Stanford Researchers Develop Tool for Reading the Minds of Mice

BY BJORN CAREY

If you want to read a mouse's mind, it takes some fluorescent protein and a tiny microscope implanted in the rodent's head.

Stanford scientists have demonstrated a technique for observing hundreds of neurons firing in the brain of a live mouse, in real time, and have linked that activity to long-term information storage. The unprecedented work could provide a useful tool for studying new therapies for neurodegenerative diseases such as Alzheimer's.

The researchers first used a gene therapy approach to cause the mouse's neurons to express a green fluorescent protein that was engineered to be sensitive to the presence of calcium ions.

When a neuron fires, the cell naturally floods with calcium ions. Calcium stimulates the protein, causing the entire cell to fluoresce bright green.

A tiny microscope implanted just above the mouse's hippocampus – a part of the brain that is critical for spatial and episodic memory – captures the light of roughly 700 neurons. The microscope is connected to a camera chip, which sends a digital version of the image to a computer screen.

The computer then displays near real-time video of the mouse's brain activity as a mouse runs around a small enclosure, which the researchers call an arena.

The neuronal firings look like tiny green fireworks, randomly bursting against a black background, but the scientists have deciphered clear patterns in the chaos.

"We can literally figure out where the mouse is in the arena by looking at these lights," said Mark Schnitzer, an associate professor of biology and of applied physics and the senior author on the paper, recently published in the journal Nature Neuroscience.

"The hippocampus is very sensitive to where (continued next page)
An exciting new core facility, in collaboration with the Stanford Behavioral and Functional Neuroscience Laboratory (SBFNL), is now available to Stanford scientists.

The Spinal Cord Injury Core Facility specifically addresses the needs of those scientists who currently use, or are interested in using, animal models of spinal cord injury in their research.

The core facility is being directed by Dr. Giles Plant, Basic Science Director of the Stanford Partnership for Spinal Cord Injury and Repair (SPSCIR) and Associate Professor of Neurosurgery, Stanford University. In additional to co-directing the partnership, Dr. Plant is also the head of his own laboratory and is a member of SINTN.

He has many years of expertise in dedicated spinal cord injury animal research and modeling, both mouse and rat, with a focus on cell-based transplantation therapies.

“We’ve been working hard to create this facility in collaboration with Dr. Mehrdad Shamloo of the SBFNL and we’re excited to see it up and running,” Dr. Plant said. “This type of modeling is very specific and needs careful planning to ensure the animals receive the best possible treatment and also to ensure the researcher’s scientific goals are being addressed through the use of these models.”

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New Capabilities—the Spinal Cord Injury Core Facility at SINTN

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The SPSCIR Facility offers two key instruments not commonly available in a shared animal resource facility: the Precision Systems and Instrumentation Infinite Horizon Impactor and the NYU Impactor. These are devices that mimic spinal cord contusion injuries in mice and rats.

The facility also offers additional injury models such as complete transections and hemisections (unilateral or bilateral). Dedicated surgery suites, post-surgery recovery rooms and behavioral rooms are available both internal and external to existing barrier facilities.

Dr. Plant and his team are available to provide advice at every stage of experimental planning, including:

- selection of appropriate injury models
- animal care and husbandry
- behavioral analysis
- histological processing advice regarding tissue removal, sectioning and staining
- appropriate tests to measure pain
- animal ethics documentation and regulations relating to spinal cord injuries

“My goal is to standardize, as much as possible, the many injuries and protocols available for spinal cord injury modeling in rodents,” Dr. Plant explained. “Different models will suit certain drug targets or cellular therapies, and I’m aiming to help researchers find the optimal combination for their experiments.”

New Capabilities—the Rodent Animal Neurophysiology (RAN) Core Facility

BY DR. BRENDA PORTER

As part of a new collaboration between the Stanford Behavioral and Functional Neuroscience Laboratory (SBFNL) and Dr. Brenda Porter (Associate Professor of Neurology), Stanford scientists now have access to the new Rodent Animal Neurophysiology (RAN) Core Facility. One of the key services of the RAN Core is video-electroencephalography (VEEG) monitoring. VEEG is a specialized form of EEG that allows for monitoring of the EEG and behavior for up to several months in a mouse or rat.

The advantage of this over traditional EEG testing is the ability to directly observe brainwave activity when a seizure is occurring.

The RAN will be using VEEG as the optimal tool to assess rodents for seizure activity; it has broad applications to many areas of research as there are a variety of disease that include epilepsy as one of its symptoms.

Assessment of the background EEG allows for identification of EEG abnormalities such as sharps and spikes that are markers for epilepsy.

VEEG allows for quantification of number, duration and localization of seizures using both electrographic and behavioral characteristics.

In addition to using VEEG for seizure analysis it can also be used to assess sleep stages, duration of different sleep states and the overall power spectrum of the EEG can be used to assess global network function.
Relief, Tolerance or Dependence? New SINTN Recruit Explores the Complexities of Chronic Pain and Opioid Analgesics

BY CHRISTINE PLANT

We all have experienced pain in one form or another. Many different factors contribute to an individual’s perception of pain, and the circumstances in which pain is experienced.

For some it may be the acute pain of a migraine, for others it may be the chronic pain associated with lower back problems. Opioids are narcotic pain relievers used for both acute and chronic pain; that is, they are a type of drug that work to block the transmission of the pain signal to the brain.

Even though opioids have been used for centuries for effective pain relief, they have many side effects that can be detrimental. Their continued use can ultimately lead to a decrease in the quality of pain relief that is obtained, and tolerance, dependence or addiction can result.

So how do opioids really work to control our experience of pain? And when they don’t work…why?

SINTN’s latest recruit, Dr. Gregory Scherrer is intrigued by these questions, and welcomes the challenges associated with this complex field. His career started at the University Louis Pasteur in Strasbourg, France where he studied Pharmacology and Cellular and Molecular Biology.

However, it was his postdoctoral research into the neurobiology of pain that sparked a great interest in researching chronic pain and its control by opioids. He wanted to test his theory: first, that distinct opioid receptor subtypes are present on different neurons of pain pathways and secondly, that they have specific functions in the control of distinct types of acute or chronic pain.

He moved to the University of California, San Francisco (UCSF) in 2006 to study the neurobiology of pain, and then undertook further postdoctoral training in New York at Columbia University to study physiology of the spinal cord dorsal horn. Dr. Scherrer welcomed the opportunity to move back to California when he became an Assistant Professor of Anesthesia and (by courtesy) of Molecular and Cellular Physiology and a faculty member of SINTN.

“I love it here,” Gregory said. “I enjoyed my previous time in California, and it’s such an amazing opportunity to work here at Stanford University. The research facilities and educational opportunities, as well as the intellectual environment, made it an easy decision for me.”

He credits much of his success to his trusted mentors: Professor Brigitte Kieffer, Professor Allan Basbaum and Professor Amy MacDermott.

“I feel very fortunate to have been trained by three outstanding mentors, each of them world-renowned experts in their respective fields,” Gregory explained.

From Professor Kieffer, he learned many molecular biology techniques and about mouse genetic animal modeling. She was the first person to clone the genes encoding opioid receptors in the late 1990s; it was from this significant discovery that allowed pain research scientists to study the structure and function of these receptors, and their interactions with opioid ligands.

Professor Allan Basbaum, a highly regarded pain research scientist at UCSF, taught Gregory neuroanatomical and behavioral techniques that allow a greater understanding of how opioids regulate the transmission of pain signals from the body to the brain. Professor Basbaum’s research, together with Professor David Julius (UCSF), has significantly broadened current knowledge of pain circuit anatomy and of the molecules involved in the perception of pain.

Gregory completed his postdoctoral training with Professor Amy (continued next page)
The image above is the result of a co-labeling experiment with two fluorescent antibodies to identify the pain sensory neurons on which the morphine receptor is present (mu opioid receptor, MOR) (green, middle panel). The vast majority of the cells that contain the heat sensor TRPV1 (red, left panel) appear in yellow in the merged image (right panel), indicating that these heat sensitive pain neurons also contain MOR, and explaining why morphine can be used to reduce pain induced by high temperatures. Scale bar = 50 microns. (Image courtesy of Dr. Gregory Scherrer)

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MacDermott at Columbia University. She is a very talented electrophysiologist and an expert in the physiology of the spinal cord dorsal horn, a region responsible for the coding and integration of the pain signals that get transmitted to the brain.

“I enjoyed my postdoctoral research with Allan very much and it was an extremely difficult decision to leave his laboratory in 2009,” Gregory said. “But I’m so glad that I had the chance to work with and learn electrophysiology from Amy. It’s a critical skill to have in our arsenal to dissect further pain circuits and opioids mechanisms of action”.

Gregory has published a number of research articles, including first-author articles and reviews in the highly regarded Cell and Proceedings of the National Academy of Sciences (PNAS) journals.

In particular the article published in Cell in 2009 described the distribution of opioid receptor subtypes in the nerve fibers responsible for detecting pain stimuli. This finding contrasted against popular opinion at the time.

“Understanding the organization of the opioid system has been challenging because opioid receptors are very difficult to localize in tissues with traditional histological techniques,” Gregory explained.

Instead, he employed a different approach and generated a mouse strain that expresses a fluorescent green version of the delta opioid receptor, making it possible to visualize it in pain neural circuits.

He found as a result that certain subtypes of opioid receptors are present on specific types of pain fibers; that is, delta opioid receptors are present on pain fibers that generate pain as a result of mechanical stimuli such as pressure. Conversely, pain fibers that have mu opioid receptors are the ones responsible for generating pain sensation caused by high temperatures and injuries to deep tissues or internal organs.

From these differentiations, Dr. Scherrer proposed a new organization model that allowed a new approach to opioid targeting of nerve fibers and therefore a new understanding in how different types of pain might be targeted with more specific drugs.

“This is clinically relevant, because all opioids currently used in the clinic such as morphine or fentanyl target the mu opioid receptors,” Gregory said. “Our findings indicated that pain neural circuits are made up of different opioid receptor subtypes. This explains, at least in part, why some types of pain are not very sensitive to morphine. So, other subtypes of opioid receptors could be targeted to more efficiently relieve morphine-resistant types of pain.”

Dr. Scherrer believes that different mechanisms are responsible for opioid analgesia (pain relief), opioid tolerance, and opioid-induced hyperalgesia (increased sensitivity to pain through long-term opioid use and/or abuse); further, that analgesia and side effects such as constipation, respiratory depression, and dependence can be dissociated.

“I am convinced that we need to better understand, at the molecular level, how and where opioids act in neural circuits. This will help us to distinguish between opioid-induced analgesia and its side effects, whilst at the same time developing more efficient and safer analogesics,” Gregory stated.

With twelve years of diverse experience, Dr. Scherrer and his laboratory team are well equipped to make impressive strides in this specific area of pain research.

“I’m using molecular biology, neuroanatomical, electrophysiological and behavioral techniques to investigate the cellular and molecular mechanisms of pain and its control by opioids,” Greg said. “So many of the questions I’m interested in require the combined use of these techniques in order to be rigorously addressed, and it’s wonderful to now be able to do that in my own laboratory at Stanford.”

Dr. Scherrer also has big plans for the future, starting with the aim of mapping pain neural circuit organization and the opioid peptides and receptors in these circuits. From this he will be able to assess whether certain painful medical conditions may in fact be sensitive to opioids, as well as uncover new strategies to treat morphine-resistant types of chronic pain.
Regeneration, Repair and Restoration of Function after Spinal Cord Injury—a Review of the 2012 Symposium

BY CHRISTINE PLANT

An inaugural symposium on spinal cord injury was held on November 16-17, 2012 at Stanford University, as part of the University’s commitment to the Stanford Partnership for Spinal Cord Injury and Repair.

Generously sponsored by SINTN and with support from the Stanford Center on Longevity and the Rick Hansen Institute in Canada, this meeting brought together basic scientists and clinicians who are passionate about resolving the multifaceted issues arising from spinal cord injury. The meeting was grouped into four distinct yet related sections to provide a comprehensive viewpoint on the issues and challenges facing this research:

Laying the foundation: translating the basic science
Advances in basic science research and translational goals comprised the first section of the symposium. Presentations ranged from the immune response in animal models following traumatic SCI by Dr. Phil Popovich (Ohio State University), to understanding the endogenous plasticity of the CNS in nonhuman primates by Dr. Corinna Darian-Smith (Stanford University).

Preclinical cell transplantation studies were also a focus; Dr. Giles Plant (Stanford University) and Dr. Aileen Anderson (UC Irvine) presented their research in examining the use of human mesenchymal progenitor cells and neural stem cells as therapies in subacute and chronic animal models of spinal cord injury.

Mileposts along the way: lessons learned from recent SCI clinical trials
This section of the symposium focused on the lessons learned from clinical spinal cord injury trials and highlighted the need for standardization of outcomes measures, models and efficacy in such trials.

Dr. Brian Kwon (University of British Columbia, ICORD) presented survey results from basic and clinical SCI researchers; they showed that certain research areas are of critical importance, such as the choice of appropriate animal and injury models, and the need for appropriate experimental designs based on clinically meaningful efficacy.

A particular highlight was the Q&A session between past Geron trial participant Katie Sharify and her physician Dr. Stephen McKenna. Ms. Sharify generously spoke of her experiences with the Geron clinical trial and the obstacles she faced, in the hope that her experience would serve as counsel for other spinal cord injury patients who are also considering becoming involved in clinical trials.

Fuel for the journey: communication and collaboration
Successful “bench to bedside” translation of spinal cord injury treatments requires close collaboration between basic research scientists and specialist clinicians. This section of the symposium examined the building blocks of past models, research consortia, international networks and funding initiatives, in order to design successful future preclinical and clinical spinal cord injury trials.

Obstacles along the way: the future of clinical trials in SCI
An interactive session, led by Dr. John Steeves (ICORD), discussed the future of spinal cord injury clinical trials. Participants also heard from representatives of clinical research networks that all agreed on the need for centralized coordination, as well as the harmonization of protocols and institutional research ethics boards.

Further information about the symposium can be found at http://www.sci2012.org/, or you may wish to contact the Stanford Partnership for Spinal Cord Injury and Repair:
Clinical Director - Dr. Graham Creasey (gcreasey@stanford.edu)
Basic Science Director - Dr. Giles Plant (gplant@stanford.edu)
Updates on SINTN’s Core Service Centers

Each quarter, we’ll be bringing you an update on each of SINTN’s three core service facilities. The GVVC, SBFNL and NMS are part of a wider group, the Stanford School of Medicine Scientific Service Centers (http://corefacilities.stanford.edu/). These specialized centers are available to all Stanford University faculty and laboratory members; simply contact the directors or managers to discuss your experimental requirements.

THE NEUROSCIENCE GENE VECTOR AND VIRUS CORE (GVVC) - STOCK AAV SEROTYPES

BY DR. MICHAEL LOCHRIE

The last update introduced the many advantages of stock viruses (premade and precharacterized viruses) that can be obtained through the GVVC. Please see http://neuroscience.stanford.edu/ngvvc/stockviruses.html for a complete list of stock viruses that are currently available.

AAV serotypes
The GVVC has produced 14 different AAV serotypes to date, with plans for additional serotypes in the future. Each serotype consists of a different AAV capsid (the virus protein shell) that packages the AAV genome. Since the AAV capsid binds to specific receptors that mediate the initial steps of infection, it is the capsid that determines AAV “tropism”; that is, determines the specific cell type and species that the AAV is able to infect. AAV capsid sequences on the outside of the virus are highly variable and so the infectious properties of different AAVs can also be highly variable. As such, certain AAVs are more suitable for infecting some cells than others.

Specific technical examples of AAV stock virus applications
Two of the more popular AAV stock viruses either express cre, or are cre-dependent. Examples of applications of these types of stock viruses, AAV-DJ eGFP-cre and AAV DIO, are given below:

AAV-DJ eGFP-cre
AAV-DJ eGFP–cre can be used in any circumstance where it is desirable to express the cre recombinase via viral infection. In the stock virus cre is fused to eGFP, which then enables the researcher to determine which cells express cre. AAV-DJ eGFP–cre can then be used to activate or inactivate expression of floxed genes in cultured cells or in transgenic mice in specific areas of the brain where the virus in injected. It can also be used to test gene expression from viruses or plasmids with cre-dependent genes (see figure). An AAV-DJ eGFP-Delta cre virus, which expresses a deleted, inactive cre recombinase, is also available as a control.

AAV DIO
A common problem with using viruses to deliver genes to specific cells, is that very few viruses have cell type-specific infection capabilities. Most recombinant viruses used in research infect a wide variety of cell types and species. This problem is typically solved by using cell type-specific promoters to express genes even when the virus itself has little or no cell type specificity. Another way to achieve cell type-specific expression is to inject viruses that express cre-dependent genes into transgenic mice engineered to express cre in specific cell types.

Several vector configurations have been used to render genes cre-dependent, but one configuration that has become popular is often referred to as DIO (double-floxed, inverted orientation) or FLEX (flipped, excised). Typically in these configurations, the coding region for the gene of interest is inverted relative to the promoter, which usually results in very low background expression. In the presence of cre the orientation of the coding region is inverted to the correct orientation resulting in gene expression. An example of a cre-dependent AAV - AAV-8 EF1a DIO mCherry - can be seen in the figure.

For further information about the GVVC, visit http://neuroscience.stanford.edu/ngvvc/index.html or please e-mail Dr. Michael Lochrie (mlochrie@stanford.edu).
Updates on SINTN’s Core Service Centers

THE NEUROSCIENCE MICROSCOPY SERVICE (NMS) CORE - LONG-TERM TIME LAPSE IMAGING DEMONSTRATION AT THE NMS

BY DR. ANDREW OLSON

Do you need long-term time-lapse imaging capabilities for your research experiments? The Neuroscience Microscopy Service (NMS) is arranging for an on-campus demonstration of the IncuCyte Zoom live cell imaging system from Essen BioScience. It's an automated microscope for imaging cells inside a standard tissue culture incubator. The system can accommodate up to six multi-well plates, and also handles tissue culture flasks and dishes of various sizes.

The microscope performs high-quality phase imaging, and also two-color fluorescence (green and red). The system is network-connected, so you can monitor the imaging results from your laboratory computer!

It uses a single objective, which can be 4x, 10x or 20x. Essen BioScience has developed software modules for monitoring cytotoxicity, cell proliferation, cell migration, and neurite outgrowth. Images can be saved to standard formats (such as JPEG, PNG, TIFF, and RAW) for analysis with your own software.

If you are interested in getting some time-lapse imaging data from your cells, please contact Dr. Andrew Olson at nismicro@stanford.edu or aolson6@stanford.edu for information on the full range of microscopy and image analysis services available to Stanford University researchers.

THE STANFORD BEHAVIORAL AND FUNCTIONAL NEUROSCIENCE LABORATORY (SBFNL) CORE - LPS INDUCED COGNITIVE IMPAIRMENTS AS A NEW TEST MODEL FOR COGNITIVE ENHANCERS

BY DR. MEHRDAD SHAMLOO AND LILLIAN TO

At the SBFNL, we have established an LPS toxicity protocol followed by a Fear Conditioning assessment to explore deficits in retrieval of cued and contextual memory in mice.

Lipopolysaccharide (LPS) is a large molecule that prevents Gram-negative bacteria from losing structural integrity during chemical attacks. Intraperitoneal injection of LPS (at 250 µg/kg) in rodents induces a neuroinflammatory response and memory impairment determined by various behavioral measures.

It has been suggested that repeated injection with LPS results in an accumulation of Aβ1-42 in the hippocampus and cerebral cortex of mice brains through increased β- and γ-secretase activities and Alzheimer’s Disease (AD)-like acute pathology. These pathological changes are accompanied by behavioral deficits characteristic of an AD brain.

The image above shows two embryonic stem cell-derived cardiac myocytes, fixed and stained for troponin-T (red) and DNA (blue). The scale bar corresponds to 5µm. (Image courtesy of visiting graduate student Marc Engels and postdoctoral fellow Dr. Rajarajan Kappusamy, both in the laboratory of Assistant Professor Sean Wu)

More laboratories are using NMS’s structured illumination super-resolution microscope, but remember - it’s still easier to find time on the SIM microscope than on the confocal microscope! If you have thin samples, why not give it a try? Contact the NMS to arrange a time and/or training on the SIM.

The image above shows that intraperitoneal injection of LPS at 2.5mg/kg induces significant deficits in both context and cue dependent memory retrieval in mice (values represent mean ± SEM. *P<0.05). (Image courtesy of the SBFNL)
We have shown that increasing LPS dosages elicits deficits in both contextual and cued memory retrieval in wild type C57Bl/6J mice (as shown in the figure). Thus, LPS toxicity in mice could be used as yet another model for testing the efficacy of experimental therapeutics and genetic models for AD and many other CNS disorders.

If you are interested in finding out more about this new cognitive test model, or for more information about the SBFNL, please contact the Laboratory Manager via email at neurobehavior@stanford.edu or telephone (650)-725-3152, or visit http://sbfnl.stanford.edu/.

BY CHRISTINE PLANT

In a recent study led by Dr. Lu Chen of SINTN, researchers found that when neural circuits in the brain become inactivated, the brain is able to change and compensate for the loss by creating silent synapses and switching them on quickly during synaptic plasticity.

The brain’s ability to change is known as neural plasticity and allows the neurons to compensate when changes in its environment occur, for example through injury or disease.

The results showed that when a neural circuit becomes inactivated, long-term potentiation (LTP) is enhanced. LTP refers to the increase in signal transmission between two neurons, which leads to increased synaptic strength and the formation of learning and memory.

Chen and colleagues revealed that two mechanisms are at work. Firstly, that the network reacts by increasing existing synapse strength. Secondly, that new silent synapses lacking a specific receptor (AMPA) are created when neural activity is blocked. Inducing LTP then causes these silent synapses to become active by inducing AMPA receptor insertion.

Having the ability to rapidly switch on synapses by converting them from “silent” to “active” means that stronger LTP can occur to neuronal inputs after prolonged loss of activity, potentially allowing recovery of circuit function.

So in instances where a neural circuit becomes compromised and decreased synaptic activity results, the network is able to not only increase the strength of its existing synapses but also promote the emergence of new functional synapses to compensate.

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**Want to know more? Find us at:**
http://neuroscience.stanford.edu/

This research was published in the Journal of Neuroscience:
To view the full article, please go to:

The image above shows that a neural network compensates for the lack of synaptic activity in two parallel ways: 1) increasing existing synaptic strength and 2) promoting new synapse formation and/or emergence. (Image courtesy of the Chen laboratory)