Title: Nucleus accumbens dopamine D3 receptor regulates mesolimbic information processing to drive motivated behavior

Authors: *J. ENRIQUEZ TRABA[1], H. E. YARUR[1], R. J. FLORES GARCIA[1], S. ROY[2], T. B. USDIN[2], R. MORATALLA[3], H. A. TEJEDA[1];
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Abstract: Adaptations in motivated behavior are prominent in a plethora of neuropsychiatric disorders. However, the neuronal mechanisms underlying this process have not yet been fully elucidated. One of the key brain regions mediating motivated behavior is the nucleus accumbens (NAc), a major target of dopamine signaling underpinning motivation control. Dopamine D3 receptor (D3R) is highly enriched in the NAc, and alterations in its function have been postulated as a prominent pathophysiology of several mental disorders. How these receptors modulate processing of motivated behavior and information processing in the NAc is yet poorly understood. By using a combination of viral-mediated anatomical tracing, genetic ablation and fiber photometry, we show that D3R regulation of information processing in the NAc is essential for the development of motivated behavior in mice. In-situ hybridization revealed that that D3R colocalizes with D1R but is largely absent in D2R-expressing medium spiny neurons (MSNs). Our results also suggest that D3R-MSNs project to the ventral pallidum, lateral hypothalamus and the ventral-tegmental area, similar output regions as D1R-MSNs. Patch-clamp electrophysiology and optogenetics suggest that local, while efferent inhibitory transmissions are inhibited by D3R activation in a pathway-independent manner, further supporting our anatomy results. Genetic ablation of D3R signaling blocked motivated behavior in three different models

of motivation. This effect is also recapitulated when D3R is selectively deleted from neurons projection to each of their three NAc output regions. By using a combination of pharmacological antagonism and genetic ablation, we were also able to show that this behavioral output is mediated by a local D3R activation within the NAc. D3R-expressing NAc MSNs are activated in response to reward-related stimuli and chemogenetically silencing them blocks motivation. Collectively, our work delineates how D3R is embedded within NAc circuits and functions, in addition to identifying NAc D3R as a potential therapeutic target for the mental disorders caused by alterations in motivated behavior.

Disclosures: J. Enriquez Traba: None. **H.E. Yarur:** None. **R.J. Flores Garcia:** None. **S. Roy:** None. **T.B. Usdin:** None. **R. Moratalla:** None. **H.A. Tejeda:** None.

Nanosymposium

682. Neuronal Circuits Driving Reward and Motivated Behavior

Location: SDCC 23

Time: Wednesday, November 16, 2022, 1:00 PM - 3:15 PM

Presentation Number: 682.09

Topic: G.03. Motivation

Title: Superior Colliculus neurons encode position and object value information.

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Abstract: Value information is critical for all animals to make appropriate decisions. Interestingly value may be associated with certain objects or with specific locations in the environment. It is not clear if there exist separate circuits and representations in the brain to process these two types of value information. To test this, neurons from the superior colliculus (SC) were recorded while monkeys performed two different value tasks that involved making similar visually guided saccades to fractal targets. In the object task, 4 objects were associated with larger volumes of juice reward (good objects) while the other 4 gave smaller volumes (bad objects). In the position task, objects appeared in 2 locations; one of which provided greater volumes of juice reward (good position) compared to the other location (bad position). The high reward positions switched to the opposite side after a block of 25-30 trials. The saccades directed to good objects and positions had faster reaction times and greater peak velocities compared to their bad counterparts suggesting that the animal formed value associations in both tasks. We found that value modulation (difference between good vs bad) due to the position is seen in SC neurons during the baseline and the initial visual transient after target onset. In contrast value modulation due to objects is seen during the later phases i.e. 80ms after the target onset. These data suggest that SC neurons encode value information based on both position and object. Further, we observed faster saccade reaction times in the position task compared to the object task consistent with the earlier onset of value modulation in neurons during this task The

preliminary analysis points to different groups of SC neurons separately encoding position and object value information which we hypothesize to be sent from Caudate Head (CDh) and Caudate Tail (CDt) respectively.

Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.01

Topic: H.09. Spatial Navigation

Title: Medial temporal lobe error signals mediate developmental differences in spatial memory precision

Authors: *H. E. ROOME[1], K. R. SHERRILL[2], K. V. NGUYEN[3], A. B. KARAGOZ[4], C. COUGHLIN[5], A. R. PRESTON[2];
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Abstract: When we move to a new city, we build spatial maps by learning routes from our home to new locations. With experience, we are able to update such maps by refining the learned relationships between landmarks, streets, and locations. In adults, formation and updating of spatial maps are supported by the medial temporal lobe (MTL), particularly the hippocampus (HPC), that integrates novel route information with prior spatial knowledge, as well as the medial parietal cortex that tracks location and orientation within an environment. Notably, spatial learning has a prolonged developmental trajectory. Even through middle childhood, children are less accurate at spatial memory and navigation relative to adults, suggesting children's spatial maps distinguish individual locations from one another less precisely. Here, we tested for developmental differences in the precision of neural representations of space and how they may arise from age-related differences in error-related memory updating during active navigation. Children (6-12y) and adults (18-33y) learned the locations of different objects within a virtual arena while undergoing functional MRI scanning. During test trials, participants were cued with an object and had to navigate to its remembered location. Immediately following navigation, participants received feedback about an object's actual location and navigated from their selected location to the correct goal location. This active feedback provided participants with the opportunity to update previously learned object-location mappings to improve performance on the next trial. We found that across learning blocks, HPC activation patterns elicited by each object became more distinct in adults relative to children, suggesting that adults formed more refined spatial maps, which differentiated the locations of individual objects from one other.

Moreover, we found that adults engaged HPC, entorhinal cortex, parahippocampal cortex and retrosplenial cortex during feedback more so than children, with the degree of error response further tracking spatial memory improvements on the next trial in adults, but not children. Together, these results indicate that developmental differences in error-related memory updating, guided by MTL and medial parietal regions, led to age-related differences in the precision of HPC spatial maps. In particular, children may be less likely to use feedback signals to learn from their navigational mistakes as a means to build a refined spatial map of their environment.

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Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.02

Topic: H.09. Spatial Navigation

Support: NIH Grant R21AG063131

Title: How episodic memory and acute stress influence spatial learning and value-based decision-making in spatial navigation

Authors: *T. BROWN, Q. HE, E. BEVERIDGE, L. LIU, V. VARGAS, A. SALEN, L. ESCHAPASSE;
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Abstract: Much of our decision-making during spatial navigation includes risk and uncertainty (e.g., a novel shortcut may save time and gas money - but only if I don't get lost) which interacts with our goals to determine the expected value of different navigational choices. Our past experiences inform our both our navigational goals and our uncertainty about different ways of achieving them. The presented studies ask two critical questions: 1) how do people integrate episodic memories into their current navigational decisions, and 2) how does stress and uncertainty affect the way memory is utilized from such learning experiences to inform decision-making in spatial navigation? We developed a virtual navigation paradigm in which participants learned to find locations of objects in an environment from a fixed starting location ("rigid" spatial learning), and from unpredictable starting locations (flexible learning under heightened uncertainty). Participants then performed a value-based decision-making task in which they decided whether to reach goal objects from the fixed or unpredictable starting locations, with different penalties associated with each option. We developed computational models to 1) quantify the temporal scope with which prior experiences are integrated into a navigator's current decision, and 2) test whether, when given an object to find, spatial decisions reflected past performance specific to that goal (Target-specific model) or integrated memory from performance with all goals in the environment (Target-common model). We found that

participants made better decisions when adhering to a Target-specific model, but most participants' decisions were better fit by the Target-common model, and this integrative tendency may be tied to concurrently greater performance variability with each goal. Moreover, greater success on our task was predicted by an interaction between the ability to estimate probabilities relevant to decision-making and one's sense of direction. Finally, when introducing experimentally-manipulated stress, we show this impairs rigid learning (in females), but can even improve flexible learning when the performance with rigid learning is controlled for. Critically, our model reveals that stress reduces memory integration in both genders, making participants focus more on recent episodic memory and less likely to integrate information from other related sources in decision-making. Collectively, our results elucidate individual differences in navigational decision-making and show how acute stress impacts different memory systems and the translation of episodic memory into our navigational choices.

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Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.03

Topic: H.09. Spatial Navigation

Support: Marcus and Amalia Wallenberg Foundation
Stanford Center for Cognitive and Neurobiological Imaging

Title: Representational integration and differentiation in the human hippocampus following goal-directed navigation

Authors: *C. FERNANDEZ[1,2], J. JIANG[4], S.-F. WANG[3], H. CHOI[3], A. WAGNER[2,3];
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Abstract: Dynamic memory processes build structured knowledge across our experiences as we learn. Such knowledge enables the formation of internal models of the world, or 'cognitive maps', that we use to plan, make decisions, and act. Recent theorizing posits that mnemonic mechanisms of differentiation and integration - which at one level may seem to be at odds - both contribute to the emergence of structured knowledge. We used fMRI to test this possibility as human participants (n = 23) learned to navigate within local and global virtual environments over the course of three days. Participants first learned to navigate between cued-goal locations on three distinct tracks (Local Navigation Task). The separately learned tracks were then connected, and participants learned to navigate across tracks to reach cued goals (Global Navigation Task). Neural pattern similarity analyses in the hippocampus and entorhinal cortex quantified the ways in which experienced trajectories through virtual space transformed the similarity between

features of the environment. Our findings reveal evidence that differentiation and integration work concurrently to build local and global environmental representations, and that variability in integration relates to differences in navigation efficiency. These results offer new insights into the neural machinery and the underlying mechanisms that translate experiences into structured knowledge that allows us to navigate to achieve goals.

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683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.04

Topic: H.09. Spatial Navigation

Title: Prefrontal Cortex and the Disambiguation of Overlapping Paths

Authors: *K. ISENBURG[1,2], S. S. P. LIAPIS[1,2], J. GUO[1], T. I. BROWN[4], J. T. MCGUIRE[3,2,1], C. E. STERN[1,2,3];
[1]Cognitive NeuroImaging Ctr., [2]Grad. Program in Neurosci., [3]Dept. of Psychological & Brain Sci., Boston Univ., Boston, MA; [4]Sch. of Psychology, Georgia Inst. of Technol., Atlanta, GA

Abstract: The hippocampo-prefrontal pathway is an important circuit in both cognition and memory processes. Mounting evidence suggests that regions within the medial temporal lobe (MTL) and prefrontal cortex (PFC) are crucial for the disambiguation of overlapping (OL) memories (Brown et al., 2013, Chanales et al., 2017, Epstein et al., 2017, Brunec et al., 2021). The hippocampus and parahippocampal cortex have been identified in navigational path disambiguation, while the PFC has an established role in decision making and schematic representation of partially overlapping environments (Zheng et al., 2021). Given known interactions between MTL and PFC regions, we hypothesized that the PFC would be important for contextual spatial representation, integrating information from MTL in order to represent decision points along pairs of overlapping paths. Additionally, we hypothesized that these representations would differ from those of non-OL paths, and mismatched non-pairs of OL paths, specifically during task learning. This analysis used data from a task-based neuroimaging study using functional Magnetic Resonance Imaging (fMRI) during the navigation of novel and familiar mazes, some sharing overlapping paths. 16 healthy participants attended one training session and one fMRI session, consisting of 10 scans. Brain masks were generated for 3 regions of interest (dorsolateral PFC, ventrolateral PFC, and orbitofrontal cortex) using the Desikan

atlas. Single-trial voxelwise activity patterns for each ROI and event of interest were extracted via beta-series regression for pattern similarity analyses (Mumford et al., 2012). Results from all ROIs showed that patterns for the first OL decision point in pairs of OL paths were anti-correlated during learning and became positively correlated and significantly more similar once participants had learned the paths. Additionally, as these patterns increased their similarity after learning, they became equally as similar as patterns extracted from non-OL paths. This suggests that within the PFC, OL paths are strongly disambiguated from one another during learning, and despite increases in similarity once learned, remain differentiated enough to be disambiguated. Interestingly, mismatched OL paths were significantly less similar than matched pairs after learning. This suggests that the PFC is able to decode spatial context such that prefrontal representations of OL pairs are kept distinct from one another during learning, and become more grouped together when learned, more-so than mismatch pairs in which differences in spatial context encoded in the representation further disambiguate them from one another.

Disclosures: **K. Isenburg:** None. **S.S.P. Liapis:** None. **J. Guo:** None. **T.I. Brown:** None. **J.T. McGuire:** None. **C.E. Stern:** None.

Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

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Presentation Number: 683.05

Topic: H.09. Spatial Navigation

Support: NSF BCS – 1829398
Institute for Collaborative Biotechnologies
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California Nanosystems Institute

Title: Evidence for a distributed head direction and travel trajectory system in the human brain during active navigation

Authors: *Y. CHENG[1], S. LING[2], C. E. STERN[2], E. R. CHRASTIL[1];
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Abstract: Head and travel directions are crucial in human wayfinding, but we do not yet know whether these signals can be classified in the brain during active navigation or how they relate to navigation performance. In an fMRI study, we tested a large group of people (N = 98) actively navigating in a complex virtual environment. The navigation task consisted of an exploration and a test phase. During the 16-minute exploration phase, participants freely explored a maze to locate 9 objects, and were instructed to remember their locations. For each of the 48 test trials, participants started at one object and were directed to go to another object, without feedback and with limited time. We conducted an intra-subject multivariate pattern classification for different types of head and travel directions in distributed regions of interest (ROIs) including the

hippocampus, basal ganglia (caudate, putamen, pallidum, nucleus accumbens), visual cortex (extrastriate cortex and early visual cortex), thalamus, and parietal lobe (retrosplenial cortex, precuneus). We were able to successfully classify both egocentric (viewer-centered) and allocentric (world-centered) signals for head and travel direction. Moreover, during the exploration phase, we observed no correlation between classification accuracy and individual navigation performance, suggesting behavior-independent common mechanisms for direction representation. In contrast, during the test phase, we observed correlations between classification accuracy and navigation performance, revealing behavior-dependent variation in direction representation. Notably, good navigators had better brain signatures related to future and past egocentric movements, suggesting that they have converted directional information into actionable egocentric trajectories, particularly when considering their previous and upcoming movements.

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Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.06

Topic: H.09. Spatial Navigation

Support: EHR 1660996
                  EY031286

Title: Individual differences in spatial representations used for goal-directed navigation

Authors: *I. K. BRUNEC[1,2], M. PEER[2], K. V. NGUYEN[1], S. A. HENDRICKS[1], R. A. EPSTEIN[2], N. S. NEWCOMBE[1];
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Abstract: There is a substantial amount of variability in the accuracy of different individuals' internal representations of space (cognitive maps). However, we know little about how such variability is reflected in neural activity, and whether these differences arise from different navigational strategies. There are two different spatial representations people might be using when navigating to goals: 1) a path-based representation, encoded as a sequence of road segments and turns, and 2) a map-based representation, encoded as Euclidean (straight-line) distances between locations. We designed a city-like virtual reality environment comprising only one-way streets, allowing us to disambiguate between Euclidean and path distance. We recruited participants along a spectrum of navigational ability, assessed on a separate navigation task (Virtual Silcton). Participants were taught the layout of the city and the locations of 16 objects, each placed at one of the intersections. They were first guided along routes by following route markers. The routes were designed to be either direct (similar Euclidean and path distances) or indirect (very different Euclidean and path distances). After three blocks of guided navigation,

participants were asked to navigate along the same routes independently, following the one-way road system. Their Euclidean spatial knowledge was then assessed through a judgment of relative direction (JRD) task: on each trial, they were asked to imagine standing at one object and to indicate the relative Euclidean direction of another object. Their path knowledge was tested by querying their memory for the one-way street network, and they were also asked to construct a bird's eye view map of the environment. We find that these measures are inter-related but that path- and Euclidean distance representations are partially dissociable. Importantly, we also found a moderate relationship between participants' performance on Virtual Silcton and the JRD task, suggesting stable individual differences across different environments and task demands. These results suggest that variability in cognitive map accuracy may be linked to the types of representations individuals use in goal-directed navigation, and highlight the importance of testing multiple alternative cognitive map models in the same individuals.

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683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.07

Topic: H.09. Spatial Navigation

Title: Event-specific gaze behavior and brain activity during movie viewing and recall

Authors: *M. NAU[1], H. TARDER-STOLL[2], A. GREENE[1], J. CHEN[3], C. BALDASSANO[2], C. I. BAKER[1];
[1]NIMH, Bethesda, MD; [2]Columbia Univ., New York City, NY; [3]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Viewing and recall engage overlapping neural systems in the human brain. They are further linked through gaze reinstatement, the recapitulation of encoding-related gaze patterns during recall. Here, we characterize the relationship between these phenomena for continuous narratives. Specifically, we investigate whether eye movements reflect the event structure of a movie, and whether the observed neural overlap is grounded in shared gaze patterns. We tested this by combining eye tracking and model-based gaze predictions with fMRI data acquired while participants watched and recalled an episode of the BBC show Sherlock. First, we found that gaze patterns during movie viewing were indeed event specific. Further, these patterns were consistent across participants and in-scanner and out-of-scanner eye tracking, predicted by frame-wise saliency, and predictive of how memorable each event was. Second, we reconstructed the movie event structure during recall using a hidden Markov model trained on

the MR-signal of the eyeballs during viewing, suggesting that encoding related gaze patterns were partially reinstated during recall. Finally, we related the eyeball multi-voxel pattern to brain activity and found substantial overlap in gaze-dependent activity between viewing and recall, with the latter engaging the medial temporal lobe. Collectively, our results suggest that gaze patterns are inherently linked with how we remember events, and that behavioral and cortical reinstatement jointly support recall of episodic memories.

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Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.08

Topic: H.09. Spatial Navigation

Title: The Temple Tour: Neural coding of episodic and spatial representations in children and adults

Authors: *K. V. NGUYEN, J. J. ERARDI, H. POPAL, I. K. BRUNEC, I. R. OLSON, N. S. NEWCOMBE;
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Abstract: Navigation and episodic memory are two fundamental cognitive processes that guide mature decision-making. Conceptually, they are linked by reliance on accurate retrieval of spatial and temporal context and coding within medial temporal lobe structures However, the extent and nature of interdependence at behavioral and neural levels is unclear, with some recent evidence suggesting they are mechanistically distinct or mediated by scene construction. **In this study, we investigate how spatial navigation and episodic memory relate to each other behaviorally and how they are represented in the medial temporal lobe in children and adults.** We developed a real-world tour task in which children (8-13 years) and young adults took a guided walk through a novel environment and encoded sixteen distinct events. Next, they experienced a second encoding event in a testing room that was episodically rich but devoid of a spatial component. We assessed knowledge of the environment (only tour encoding) and episodic recollection of the events (both tour and room). On the second day, participants received an fMRI scan while viewing images of the tour and room objects, along with brand new objects. Preliminary linear modeling shows an interaction of age on the relationship between autobiographical episodic memory and spatial performance, with a significant relationship in children. In ongoing analyses, we will isolate areas of BOLD activation and contrast neural representations of the tour, room, and new objects, teasing apart nuances in the neural

representation of the spatial (tour v room) and episodic components (room v new). We will focus specifically on the hippocampus, a region historically implicated in both episodic memory and spatial coding, isolating the long-axis and subfield segmentations to inform possible variable coding of these closely related systems. We will present data from a full adult sample (N=40) and a partial child sample (N~20). These data will inform the developmental trajectory of the neural coding underlying spatial and episodic memory systems and tease apart how they relate both overall and componentially.

Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.09

Topic: H.09. Spatial Navigation

Title: Breaking the space-time continuum: How spatial boundaries structure our event memories

Authors: *S. LEE;
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Abstract: The brain is faced with the formidable task of adaptively organizing and storing the continuous stream of information that we experience daily. One way in which we mentally break the spacetime continuum is by chunking events into discretely organized sequences. Research across various fields of brain and cognitive sciences has shown that environmental boundaries such as walls and doorways not only provide crucial input to the representation of spatial structure but that they also influence the representation of temporal structure. A limitation of earlier studies, however, is that it is not clear whether boundary representations that subserve navigation (i.e., spatial mapping) are the same ones that influence the temporal organization of episodic memory.
To address these issues, we conducted a series of experiments that probed episodic memory using a what-where-when object placement task. First, we found that not only does spatiotemporal binding develop early in children, the effect of spatial boundaries on this binding is at first limited to 3D terrain-like boundaries that younger children can navigate by; then, as children's representation of navigational boundaries becomes increasingly flexible over development (to include abstract boundaries such as a row of objects or a 2D line), the types of boundaries that guide their event memory also changes accordingly.
In adult participants (n=35, mean age 24.3, 18 male) performing a computerized fMRI version of

the task, we found that not only is the binding of space and time fundamental to the successful engagement of the episodic memory network, an enhancement of sequential episodic memory in environments with an additional boundary is correlated with boundary-dependent activation of the medial temporal lobe (hippocampus and entorhinal cortex) and scene-processing cortical regions (e.g., OPA and PPA). We also found that individual differences in the recruitment of these brain regions were reflected in the participants' boundary utilization behavior. These results suggest that the influence of spatial representations (e.g., boundaries) on episodic memory goes beyond simply providing a contextual framework and extends to providing a temporally organized structure for our memories.

Disclosures: S. Lee: None.

Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.10

Topic: H.09. Spatial Navigation

Support: NIH R01 MH100121-01
NINDS F32 NS098808

Title: Emergence of hippocampal and ventromedial prefrontal cortex context-dependent coding during virtual navigation

Authors: *K. R. SHERRILL[1], H. E. ROOME[2], A. B. KARAGOZ[1], J. M. LONG[1], A. R. PRESTON[1];
[1]Univ. of Texas at Austin, Austin, TX; [2]Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Decision-making in everyday life depends on the context in which choices are made. Prior rodent work indicates that after learning, hippocampus (HPC) represents events hierarchically based on shared context, value, and spatial position. Ventromedial prefrontal cortex (vmPFC) then exerts top-down control of context-dependent HPC representations in service of optimal decision-making. Here, we quantify the emergence of such context-dependent coding in the human brain during learning. While undergoing fMRI scanning, participants learned about objects with context-dependent reward values in a naturalistic, virtual environment consisting of an elongated, contextually-varying hallway with decision points on either end. During learning, participants learned how the reward value of objects varies as a function of the spatial context during the hallway period. Participants initially learned one set of object-reward pairings before a new set of object-reward pairings was introduced. We hypothesized that prior knowledge of the hierarchical reward structure would transfer, or generalize, to the new set of objects. Early in learning, participants gradually learned the context-dependent object-reward pairings. Upon introduction to the new set of object-reward-pairings, participants were accurate

at selecting objects associated with the highest reward values, indicating rapid generalization of reward structure to the new set of objects. We trained a pattern classifier to decode reactivation of individual objects as participants navigated the hallway to the decision point. Early in learning, HPC reflected reactivation of object representations associated with the highest value in the current context, with decoding predicting individuals' choices. During transfer, such predictive object decoding further emerged in vmPFC, suggesting a shift in context-dependent representation from HPC to vmPFC with the accumulation of experience. Moreover, vmPFC decoding showed a prospective-retrospective interaction. Upon entering the hallway, vmPFC patterns reflected the object that was most recently selected; as participants moved toward the next choice point, vmPFC prospectively reactivated the high-value object for the current context. These findings show that HPC and vmPFC representations distinguish objects based on how valuable they are in a given context, with HPC context-dependent codes emerging during initial learning, while vmPFC representations may support generalization of learned experience. Such representations are critical to adaptive decision making, promoting selection of the right action in any given context.

Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.11

Topic: H.09. Spatial Navigation

Title: Evidence for grid-like organization of memory palaces

Authors: *A. O. CONSTANTINESCU, E. PATEL, J. BISBY, A. CASTEGNARO, N. BURGESS;
UCL Inst. of Cognitive Neurosci., London, United Kingdom

Abstract: The memory palace technique, also known as "the method of loci", is a mnemonic training strategy which uses mental visualizations of a familiar spatial environment to enhance the encoding and recall of a large amount of non-spatial information in order. Grid cells organize spatial and non-spatial knowledge into cognitive maps by using a hexagonally symmetric code. FMRI has been used to detect grid-like signals in a network of regions in the human brain including the medial entorhinal and medial prefrontal cortices. Here we aimed to test if grid-like codes organize memory for long lists of words in a memory palace. To ensure participants used the same spatial structure, we created a virtual reality (VR) memory palace inspired by Harry Potter: a square room with salient landmarks on each wall, and 36 magical objects arranged in a

6*6 spatial layout. We trained participants with a series of tasks. First, they learnt the precise location of the objects in the VR. Second, participants learnt two simple routes: one route connected all 36 objects along one direction (an East-West raster pattern), whereas the other route connected the objects along an orthogonal direction (a North-South raster pattern). Third, participants used the memory palace technique to learn multiple lists of 36 words in order. Each word was individually presented on a blank screen, outside the VR. Participants were instructed to imagine a vivid association between the word and its corresponding object along one of the routes. After training, we scanned their brain with 3T fMRI during VR navigation and while learning two novel lists of words each aligned with one of the routes. We hypothesized that fMRI activity during list learning could be predicted from stable grid angles (φ) estimated during VR navigation. To find the most reliable φ, we split the navigation data in half by carefully balancing the distribution of trajectories in each half, did a quadrature filter analysis, and selected entorhinal voxels with high magnitude and temporally stable φ. This φ was also consistent with grid signals in the medial prefrontal cortex, orbitofrontal cortex and amygdala. Moreover, φ predicted fMRI activity during list learning in the medial and orbitofrontal cortices as a function of the alignment of the two routes with the grid angle from navigation. Our results suggest that grid-like signals organize memory palaces by using the orientation of the spatial map in the entorhinal cortex to inform the prefrontal cortex in remembering long lists of words anchored to the same spatial structure.

Disclosures: A.O. Constantinescu: None. **E. Patel:** None. **J. Bisby:** None. **A. Castegnaro:** None. **N. Burgess:** None.

Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.12

Topic: H.09. Spatial Navigation

Title: Human grid-cell-like representations encode the location and movement of others

Authors: *M. STANGL[1], S. L. L. MAOZ[1], U. TOPALOVIC[1], C. S. INMAN[2], S. HILLER[1], N. HASULAK[3], V. R. RAO[4], C. H. HALPERN[5], D. ELIASHIV[1], I. FRIED[1], N. A. SUTHANA[1];
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Abstract: When we navigate through an environment shared with other people, keeping track of another individual allows us to make moment-by-moment predictions about their future location, for example to anticipate and avoid bumping into them. This ability requires an estimate of the other individual's position, and the continuous updating of this estimate by integrating their speed and movement direction. Grid cells, a spatially-modulated neuron type predominantly located in medial temporal lobe (MTL) regions, provide a conjunctive representation of one's own location, movement direction, and self-motion speed. Grid cells are thus an ideal candidate to encode motion-related information not only for self-movement, but also for other individuals. Previous work has shown that the firing activity of grid cells in the human brain can be measured not only on the single-neuron level, but also in the firing signature of large grid cell populations (commonly referred to as grid-cell-like representations), in the form of a hexadirectional modulation of brain activity depending on one's movement direction. Using intracranial electroencephalography (iEEG) recordings from the human MTL during real-world navigation and observation tasks, we investigated whether oscillatory grid-cell-like representations encode motion-related information for oneself and others. We found a hexadirectional modulation of low-frequency oscillations (around 5-12 Hz), indicative of grid cell population activity, in MTL recording channels during self-navigation as well as during the observation of another individual. While the precise origin of these signals remains to be determined, hexadirectional modulation of theta power was present predominantly in close proximity to the entorhinal cortex and subiculum, known to be primary locations of grid cells in the rodent brain. Together, these findings suggest that human grid cells and their population-level firing signature encode motion-related information not only during self-navigation, but also serve to keep track of another person in a shared environment.

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Nanosymposium

683. Memory Encoding and Spatial Navigation

Location: SDCC 24

Time: Wednesday, November 16, 2022, 1:00 PM - 4:15 PM

Presentation Number: 683.13

Topic: H.09. Spatial Navigation

Support: CIHR
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Title: Mechanisms of spatial navigation in the hippocampus of the common marmoset

Authors: *D. PIZA[1], B. W. CORRIGAN[3], R. A. GULLI[4], L. E. MULLER[2], J. C. MARTINEZ-TRUJILLO[5];
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Abstract: The hippocampus is a specialization of the mammalian brain that plays an important role in spatial navigation. Most of our knowledge of the hippocampus physiology comes from studies in freely navigating nocturnal surface dwellers such as mice and rats. Studies in freely 3D navigating primates with sensory adaptations to a diurnal lifestyle are scarce. Here we test the hypothesis that a diurnal primate with high resolution foveal stereo-color vision, the common marmoset, uses visual navigation to explore the environment, and that such strategy has shaped the mechanisms enabling spatial navigation in the hippocampus. We show that during foraging marmosets navigate the environment through alternations of full body displacements and stops. During stops, marmosets navigate the environment visually, through rapid head-gaze shifts, which allows exploiting their far sensing capabilities (e.g., vision) to build cognitive maps of the environment without visiting locations and landmarks. On the other hand, during foraging rats predominantly use body displacements to visit locations and landmarks, which allows exploiting their near sensing capabilities (e.g., whiskers). In the marmoset hippocampus CA3 and CA1 regions, neurons were predominantly selective for head-view orientation, rather than spatial location or place as commonly found in the rat. Moreover, most of narrow spiking interneurons in marmosets were tuned to head angular speed rather than to body speed as in rats. Finally, theta oscillations were considerably less frequent during locomotion in marmosets than in rats, and were rather coupled to rapid head-gaze shifts (head phase resetting). Our results demonstrate that the mechanism of spatial navigation in the common marmoset hippocampus have evolved relative to those documented in rodents such as rats and mice, likely reflecting evolutionary adaptations to diurnal life styles.

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Nanosymposium

684. Epigenomic and Transcriptomic Cell Type Atlas of the Whole Mouse Brain

Location: SDCC 1

Time: Wednesday, November 16, 2022, 1:00 PM - 2:45 PM

Presentation Number: 684.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 5U19MH114830-05

Title: A whole-brain mouse spatial transcriptomics atlas

Authors: *M. KUNST[1], D. MCMILLEN[3], J. CAMPOS[3], B. R. LONG[4], J. L. CLOSE[2], N. MARTIN[3], Z. MALTZER[3], M. HUPP[3], Z. YAO[1], J. D. WATERS[4], H. ZENG[4];
[2]Imaging, [1]Allen Inst. for Brain Sci., Seattle, WA; [3]Alle Inst. for Brain Sci., Seattle, WA; [4]Allen Inst. For Brain Sci., Seattle, WA

Abstract: In recent years the advent of single-cell RNA sequencing (RNAseq) technologies gave scientists an unprecedented ability to define cell types by their gene expression profiles. However, the spatial information retained does not go beyond individual brain regions, and finer details (i.e subnuclear or layer specific localization and gradients) are lost in the dissociation process. Spatial transcriptomics has the potential to fill in this gap. In order to map transcriptomically defined cell types to their precise location in the mouse decided to generate a spatial transcriptmics atlas of the whole mouse brain using Multiplexed Error Robust Fluorescence In Situ Hybridization (MERFISH) using a commercially available platform (MERSCOPE). We sectioned multiple brains coronally at regular intervals between 100 and 200 μm in its entirety to evenly cover all brain regions. To relate cell type gene expression pattern to a spatial location within the mouse brain we measured the expression of 500 genes carefully selected to maximize our ability to differentiate all clusters described in our whole brain annotation. The generated data are then undergoing a rigorous processing pipeline to ensure that the quality of cells used for mapping. We verify our results by comparing gene expression pattern of select genes to the Allen ISH-atlas. In addition, we could confirm previous described cell type localization (i.e. layer and region specific localization of glutamatergic clusters in the isocortex). In addition, we are investigating the distribution of cell types in various midbrain regions (i.e superior colliculus, inferior colliculus, periaqueductal gray) of which detailed description of cell type composition are scarce.

Disclosures: M. Kunst: None. **D. McMillen:** None. **J. Campos:** None. **B.R. Long:** None. **J.L. Close:** None. **N. Martin:** None. **Z. Maltzer:** None. **M. Hupp:** None. **Z. Yao:** None. **J.D. Waters:** None. **H. Zeng:** None.

Nanosymposium

684. Epigenomic and Transcriptomic Cell Type Atlas of the Whole Mouse Brain

Location: SDCC 1

Time: Wednesday, November 16, 2022, 1:00 PM - 2:45 PM

Presentation Number: 684.02

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant U19MH114830

Title: A molecularly defined and spatially resolved cell atlas of the whole mouse brain

Authors: *M. ZHANG[1], X. PAN[1], W. JUNG[1], Z. YAO[2], H. ZENG[2], X. ZHUANG[1];
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Abstract: A mammalian brain comprises tens of millions to billions of cells intricately organized to enable a wide range of brain functions. The enormous cellular diversity and complex spatial organization of the brain have so far hindered our understanding of the molecular and cellular basis of its function. Recent advances in spatial transcriptomic techniques have allowed systematic spatial mapping of cell types in complex tissues. However, due to the

challenges associated with scaling up the measurement throughput, these approaches have only been applied to construct cell maps of a few brain regions. A comprehensive cell atlas of the whole brain is still missing. Here, we image >1100 genes in millions of cells across the entire adult mouse brain using multiplexed error-robust fluorescence *in situ* hybridization (MERFISH) and perform spatially resolved single-cell gene-expression profiling at the whole-transcriptome scale by integrating MERFISH data with single-cell RNA-sequencing data. Using this approach, we generate a comprehensive cell atlas of thousands of transcriptomically distinct cell types for the whole mouse brain with high molecular and spatial resolution. Our data provide a global view of the hierarchical organization of cell classes, subclasses, and types. The high molecular and spatial granularity of our cell atlas reveals distinct spatial distributions of the cells across different levels, ranging from major neuronal cell types classified by neurotransmitters to finer levels classified by transcription factors, neuropeptides, and other functionally important genes. At the finest levels, gradual molecular and spatial changes in cells are often observed, typically in a correlated manner, reflecting molecular and cellular gradients that may reflect developmental origins and/or have functional implications. In addition to a high diversity of neurons, our high-resolution cell atlas also enables us to characterize the brain-wide distribution of >100 non-neuronal cell types, with many showing regional specificity, and their cell-type-specific interactions with neurons. Overall, our results establish a comprehensive, spatially resolved whole mouse brain cell atlas, providing rich insights into the molecular and cellular architecture of the brain.

Nanosymposium

684. Epigenomic and Transcriptomic Cell Type Atlas of the Whole Mouse Brain

Location: SDCC 1

Time: Wednesday, November 16, 2022, 1:00 PM - 2:45 PM

Presentation Number: 684.03

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: A spatial and single-cell transcriptional atlas of the adult mouse brain

Authors: *J. LANGLIEB[1], J. WEBBER[1], K. BALDERRAMA[1], N. SACHDEV[1], E. MURRAY[1], N. NADAF[1], T. NORTON[1], V. GAZESTANI[1], C. R. VANDERBURG[1], V. KOZAREVA[1], C. MARTIN[1], F. CHEN[2], E. MACOSKO[1];
[1]Stanley Ctr. for Psychiatric Res., [2]Program in Cell Circuits and Epigenetics, Broad Inst., Cambridge, MA

Abstract: The function of the mammalian brain relies upon the precise specification and spatial positioning of diversely specialized cell types. Yet, the molecular identities of the cell types and their positions within individual anatomical structures remain incompletely known. To construct a comprehensive atlas of cell types in each brain structure, we paired high-throughput single-nucleus RNA-seq with cellular-resolution unbiased spatial transcriptomics across the entire mouse brain. From six million single-nucleus RNA-seq profiles, sampled from across the mouse nervous system, we identified 4,444 molecularly distinct populations. To precisely map these populations to anatomical structures, we performed Slide-seq on 110 serial coronal sections of the mouse brain, registering the data to a neuroanatomical common coordinate framework. Integration of these datasets allowed for the multi-scale analysis of cell type spatial distributions for each neuroanatomical structure across the brain, from examining cellular level adjacency networks to evaluating regional cell type composition. We also performed a brain-wide analysis of gene network modulation within and between neuroanatomical regions. In doing so, we defined rules of neurotransmitter usage in each structure, constructed a brain-wide network of neuropeptide signaling, and characterized the heritability enrichment of dozens of neurological and psychiatric phenotypes in each cell type, in each brain structure.
These data should find diverse applications across neuroscience, including the construction of interactive tools to interrogate each brain cell type. These tools will give researchers the ability to interoperate between cell types' differential expressed genes, spatial distribution, and the underlying spatial gene distribution. This flexibility will aid the prioritization of specific cell types and regional circuits in the study of brain diseases.

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Nanosymposium

684. Epigenomic and Transcriptomic Cell Type Atlas of the Whole Mouse Brain

Location: SDCC 1

Time: Wednesday, November 16, 2022, 1:00 PM - 2:45 PM

Presentation Number: 684.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant U19MH114830
          NIH Grant R01EY023173
          NIH Grant U01MH105982

Title: A whole mouse brain transcriptomic cell type atlas

Authors: *Z. YAO[1], H. ZENG[3], B. TASIC[4], C. LEE[5], K. A. SMITH[2], C. V. VELTHOVEN[2], N. DEE[2], M. KUNST[2], J. GOLDY[2], D. MCMILLEN[2], A. TORKELSON[2], N. GUIFORD[2], R. CHAKRABARTY[2];

[1]Allen Inst. for Brain Sci., [2]Allen Inst. for Brain Sci., Seattle, WA; [4]Cell and Circuit Genet., [5]Modeling Analysis and Theory, [3]Allen Inst. For Brain Sci., Seattle, WA

Abstract: To understand brain organization and function, it is essential to acquire the foundational knowledge about the cell type composition of the brain. Single-cell transcriptomics has revolutionized the way to identify and classify cell types. However, despite of many studies of cell types in individual parts of the brain, there has been a lack of comprehensive and standardized characterization of cell types across the entire brain. Here we report a transcriptomic cell-type taxonomy across the entire mouse brain, integrating several whole-brain single-cell RNA-sequencing (scRNA-seq) datasets. The datasets contain a total of ~5 million cells passing rigorous quality-control criteria. The integrated transcriptomic taxonomy contains >4,000 clusters that are organized in a hierarchical manner with groupings of classes, subclasses, supertypes and types/clusters. We annotate the anatomical location of each cell type by using brain-wide MERFISH datasets. The top level of the hierarchy features the neuronal, neural non-neuronal and non-neural divisions. Within the neuronal division, major cell classes are defined by their regional and/or developmental origins. The transcriptomic landscape of cell types across the brain is highly complex, characterized with many highly distinct neuronal and non-neuronal cell types, as well as a surprising continuous core of glutamatergic and GABAergic neuron types spanning the ventral axis of the brain from forebrain to hindbrain, suggesting that the transcriptomic relationships may reflect the inter-regional evolutionary relationships among cell types. We generate a comprehensive neurotransmitter and neuropeptide distribution map in the transcriptomic taxonomy, which reveals a high degree of correspondence with specific transcriptomic types. The MERFISH datasets not only provide accurate spatial annotation of major cell types at subclass level, but also reveal very fine resolution spatial gradients for cell types within each subclass. We also provide a comprehensive characterization of non-neural cell classes and types across the mouse brain. Finally, our study reveals the universal existence of subclass-specific sets of transcription factors, suggesting that these transcription factors may be master encoders of cell type identity.

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Nanosymposium

684. Epigenomic and Transcriptomic Cell Type Atlas of the Whole Mouse Brain

Location: SDCC 1

Time: Wednesday, November 16, 2022, 1:00 PM - 2:45 PM

Presentation Number: 684.05

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Brain-wide Correspondence Between Neuronal Epigenomics and Long-Distance Projections

Authors: *J. ZHOU[1], Z. ZHANG[1], Y. PANG[1], A. RIVKIN[1], E. WILLIAMS[1], C.-T. LEE[1], H. LIU[1], P. MIYAZAKI[1], A. BARTLETT[1], A. ALDRIDGE[1], M. VU[1], A. SALINDA[1], W. LAGOS[1], N. PATNE[1], L. BOGGEMAN[1], C. FITZPATRICK[1], J. NERY[1], R. CASTANON[1], M. RASHID[1], M. JACOBS[1], T. ITO-COLE[1], N. CLAFFEY[1], M. LIEM[1], C. O'CONNOR[1], K.-F. LEE[1], X. JIN[1], E. MUKAMEL[2], M. BEHRENS[1], J. ECKER[1], E. CALLAWAY[1];
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Abstract: Neuronal cell types are distinguished by their epigenetic state, gene expression, anatomy, and physiology. Recent advances in genomic technologies have led to the identification of large numbers of transcriptomic and epigenomic clusters corresponding to possible cell types across the entire mouse brain. A prominent and distinguishing anatomical feature of different brain neuron types is their distinct long-distance axonal projections. To link epigenomic clusters and their corresponding gene expression to long-distance projections we have conducted brain-wide epi-retro-seq (single nucleus methylation sequencing of retrogradely labeled neurons) on more than 40,000 neurons dissected from 31 different regions (sources) spanning the entire brain, with 26 different projection targets. In total, we have assayed neurons from 295 different source and target combinations. Our observations from whole brain epi-retro-seq mapping experiments suggest that evolutionarily older structures including brainstem and cerebellum are more hard-wired with close correspondences between projection targets and epigenomic clusters, while neurons in the neocortex have more flexible relationships between epigenomes and projections. Hindbrain neurons in the medulla and pons that project to the cerebellum are each in distinct clusters separated from those projecting to the brainstem. The cerebellum-projecting neurons in both pons and medulla are characterized by low methylation (linked to high gene expression) of multiple genes, including Cdk14 and Map4k3. These differences are conserved across cerebellum-projecting pons and medulla neurons, despite extensive overall methylation differences between pons and medulla. There are also many clusters of hypothalamic neurons that correspond strongly with their projections to the olfactory bulb, entorhinal cortex, amygdala, striatum, pallidum, thalamus, superior colliculus, ventral tegmental area, pons, or medulla. Notably we identify a sex-specific cluster of hypothalamic neurons that is present only in females and projects to thalamus and pons. Amygdala neurons projecting to the hippocampus or olfactory bulb are strongly enriched in Vglut1 expressing clusters and are largely separated from those that project to the VTA, pons or medulla and express Vglut2. Altogether, this large and rich dataset provides many new insights into the brain-wide relationships between epigenetics, gene expression and long-distance projection targets and can be used to predict neuronal projections from genomics assays.

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Nanosymposium

684. Epigenomic and Transcriptomic Cell Type Atlas of the Whole Mouse Brain

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: MH114831-01

Title: Comprehensive single-cell analysis of chromatin accessibility in the adult mouse brain

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Abstract: The mouse brain is composed of tens of millions of neurons and glial cells, which form complex neural circuits responsible for a wide range of cognitive behaviors and neurological functions. Advances in single-cell transcriptomics and epigenomics have led to detailed characterization of gene expression patterns and epigenome at unprecedented resolution indifferent mouse brain regions, but our knowledge of cell-type specific gene regulatory programs in the mouse brain is still incomplete. Here we report a comprehensive epigenetic atlas of open chromatin in the adult mouse brain by combining our previous dataset from 45 cerebral regions(including isocortex, olfactory bulb, hippocampus, and cerebral nuclei) with an additional 1.5million cells from 72 regions in amygdala, thalamus, hypothalamus, midbrain, pons, cerebellum and medulla. Integrating the 2.3 million total cells from the entire mouse brain, we defined >140new brain cell types. We characterized the spatial and cell-type-specific state of ~922,000candidate cis-regulatory elements (cCREs), nearly doubling the number of mouse brain cCREs characterized to date. The newly identified elements represent not only more detailed brain regions, but also a more comprehensive survey of rare cell types. We find a high specific spatial distribution of not only neuronal types, but also a subset of glial cell types. In particular, both telencephalon and non-telencephalon astrocytes show gradient difference across brain regions. We inferred transcriptional regulators in cell types and characterized the gene regulatory sequences associated with regional specificity. We further assessed the evolutionary conservation of these elements in the human genome, and identified significant associations between 85 genome-wide association study (GWAS) traits with distinct brain cell types. Finally, we provide annotation for >1.8 million noncoding risk variants associated with 14 neurological and psychiatric disorders at cell-type resolution. Our results provide the most comprehensive

view of cell type specific gene regulatory programs in the mammalian brain, facilitating the annotation and analysis of gene regulatory programs in the human brain.

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Title: Dna methylome and 3d chromatin landscape of cell types across the mouse brain

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Abstract: Cytosine DNA methylation is a critical epigenetic regulator involved in brain development that has been implicated in a variety of neurological disease states. Understanding methylation diversity across the whole brain in the context of the brain's 3D spatial organization is a fundamental step toward building a complete molecular atlas of brain cell types and understanding their gene regulatory landscapes. We used optimized single-nucleus methylome (snmC-seq3) and multi-omic (snm3C-seq) sequencing technologies to collect 310,605 methylomes and 176,740 chromatin conformation/methylome joint profiles from 117-dissected regions across the entire adult mouse brain. Through iterative clustering, we constructed a DNA methylation-based cell type taxonomy containing ~2,000 distinct clusters and identified millions of differentially methylated regions between the clusters, representing sites of cell-type-specific regulation between the clusters. Notably, we found massive cellular diversity in the brain stem

associated with diverse patterns of DNA methylation at binding sites of specific groups of transcription factors, allowing the prediction of a set of master regulators important for specific brain region structures. We also observed strong cytosine methylation gradients on both genes and regulatory elements in cell types along the anterior-posterior axis within and across brain regions. By integrating cell profiles of DNA methylation and genome architecture data with companion whole-brain chromatin accessibility and gene expression information, we developed an "Activation, Repression in 3D Conformation" (ARC) model, an interpretable machine learning model that weights active (accessibility) and repressive (methylcytosine) epigenetic signals with cell-type-specific 3D chromatin conformation loops to predict gene expression for thousands of cell-types. This model enables the prediction of candidate cell-type-specific enhancers and transcription factors for each gene. Together, our study establishes the first brain-wide, single-cell resolution DNA methylome and 3D multiome atlas, providing an unprecedented resource for understanding cellular-spatial and regulatory genome diversity in the mouse brain.