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New Technologies Expand Possibilities for Studying and Treating the Brain

Innovations offer ways to probe the brain more safely, efficiently, and completely

SAN DIEGO — Advances in technology and increased understanding of the properties of neurons are paving the way for future neuroscience breakthroughs. Researchers are both improving common techniques in efforts to make treatments safer and refine animal models, as well as creating novel ways to stimulate and map the brain.

These projects were presented today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Today's new findings highlight:

- A nonsurgical way to stimulate structures deep within the brain, a less risky alternative to current treatments for Parkinson's disease (Nir Grossman, abstract 116.06, see attached summary).
- A technique to map the cortical projections of single neurons in an entire mouse brain (Longwen Huang, abstract 367.11, see attached summary).
- A potentially safer method for editing cells' genomes, moving science one step closer to curing some forms of genetic diseases (Brett Staahl, abstract 317.09, see attached summary).
- An approach to create genetic animal models with more precision and specificity (Stefan Blankvoort, abstract 183.12, see attached summary).

Other recent findings discussed illustrate:

- A way to control specific brain regions with magnetic fields, a breakthrough that could replace electronic-based stimulation treatments (Galit Pelled, presentation 95.01, see attached speaker summary).

"The innovations described today make studying the brain easier, safer, and more comprehensive," said press conference moderator Minmin Luo, PhD, of the National Institute of Biological Sciences, China, and an expert in electrophysiology and optogenetics. "These exciting advances bring us closer to a better understanding of the brain and better ways to treat diseases."

The research was supported by national funding agencies such as the National Institutes of Health, as well as other public, private, and philanthropic organizations worldwide. Find out more about the brain and new technologies for exploring its mysteries at *BrainFacts.org*.

Related Neuroscience 2016 Presentation
Minisymposia: Multiscale Connectomics: Maps, Models, and Mechanisms
Wednesday, Nov. 16, 2016 1:30-4 p.m., SDCC 6E

Abstract 116.06 Summary

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Scientists Develop New Strategy for Stimulating Deep Brain Structures Without Surgery

Technique uses the intersection of multiple electrical fields to target specific areas

A new noninvasive way to stimulate regions deep within the brain may provide a possible alternative to electrical stimulation therapies requiring surgery. The technique, tested in mice, was unveiled today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Deep brain stimulation (DBS) can alleviate the tremors and movement issues associated with Parkinson's disease in patients whose symptoms aren't adequately controlled with medication. However, for many patients, the surgery required to implant an electrode into the brain and target the affected region carries too much risk. Current noninvasive types of stimulation, which apply electric fields to the outside of the brain, lack the precision needed to be an alternative to DBS.

This study highlights a new noninvasive technique that delivers targeted deep brain stimulation without surgery or affecting other areas. Called temporal interference stimulation, the technique builds on the discovery that neurons can sense the difference between two frequencies. For example, if both a 10,010 hertz and 10,000 hertz stimulation hit a neuron, the neuron interprets the frequency as 10 hertz. Importantly, neurons don't respond to single high frequencies, so 10,000 hertz stimulation alone has no effect. In this new method, the researchers position multiple electric fields around the brain in such a way that they only overlap deep inside the brain. The outer layers of the brain aren't affected by single high frequencies, but deep within the brain, frequencies from multiple sources converge, and neurons there interpret them as one low frequency and respond. Using the new technique, the researchers noninvasively activated the hippocampus, a deep brain structure involved in memory, without activating the overlying cortex. The technology currently activates volumes bigger than the structures recruited by DBS, but further engineering may be able to significantly reduce the volume activated.

"Until now, to reach a deep structure you needed to implant an electrode in the head, and that is associated with a high risk," said lead author Nir Grossman, PhD, of Imperial College London. "We believe we've opened the way to do it in a noninvasive manner. And, if you do it in a noninvasive manner, you essentially revolutionize the risk-benefit ratio of such a therapy."

Research was supported with funds from the Wellcome Trust.

Scientific Presentation: Sunday, Nov. 13, 9:15-9:30 a.m., SDCC 7B

Abstract 10725, Noninvasive deep brain stimulation via delivery of temporally interfering electric fields
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TECHNICAL ABSTRACT: Electrical brain stimulation is a key technique in research and clinical neuroscience studies, and also is in increasingly widespread use from a therapeutic standpoint. However, to date all methods of electrical stimulation of the brain either require surgery to implant an electrode at a defined site, or involve the application of non-focal electric fields to large fractions of the brain. We here report a strategy for sculpting the amplitude of electric fields so as to enable focal, yet noninvasive, electrical neural stimulation. By delivering multiple electric fields to the brain at slightly different frequencies that are themselves too high to recruit effective neural firing, but for which the difference frequency is low enough to drive neural activity, we can cause neurons to be electrically activated at a focus without driving neighboring or overlying regions. We call this method temporal interference (TI) stimulation, since the interference of multiple electric fields is what enables the focality, since only the region for which the amplitude of the envelope at the difference frequency is high will experience neurally relevant frequencies of electric field. We modeled the concept using finite element methods in order to develop principles of how to sculpt fields in 3-D in the brain. We validated that neurons in the living mouse brain could follow the difference frequency electric field, but would not be entrained by the high frequency fields themselves. We further validated, in living mice, using c-fos labeling, that we could stimulate a deep region (e.g., hippocampus) without any stimulation of overlying cortex, via TI stimulation with a difference frequency of 10 Hz; in contrast, simply stimulating with 10 Hz transcranial alternating current stimulation resulted in significant cortical c-fos labeling in regions overlying the activated hippocampus. Finally, we explored the steerability of TI stimulation in the human brain as reflected by fMRI imaging of BOLD signals as we varied the sites and patterns of electric fields applied to specific electrodes on the scalp. TI stimulation may represent a new method of brain stimulation using familiar and well-tested electric fields, but able to achieve focal stimulation without the need for neurosurgery.

Abstract 367.11 Summary

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New Method Maps Cortical Connections of Single Cells

Procedure revealed projections of individual neurons within an entire hemisphere of mouse cortex

A new technique discerns the pattern of connections between individual neurons and their targets in the brains of mice, according to a study described today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. Identifying connections at the single cell level can help uncover the functions of different cortical circuits.

Excitatory neurons of the cortex send long projections to other cortical regions and areas deep within the brain. The patterns of these projections can reveal how the brain is shuttling information, which cells and regions are connected, and how the connections give rise to function. However, as yet, there are no comprehensive maps detailed enough to reveal the connections of a single cell.

To determine the patterns of these connections, researchers developed a new technique called Multiplexed Analysis of Projections by sequencing (MAPseq). Using a virus, researchers label individual neurons in the cortex of a mouse with unique RNA sequences, called barcodes. Within each cell, the RNA barcodes attach to a protein that is then transported to the axon terminal, which abuts the neuron's target. After sectioning the brain, the researchers quantified the amount of each RNA barcode in each section. Higher amounts of a particular barcode indicate a stronger projection from the corresponding neuron to that section.

"We implemented MAPseq to label neurons distributed throughout an entire cortical hemisphere and determined the full corticocortical projection map of an individual mouse in a single experiment," said lead author Longwen Huang, PhD, of Cold Spring Harbor Laboratory. "By uncovering the entire corticocortical projection structure at single-cell resolution, we may provide insight into the principles that underlie the organization and function of cortical circuits."

Research was supported with funds from the National Institutes of Health, the Simons Foundation, the Boehringer Ingelheim Fonds, and the Genetech Foundation.

Scientific Presentation: Monday, Nov. 14, 10-11 a.m., Halls B-H

Abstract 11556, Mapping brain-wide corticocortical projections at single-cell resolution by sequencing of barcoded RNA
L. HUANG, J. M. KEBSCHULL, A. M. ZADOR;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

TECHNICAL ABSTRACT: In the mammalian neocortex, excitatory neurons in different layers send long-range axonal projections to different cortical and sub-cortical targets. These distinct patterns may function to route different streams of information to appropriate targets. Even within a given cortical layer different neuronal classes may have characteristic projection patterns, but it is not known how many distinct projection classes there are. To date, there has been no comprehensive survey of the long-range connectivity of the mammalian cortex at single neuron resolution. We have recently developed MAPseq (Multiplexed Analysis of Projections by sequencing), a method that exploits high-throughput DNA sequencing for efficiently mapping the inter-areal projections of many individual neurons at single cell resolution. In MAPseq, we uniquely label each neuron in a population with a random RNA sequence ("barcode") by infecting with a viral library. The virus also encodes a protein engineered to transport the barcode to the presynaptic terminals. We then dissect projection targets of interest, extract the barcode RNA from each target, and quantify the abundance of each barcode sequence present in each area. The abundance of a particular barcode sequence in each target represents a measure of the strength of the projection of the barcoded neuron to the target. We are now scaling up MAPseq to label neurons distributed throughout an entire cortical hemisphere, and thereby determine the full cortico-cortical projection map of an individual mouse in a single experiment. After infecting the neurons in one cortical hemisphere with a MAPseq barcode viral library, we dissect the cortex into several hundred ~1 mm x 1 mm x 300 m cuboids. We then sequence the barcodes in each cuboid and determine the location as well as the projection targets of each neuron. This brain-wide MAPseq has the potential to uncover the entire cortico-cortical projection structure at single cell resolution, and will provide insight into the principles that underlie the organization of cortical circuits.

Abstract 317.09 Summary

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Researchers Modify Gene-Editing Tool to Reduce Potential for Long-Term Complications

Technique successfully tested in mice; if proven safe, gene editing could cure some genetic diseases

A tweak to a gene-editing technique may cut down on some of the potential risks associated with the process, moving the field one step closer to safe treatment for the underlying causes of some genetic diseases. This new animal research was released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

The CRISPR-Cas9 system is a gene-editing tool that uses the enzyme Cas9, a type of molecular scissors, to cut out a particular gene. The strategy could overhaul the treatment for genetic diseases by replacing or deleting problematic genes with functional versions. However, for it to work, a gene for the Cas9 enzyme is inserted into cells, and continuous expression of this gene may cause problems: It could alter other genes and possibly, over time, cause an immune response or even insert itself into the cell's DNA.

In this study, researchers sought to mitigate these possible long-term complications of Cas9 by using it in a transient way. They engineered the Cas9 ribonucleoprotein complex so that it could be inserted directly into cells as a functioning enzyme, rather than as a gene to be expressed. They then injected the complex into the brains of mice, where it successfully deleted a segment of DNA from within neurons. Unlike genetically encoded Cas9, the injected complex eventually degraded, preventing long-term consequences.

"We've taken one step forward by showing that we can edit neurons in adult animals using a non-genetically encoded Cas9 molecule," said lead author Brett Staahl, PhD, of the University of California, Berkeley. "The animal doesn't seem to have an innate immune response against our Cas9 molecule, it's well tolerated, and the scale of editing is significant."

Research was supported with funds from a Roche Postdoctoral Fellowship and Roche Pharmaceutical's Roche Alliance with Distinguish Scientists (ROADS) Fund.

Scientific Presentation: Monday, Nov. 14, 8-9 a.m., Halls B-H

Abstract 12468, Efficient protein-based genome editing by local delivery in the brain
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TECHNICAL ABSTRACT: RNA-guided genome editing triggered by the CRISPR-Cas9 DNA endonuclease has the potential to induce therapeutic changes to the DNA in patients with genetic disease. Such biomedical applications will require tissue-specific delivery of the editing molecules in vivo, a goal that is particularly challenging in the brain. Here we show non-genetically encoded Cas9 ribonucleoprotein (RNP) complexes can be used to edit post-mitotic neurons in the mouse brain by direct delivery following localized injection. Cas9 RNPs introduced locally in the brain trigger genome editing in cells including hippocampal, striatal and cortical neurons. We also show that protein engineering of Cas9 led to a ten-fold increase in the efficiency of neuronal cell editing in vivo. These advances provide a robust technology for application of genome editing in the brain to treat the underlying cause of genetic neurological diseases.

Abstract 183.12 Summary

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New Strategy Aims to Create More Reliable Transgenic Animal Models

Approach used to generate mice that express particular genes exclusively in specific cell types

A new method to express genes in specific cell types in the brains of mice may provide a way to more precisely target, manipulate, and understand the role of different cell types in health and disease. The technique was revealed today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

The brain houses more cell types than the rest of the body combined. Because some diseases arise from problems in a single cell type, understanding cells' individual roles could offer valuable insight into treating diseases. Developing animal models expressing particular genes only in specific cell types is critical for this effort. However, current methods of creating these animal models fail to reliably produce animals with the desired genetic characteristics in specific cell types.

In this study, researchers increased the reliability of creating transgenic animals by identifying noncoding bits of DNA (enhancers) that can drive gene expression in certain brain regions. By connecting an enhancer specific to the area of the brain known as the entorhinal cortex to small bits of DNA that deliver start messages to gene expression, the researchers created eight different mouse models. Six of the mice expressed the genes exclusively in the medial entorhinal cortex, demonstrating a more predictable way to limit expression to specific cell types.

"This will not only allow for greater understanding of how the brain works, but it may eventually improve the specificity and efficacy of genetic and molecular therapies," said lead author Stefan Blankvoort, PhD, of Norwegian University of Science and Technology. "It could truly revolutionize molecular therapeutics by targeting therapeutic transgenes specific to the cells that underlie the pathology, such as dopaminergic neurons in Parkinson's disease."

Research was supported with funds from the Kavli Foundation and the Norwegian Research Council.

Scientific Presentation: Sunday, Nov. 13, 9-10 a.m., Halls B-H

Abstract 10283, Enhanced transgenics: a novel means to generate neuroanatomically-specific genetic tools
S. BLANKVOORT[1], M. P. WITTER[1], J. NOONAN[2], J. COTNEY[3], C. KENTROS[1,4];
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TECHNICAL ABSTRACT: Recent years have seen the development of extraordinary molecular tools for neuroscience, from transgenes that allow the control or visualization of neuronal activity to precise and unambiguous neuroanatomical tracing systems. However, the full potential of such tools can only be realized if they are deployed with anatomical specificity that approaches the granularity at which neural circuits operate. This cell-type specificity can only be obtained by molecular genetic methods. To date this has involved using the specificity of native promoters to direct transgene expression, either by using minimal promoter constructs with viral vectors or pronuclear injections into oocytes, or by knocking the transgene directly into the native RNA transcript via homologous recombination. However, despite several initial successes, these techniques have serious limitations. Viruses and transgenic lines made with minimal promoters typically do not faithfully phenocopy native gene expression. Even knock-ins, which can do so, are limited by the fact that very few genes actually express exclusively in a single cell type. Therefore, all these approaches have fatal flaws. Leveraging precise tissue dissection techniques with ChIP-Seq of histone modifications associated with active enhancers, we have identified enhancers active specifically in particular brain regions. Combining these tissue specific enhancers with a mutated minimal promoter incapable of driving gene expression alone has allowed us to generate lines of transgenic mice, which target distinct cell types of particular brain regions. While our first proof-of-principle case targets distinct neurons of the Medial Entorhinal Cortex, this method can be used to target cells of any brain region. Ultimately, this enhancer-based approach should provide a means to deliver any transgene to any cell type in the brain, greatly enhancing our ability to understand the native circuitry of the brain at the level of granularity at which it operates.

Speaker Summary 95.01

Speaker: Galit Pelled, PhD (240) 418-1436
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From Fish Navigation to Neuromodulation

Scientific Presentation: Saturday, Nov. 12, 1-2 p.m., Halls B-H

We have developed a new technology that will allow scientists to control the activity of specific brain regions with high precision. This technology relies on a combination of a new gene that we have discovered and an external transmitter that can switch on and off neurons function in the brain. Thus, scientists can modulate neuronal function (a process known as neuromodulation) via induction with non-invasive magnetic fields.

The gene — called EPG (electromagnetic-perceptive gene) — was discovered in a fish that navigates according to the earth's magnetic field. The implications of such a technology could be significant on multiple levels; for basic scientific research, EPG technology will have an impact on understanding how the brain works and how diseases and neuronal disorders develop. It can also help in screening for new drugs in rigorously controlled laboratory models. In addition, it is our expectation that, in the future, the EPG technology could be applied to human patients and effectively replace electronic-based stimulation methods for devastating diseases such as epilepsy and Parkinson's disease.

In our study that was supported by the NIH EUREKA award (R01NS079288), we screened DNA libraries from the glass catfish, which is known to navigate according to the earth's magnetic field. Indeed, we discovered a single gene (EPG) that plays a critical role in this sensory mechanism. This is the first time that scientists have been able to find a gene in vertebrate animals that is involved in electromagnetic fields perception. Next, we expressed the EPG in mammalian cells, neuronal cultures and in the rodent brain. In EPG-expressing cells and neurons we found that magnetic stimulation increased calcium influx, which is indicative of activity. In rats, we directly injected the EPG into the motor cortex forelimb representation. In EPG-expressing rats we measured muscle activity in all four limbs, and found that magnetic stimulation controlled motor output in the limb corresponding only to the neurons that were bioengineered to express the EPG. Thus, we have compelling evidence that the EPG can modulate motor behavior in animal models.

We are now working on using the EPG technology as a neurostimulation method in rodent models of disease. In addition, we continue to study the structure and the function of the EPG protein in order to improve, optimize and make it to an even better scientific tool.

In the past decade there has been a substantial growth in the development of genetically engineered tools for neuromodulation. Our work capitalizes on previous molecular biology tools that were developed, and is one of the first endeavors to identify a magnetic-sensitive gene and develop a technology to remotely control cellular function through the expression of these genes in a mammalian system. Results from this work could revolutionizing neurostimulation methodologies, and ultimately can lead to better understanding of the human brain.

Research was supported with funds from the National Institute of Neurological Disorders and Stroke.