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Press Room, Oct. 17–21: (312) 791-6619

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OPTOGENETICS SHEDS LIGHT ON BRAIN CIRCUITS AND DISEASES

Using light to turn brain cells on and off, researchers are one step closer to mapping the brain

CHICAGO — Researchers are deconstructing previously mysterious brain mechanisms involved in learning, recall, and emotions through a relatively new technique combining light and genetics known as "optogenetics." The new findings were released today at Neuroscience 2009, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news on brain science and health.

Optogenetics uses light to turn neurons on and off and enables researchers to study specific brain circuits with unprecedented precision. Findings using this tool already provide a greater understanding of how the brain works in both health and disease, and hold potential to improve therapies.

Today's new findings show that:

- Stimulating cells with laser light in the brain region known for processing emotion helps relieve symptoms of depression, providing deeper understanding and suggesting potential therapies for sufferers who do not fully recover with antidepressant medication (Herbert Covington, PhD, abstract 286.18, see attached summary).
- The discovery of new light-sensitive proteins (from diverse ecological niches) underscores the unique interrelationships among science, the environment, and the diversity of life. These new tools improve understanding of the relationship between brain circuit activity and brain disease (Feng Zhang, PhD, abstract 806.1, see attached summary).
- Researchers have determined the specific neural connections in mice that lead them to behave as if they are drug-addicted, a profound step toward determining the brain pathways responsible for these actions (Garret Stuber, PhD, abstract 686.8, see attached summary).
- Using optogenetics, scientists have discovered that reactivation of complex memories may involve only a tiny fraction of brain cells, a concept that has been under debate (Michael Hausser, PhD, abstract 388.8, see attached summary).

In addition, researchers discussed relating findings demonstrating that:

- Optogenetics has helped establish causal relationships between defined brain circuits and behaviors in health or disease (Karl Deisseroth, MD, PhD, see attached speaker's summary).

"Although relatively new, optogenetics has already proven to be an extremely powerful tool," said press conference moderator Karl Deisseroth, MD, PhD, of Stanford University, a pioneer in optogenetics. "There are a hundred billion neurons in the human brain and countless subgroupings and intersecting populations of different cell types. Using techniques like optogenetics, we can map how, why, and when those types of neurons are used. Today's findings bring us one step closer toward unlocking the brain's mysteries and a better understanding of the origins of disease, behavior, and memory."

This research was supported by national funding agencies, such as the National Institutes of Health, as well as private and philanthropic organizations.

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Related Presentation:

Special Lecture: **Optogenetics: Development and Application**
Tuesday, Oct. 20, 2009, 8:30–9:40 a.m., Hall B1
# # #

Abstract 286.18 Summary

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New Potential Treatments for Depression

Stimulation of prefrontal cortex in animals has antidepressant-like effects

Stimulating cells in the brain region known for processing emotion helps relieve symptoms of depression, according to new animal research. Cell activation from laser light in this brain area caused antidepressant chemical and behavioral changes in mice. The findings were presented at Neuroscience 2009, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Although antidepressant medications are highly effective, less than half of people with depression in the United States recover fully with these compounds. This study explores two new treatments based on stimulating the brain's prefrontal cortex and chemically modifying DNA.

To do this, the authors intensified the chronic stress levels of mice to the point that the animals displayed signs of depression in their brain chemistry and social behavior. The researchers then attempted to alleviate the depression in two ways. In one, the prefrontal cortex was stimulated by laser light after light-sensitive proteins were produced in the brain cells. In another, the enzyme histone deacetylase was blocked for 10 days through use of a small pump that infused this brain area with a chemical compound.

"We found that both methods reversed behavioral symptoms of depression, which we measured: by levels of social avoidance, and by increased brain activity that had been reduced. The effects were similar to those observed after chronic treatment with classical antidepressants," said Herbert Covington, PhD, of Mount Sinai School of Medicine, lead author on the study.

The authors predict that depressed brain chemistry in the prefrontal cortex can be corrected by either stimulation of specific cells or by the chemical inhibitors used in this study. Once this correction occurs, symptoms of depression should soon improve.

Research was supported by National Alliance for Research on Schizophrenia and Depression, National Institute of Mental Health, and AstraZeneca.

Scientific Presentation: Sunday, Oct. 18, 2–3 p.m., South Hall A

286.18, Functional activation of the medial prefrontal cortex in depressive-like behaviors
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TECHNICAL ABSTRACT: Functional activity of the medial prefrontal cortex (mPFC) is implicated in depression. The objective of the current series of experiments is to examine the relationship between chronic stress-induced molecular modifications of the mPFC and depressive-like behaviors in mice. The functional activity of infralimbic and prelimbic cells were inferred from zif268 expression, CREB activity, and c-fos expression at early (24 hr) and late (10 days) time points after chronic social defeat stress. Deficits in mPFC functional activity persist long after social stress, and these deficits are corrected via stimulation of the mPFC using virally-mediated expression of channel rhodopsin 2 (ChR2) which when activated with a blue laser (470nm) will result in neuronal firing. Experimental stimulations with the laser, which mimic patterns of mPFC "burst" firing, not only restored functional activity, but also corrected behaviors impaired by social stress, indicating strong antidepressant-like effects of mPFC stimulation. Interestingly, persistent changes in the functional activity of cortical cells correspond with a progressive increase in histone H3 methylation and acetylation in these cells. Whether or not global changes in repressive or activational marks on chromatin initiate and maintain the changes in the functional activity of cortical cells is currently under investigation, although experimental increases in histone H3 acetylation in the mPFC (via histone deacetylase inhibition) has antidepressant-like effects, similar to rhodopsin channel-mediated stimulation. We speculate that enduring histone modifications lead to changes in the functional activity of cells that ultimately regulate behavior.

Abstract 806.1 Summary

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New Light-Sensitive Proteins Identified

Findings could give scientists more tools to map brain circuits

Novel light-sensitive proteins with the potential for controlling nerve cells in the brain have been identified. In addition to these new compounds, only three proteins are known that allow researchers to probe distinct brain circuits using laser light. The findings were presented at Neuroscience 2009, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

The use of light-sensitive proteins is integral to optogenetics, the new technique of mapping individual brain components. These proteins enable researchers to control specific neurons without affecting other brain elements, a difficult task considering the interwoven nature of the brain's nerve cells. Until now, only three major light-sensitive proteins have been reported.

The researchers searched genome databases to find new proteins suitable for controlling neurons. One protein in particular has the potential to inhibit neurons using blue light, which would give even greater control over cells.

"This protein can be used in combination with the known protein halorhodopsin, which responds to yellow light, so that researchers can use blue and yellow light to control two distinct populations of neurons in the same brain tissue," said Feng Zhang, PhD, at Stanford University, lead author on the study.

The authors have also developed several more light-sensitive proteins taken from plants. "These new findings will allow us to deconstruct previously inaccessible brain circuits even further, and will improve our understanding of the relationship between brain circuit activity and brain disease," Zhang said.

Research was supported by the National Institutes of Health National Research Service Award, the National Institute of Neurological Disorders and Stroke, and the Harvard Society of Fellows.

Scientific Presentation: Wednesday, Oct. 21, 1–1:15 p.m., Room N426

806.1, Ecological diversity and circuit optogenetics
F. ZHANG[1], C. RAMAKRISHNAN[2], K. DEISSEROTH[2]; [1]Dept Bioengineering, [2]Stanford Univ., Stanford, CA

TECHNICAL ABSTRACT: The integration of genetic, behavioral, and optical-engineering techniques has recently enabled progress toward high-resolution functional mapping of the heterogeneous cellular and circuit organization of the brain. These recent developments in optogenetic technologies (channelrhodopsins and halorhodopsins) have given researchers the potential ability to reverse-engineer intact mammalian or invertebrate neural circuits, by directly probing the necessity and sufficiency of cellular and circuit characteristics with high-speed pulses of light to directly activate or inhibit defined neurons. In the course of this work, we have identified a number of genes with novel neuroscience applications from genomes spanning enormous phylogenetic and ecological ranges, developed optical control devices suitable for use in behaving mammals, and developed precise cell-targeting techniques for restricting gene expression to specific sets of neurons in the mammalian brain. Here we report further advances in this effort with novel tools arising from ecological diversity and circuit mapping. First, based on informatic analysis, we have identified two new opsins from chlorophytes and cryptophytes with excitatory and inhibitory functions respectively. Both opsins have an excitation maxima of ~500nm and generate light-induced currents and voltage modulations (~ 5-15 mV) when expressed in neurons, and reveal clear strategies for further enhancement and optimization for neural modulation. Second, we have now developed tools to control circuit elements based not on cellular identity but on circuit topology. These "circuit optogenetics" tools involve a set of anterograde and retrograde transsynaptic transporting Cre recombinases to achieve cell-specific gene expression based on projection patterns. By combining the transsynaptic recombinases with Cre-dependent expression systems, we were able to selectively express microbial opsins in neurons upstream or downstream from a target brain structure. The combination of advanced genetic targeting techniques with the expanding set of opsin-based neuromodulators will enable increasingly potent deconstruction of previously inaccessible brain circuits, and will improve our understanding of the causal relationship between circuit element activity patterns and neuropsychiatric disease.

Abstract 686.8 Summary

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Brain Circuits for Addiction-like Behavior Mapped

Mice repeated certain behaviors to earn laser stimulation rewards

When specific neural connections in mice are stimulated, the mice behave like they are addicted to a drug, according to a new study. This is the first time researchers have determined in detail the brain connections responsible for these actions. The research was presented at Neuroscience 2009, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Researchers have previously shown that animals will repeat behaviors to earn drugs such as cocaine. But the billions of neurons in the human brain make it difficult for scientists to match actions with particular neural connections. To identify more precise brain pathways involved in addictive behaviors, the authors in this study used light to turn connections between specific neurons on and off.

Circuits in the nucleus accumbens — a brain region responsible for reward-seeking behaviors — were the focus of the study. Other neurons that send projections to this area were infected with a virus that led to the production of a light-sensitive protein, which can be activated by blue laser light. This way, only certain neurons would be stimulated and specific synaptic connections studied.

Results showed that one particular subset of synapses, when laser-activated, caused the mice to perform a behavior known as a "nose poke" (pressing a lever with its nose). When a mouse completed the action it was rewarded with an optical stimulation, prompting the animal to continue the behavior for an hour, which is similar to how drug-addicted mice act. But after the laser was turned off, the mice stopped. In addition, mice lacking the light-sensitive protein did not learn the motions required for stimulation.

"This demonstrates that the reward-seeking behavior depended on stimulation of these synapses," said Garret Stuber, PhD, at University of California, San Francisco, and lead author of the study. "Future studies in our lab will continue this work to better understand the brain circuits that underlie addiction and psychiatric disease."

Research was supported by the National Institute on Drug Abuse and by the State of California for medical research on alcohol and substance abuse through the University of California, San Francisco.

Scientific Presentation: Tuesday, Oct. 20, 4–5 p.m., South Hall A

686.8, Optogenetic control of brain reward circuitry
G. D. STUBER[1], W. A. VAN LEEUWEN[1], D. R. SPARTA[1], F. ZHANG[2], K. DEISSEROTH[2],A. BONCI[1]; [1]Dept Neurobiol, Ernest Gallo Clin. and Res. Ctr., Emeryville, CA; [2]Bioengineering, Stanford Univ., Palo Alto, CA

TECHNICAL ABSTRACT: Environmental cues associated with rewards are important stimuli capable of driving motivated behavior. While many brain nuclei orchestrate behavioral responding to cues, the specific synaptic connections within the brain that underlie this adaptive response remain poorly understood. Here, we use optogenetic stimulation of channelrhodopsin-2 (ChR2) to specifically control neural transmission from the basal lateral amygdala (BLA) to the nucleus accumbens (NAc), two brain regions critical for behavioral response to cues. Mice were stereotaxically injected with an adeno-associated virus coding for ChR2-EYFP under control of the CaMKIIα promoter to predominantly express ChR2 in glutamatergic neurons of the BLA. Optogenetic activation of BLA neurons in vitro resulted in spiking in pyramidal neurons with high fidelity at frequencies >20 Hz. Importantly, optically stimulated EPSCs from BLA-to-NAc synapses were readily detected in NAc-containing brain slices and were blocked by bath application of the AMPAR antagonist, CNQX. Furthermore, in anesthetized mice optical stimulation of BLA-to-NAc fibers resulted in firing in postsynaptic NAc neurons. In vivo optical stimulation in freely moving mice resulted in profound alterations in reward-related behaviors in mice expressing ChR2 in BLA-to-NAc synapses vs. control mice only expressing EYFP. These data demonstrate that glutamatergic transmission from the BLA to the NAc plays an important role in reward-related behaviors.

Abstract 388.8 Summary

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Small Groups of Neurons Evoke Complex Memories

Animal study shows stimulation of specific cell groups recalls past learning

Complex memories can be triggered by activating a tiny fraction of brain cells, according to new animal research. After specific groups of neurons — those associated with memory — were stimulated in mice, the animals froze in fear as they recalled frightful memories. The findings were presented at Neuroscience 2009, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Exactly how the brain retrieves memories from its circuits is a long-explored topic of research. While there has been great progress in understanding memory retrieval, researchers still know little about how events from our past can be recalled under the right conditions.

In this case, scientists used two genetic tools to find how brain circuits are involved in memory recall. One was a gene that highlighted the particular cells associated with recent brain activity, such as learning something new. The second was a gene that produced a light-sensitive protein taken from algae that responds to blue laser light. The researchers used the first gene to mark recently active neurons, while the second gene (expressing the light-sensitive protein in the same neurons) made it possible for them to be activated by blue light.

"These tools allowed us to perform a powerful experiment: We could reactivate specific groups of cells and test whether this 'spark' was enough to recall the memory the animal had learned," said senior author Michael Hausser, PhD, at the Wolfson Institute for Biomedical Research in London.

The authors discovered that stimulating neurons in the hippocampus made mice freeze with fear because they remembered prior fearful experiences. When cells unassociated with learning and memory were stimulated, the mice did not appear fearful. Results also showed that activation of only a tiny fraction of neurons in the learning brain area were needed to recall memories.

Research was supported by the Gatsby Charitable Foundation and the Wellcome Trust.

Scientific Presentation: Monday, Oct. 19, 10–11 a.m., South Hall A

388.8, Memory recall driven by optical stimulation of functionally identified sub-populations of neurons
M. RIZZI, K. POWELL, J. HEFENDEHL, **M. A. HAUSSER**; Wolfson Inst. for Biomed. Res., Univ. Coll London, London, United Kingdom

TECHNICAL ABSTRACT: The mammalian brain is capable of storing information in sparse populations of neurons encompassing several brain areas. Immediate recall of this information is possible upon presentation of a cue or context. Most aspects of this process remain unresolved: are the cells involved in information storage also responsible for its recall? What portion of this distributed circuit needs to be reactivated, in order to achieve successful recall? To answer these questions we selectively expressed a genetically encoded optogenetic probe (Boyden et al., 2005) in neurons engaged during the learning of a specific association. A plasmid encoding channelrhodopsin-2 and EGFP under an immediate early gene promoter (c-fos-ChR2-IRES-EGFP) was electroporated in vivo into granule cells (GCs) of the dorsal dentate gyrus of anaesthetized C57BL/6 mice. Mice were allowed to recover, and then underwent classical delay fear conditioning (consisting of 10-20 pairings of a 5 second auditory tone and a 2 second footshock). An optic fiber was implanted intra-cranially to allow optical stimulation of transfected neurons. Light stimulation (λ = 530 nm; 5 Hz) successfully induced recall of the fear memory, measured as freezing behaviour (n = 27 animals). Post-hoc analysis of the transfected tissue revealed that a remarkably small subpopulation of GCs (<~100 cells) was sufficient to cause this effect. We then tested whether any, comparatively sized, subset of GCs could be equally effective. We transfected neurons with a plasmid encoding ChR2 expression under a general promoter (pCAG-ChR2) to obtain ChR2 expression in a random population of cells. Interestingly, optical stimulation of this population was insufficient to induce memory recall (population data: n=30). Our results therefore suggest that recall of a learned association, sparsely stored in neuronal circuits distributed over several brain areas, can be achieved by the simple reactivation of a very small subset of neurons involved in learning this association. Furthermore, our strategy may also be useful for dissecting the complexities associated with memory storage and recall.

Speaker's Summary

Speaker: Karl Deisseroth, MD, PhD (650) 736-4325
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Special Lecture: **Optogenetics: Development and Application**
Tuesday, Oct. 20, 8:30–9:40 a.m., Hall B1

Optogenetics implements temporally precise control of defined electrical and biochemical events within specific cell types in living circuits, including those within freely behaving mammals. This lecture describes the development of optogenetic tools, along with applications to cells and circuits involved in synchrony, locomotion, awakening, and reward. Applications to disease questions will also be covered, where optogenetics has allowed establishment of causal relationships between precise activity patterns in defined cells and behavioral pathophysiology.