Keck Center scientist John Meitzen announced today his startling discovery of a new small group of neurons in the medial region of the brain’s ventral striatum. The neurons, which colleagues have already dubbed “Meitzen neurons” have extensive connections throughout the brain. Meitzen identified these neurons by using a particular fluorescent driver that specifically lights up those cells. Linking the reporter construct to a magnetic enhancer enabled Meitzen to visualize and monitor activity of this set of neurons in real time and at high resolution in the human brain.

In his paper in the journal “Open Neuroscience” published by the Swedish Royal Academy, Meitzen reports a preliminary study in which activity of the Meitzen neurons was monitored in two controlled volunteer populations from Raleigh, North Carolina. One population consisted of 10 veterinarians from NC State’s College of Veterinary Medicine and the second population consisted of 10 volunteers from the North Carolina State legislature. Compounds were delivered orally, as they readily cross the blood brain barrier and brain activity was monitored using high resolution nuclear magnetic resonance, while subjects responded to a range of questions, including gun control, support for higher education, and genomic networks. The results were surprising. Strong activity of the Meitzen neurons was observed in all but one of the veterinarians, whereas virtually no activity was evident in the brains of any of the legislative volunteers. This led Meitzen to conclude that the Meitzen neurons are essential for mediating intelligent decision making.

“This is an exciting new function of the ventral striatum,” says Meitzen. However, he cautions that these experiments, although encouraging are still preliminary and will require a larger number of samples. “This is a Nobel prize worthy discovery”, says Center Director, Robert Anholt. “Let us congratulate John on this marvelous achievement.”
The Good Cone Snail Hunting

by Xiaohu (Tiger) Xie

On March 12\textsuperscript{th}, 2015, the W. M. Keck Center for Behavioral Biology, hosted Dr. Baldomero M. Olivera from the University of Utah, where he is appointed as Distinguished Professor of Biology. His distinguished seminar was titled "Fish hunting cone snails: What they teach us about drug design and the cellular complexity of nervous system”

Olivera began by introducing one of the cone snails, \textit{Conus magus}, which is a fish hunter, and can be found in the Western Pacific. It is so common in some of small Pacific islands, especially in the Philippines, that it is routinely sold in the market as food. This magician’s cone attacks its fish prey by sticking out its light yellowish proboscis, from which venom is pushed through a harpoon-like tooth. It hunts by the hook-and-line method and will engulf its prey after it has been paralyzed.

Cone snails are highly toxic and can be fatal to people. Olivera went on to elaborate how he and his colleagues started getting interested in analyzing the venom of the magician’s cone. It turned out that there are many different components in it, including toxins that act similar to snake toxins that block acetylcholine receptors as well as toxins that act like tetrodotoxin on voltage gated sodium channels. Thus, when you get stung by a cone snail, it is as if “you are bitten by a snake while consuming a puffer fish.” The venom from a single \textit{Conus} species contains more than 80 active peptides, which fall into over 15 classes.

Olivera’s laboratory has characterized a set of unique toxins, the omega-conotoxins, which irreversibly bind (and block) calcium channels. Surprisingly, one of these omega-conotoxins was discovered to have a unique pharmacological activity by blocking a specific N-type calcium channel, which is critical for conveying pain signals from nerve cells. Thus, blocking such type of calcium channels will allow the pain signals not to be perceived by the brain. Later, this $\omega$-conotoxin was developed as a pain management drug, and is now chemically synthesized and sold under the trade name \textit{Prialt}. This drug is given to patients who have very severe chronic pain that is not alleviated by morphine.

Building on the findings from these cone snails, Olivera further explained why it will be promising to use these toxins discovered from various cone snails as a great tool to study key molecules in the brain. First, ion channels and receptors, especially nicotinic acetylcholine receptors have so many different isoforms, which are hard to study \textit{in vivo}. However, Olivera and his colleagues have isolated numerous neurotoxins from the venoms of the predatory cone snails, which can target specific isoforms of nicotinic acetylcholine receptors. These toxins will be great tools for pharmacologists and neuroscientists to study specific nicotinic acetylcholine receptor subtypes.

Furthermore, some cone snails are extremely variable with respect to their shell patterns. Different specimens of \textit{Conus magus} collected from different islands will look different from each other. Although it is not well understood why some species of \textit{Conus} are very constant in their shell pattern, the shells of other species, such as \textit{Conus magus}, are extremely variable. Olivera believes that such variability and diversity in cone snail species may render them a large treasure box for scientists in biomedical research.
How the Nose Knows

by Leslie Wilson

On March 23rd, the W. M. Keck Center was honored to have Dr. Stavros Lomvardas of Columbia University, winner of The Vilcek Prize, give a seminar titled “Understanding Olfactory Receptor Gene Choice”.

Lomvardas began by highlighting that he uses genetic tools to study regulation of expression of olfactory receptors within mouse olfactory neurons. He explained that development is orchestrated by genetically hardwired signaling gradients, which in different tissues activate sets of transcription factors that give rise to the various types of cell identities. It is this deterministic mechanism which is mostly responsible for an organism’s development from a single fertilized egg to a multicellular organism. However, the olfactory system requires an increased level of cellular diversity to enable olfactory discrimination, and therefore the deterministic model cannot produce the desired level of specificity. The type of olfactory receptor (OR) that is expressed in the neuron determines its specificity to odorants.

Lomvardas explained that the beauty of the OR singularity in the olfactory system comes from two distinct factors. First, the vast amount of OR genes found along the mouse chromosomes are expressed explicitly within the olfactory neurons because the promotors have regulatory information, such as lower guanine-cytosine content, which distinguishes them from the rest of the mammalian genome. Second, only one OR is expressed in each neuron, even though there are about 3,000 olfactory receptor alleles within the genome. Lomvardas’ research aims to understand why only one OR allele of the about 3,000 alleles is transcribed while all others remain silent. The remainder of the talk was aimed at elucidating this problem.

The first step of Dr. Lomvardas’ research was to determine why 2,999 OR alleles within the mouse genome are silent, despite the fact that the transcription factors are present. He hypothesized that most alleles are silent due to repressive chromatin signatures on the OR genes. Dr. Lomvardas verified this hypothesis through utilization of DNA fluorescence in situ hybridization (FISH) using sequence capture approaches, and soft X-ray tomography.

Next he investigated why only one OR allele within the neuron is transcribed despite the fact that all the other alleles are silent. The results indicated that the transcribed allele sits in a region of the nucleus which is outside of the repressive chromatin. Additionally, transcription of OR alleles is governed by unique sequence enhancers, which Lomvardas named after Greek islands. These observations were verified through the use of hydrophobic interaction chromatography (Hi-C), DNA FISH analysis, and immunofluorescence.

Lastly, Lomvardas showed that a singularly transcribed allele is stabilized by a feedback pathway that downregulates all other transcription factors while leaving the one allele being actively transcribed alone. This is performed by a kinase in the endoplasmic reticulum named Perk. When Perk is activated it leads to a signaling pathway which leads to the translation of ATF5. ATF5 is a protein that is transcribed at the onset of OR translation, which initiates the LSD1 protein, which releases the epigenetic lock of one OR gene, but is then inactivated, thereby preventing transcription of other OR alleles. This results in the expression of only one OR allele in each olfactory neuron.

In summary, Dr. Lomvardas explained that the result of both repressing and activating interactions create the specificity of ORs which is observed within the olfactory neurons. Only one OR is expressed within one neuron due to several layers of specificity. First, the repression of about 2,999 alleles, then activation of only one OR gene through the aggregation of sequence enhancers and a feedback pathway which, once a specific allele is chosen, shuts off transcription factors for other alleles. The result is an olfactory neuron that expresses only one OR out of the 3,000 alleles present in the genome.
Brain Day at the Museum

Clockwise from left to right: John Meitzen demonstrates a human brain; The Daily Planet Theater was the site of the keynote address; John Shorter gives a talk about aggression; more than 200 visitors demonstrated the exhibition booths; The Brain in Love was a popular exhibit; voles were brought in to demonstrate the partner preference test.

This year’s Brain Awareness celebration in the North Carolina Museum of Natural Sciences was a big success thanks to the efforts of numerous students and faculty of the Keck Center, who organized exhibits and presented short lectures to the public. Christie Lee, Sheryl Arambula, John Shorter, and Jonathan Douros gave excellent presentations in the state-of-the-art Daily Planet Theater. Thanks also to John Meitzen, Lisa McGraw, Hatcher Patisaul, Leslie Sombers, Coby Schal, Ayako Katsumata, John Godwin, Troy Ghashghaei and many students who manned the booths. Apologies to those who are not mentioned here. For a more detailed account of Brain Day and the keynote lecture, see the article by Jaime Willett on page 5.

Exhibits included demonstrations of pheromone recognition, fluorescently labeled cells in the brain and chemosensation in cockroaches.
On March 12th, the Keck Center for Behavioral Biology had the honor of hosting distinguished speaker Dr. Baldomero Olivera from the Department of Biology and Interdepartmental Program in Neuroscience at the University of Utah for a seminar entitled “Fish-Hunting Cone Snails: What They Teach Us About Drug Design and the Cellular Complexity of Nervous Systems” and a Science Café talk on predator strategies for prey capture in the Daily Planet Café at the NC Science Museum featured as a part of a successful Brain and Behavior Awareness Night.

Brain and Behavior Awareness Night drew an even larger crowd this year and featured many interesting exhibits and student talks showcasing the variety of local neuroscience research currently underway while allowing the public to engage in hands-on learning and demonstrations. “Oohs” and “ahs” were heard often around the Meitzen lab exhibit where human brains were available for the public to view and hold. Many exhibits offered fun activities, such as the mirror drawing task and prism goggles bean bag toss. Other exhibits offered examples of how neuroscience research is conducted. The McGraw lab demonstrated a partner-preference test with voles while explaining their findings regarding the brain in love. It was an atmosphere of excitement and fascination as the public was offered a glimpse into neuroscience research.

During the day, Olivera presented two captivating lectures which introduced cone snail predatory mechanisms as an informative system for studying the complexity of toxin action in the nervous system and the potential biomedical applications which stem from his findings.

Olivera grew up in the Philippines, which is home to about 800 species of cone snail. About 100 of these species prey upon fish and the venom of one of these species, *Conus geographus*, is potent enough to killing people.

In his talks, Olivera describes the various chemical strategies cone snails have evolved in order to subdue the fish, worms, and other snails that they prey upon. In his study of cone snail prey capture mechanisms, he identified two peptides responsible for the lethality of cone snail venom: α-conotoxin G1, which is similar to cobra toxin and causes muscle paralysis through blockade of the connection between the motor neuron and skeletal muscle and μ-conotoxin, which is similar to tetrodotoxin in pufferfish and blocks voltage-gated sodium channels. Thus, he summarized that a dose of cone snail venom is equivalent to simultaneously receiving a cobra bite and a lethal dose of tetrodotoxin.

Olivera’s analysis of the individual components comprising cone snail venom identified nearly 100 different compounds. Injection of each of these components into the central nervous system of a mouse revealed a complex story of action, with each contributing a different effect. He went on to explain how distinguishing these individual effects has led to their application in the development of various pharmaceuticals such as the use of ω-conotoxin MVIIA in Prialt to treat intractable pain.

Ultimately, Olivera’s research identified 3 major cabals, or groups of peptides, which are employed to paralyze and capture prey. The “motor” cabal comprises 5 paralytic toxins, any one of which is sufficient to cause paralysis in fish but act via different mechanisms at the neuromuscular junction. The “lightning strike” cabal acts as a taser to immediately immobilize prey through induction of excitotoxic shock and flaccid paralysis by blocking potassium channels and keeping sodium channels open. Olivera explained that these cabals can be tailored to suit different ecological niches and each cone snail species features their own specialized set of peptides.

To demonstrate how cone snails deliver their venom cocktail to prey, Olivera showed an impressive video in which a cone snail captures a fish through use of a harpoon tooth, which acts as harpoon and hypodermic needle to both tether the fish and inject venom. Alternative mechanisms also exist. For example, certain cone snails use a “nirvana” cabal which causes sensory-deadening and sedation through action on nicotinic receptors. This facilitates capture of multiple prey simultaneously for cone snails that feed upon schools of fish. Olivera concluded that cone snail venom is a powerful and complex chemical cocktail, which can vary greatly in composition and effects.

Brain and Behavior Awareness Night was a huge success and an event enjoyed equally by both those attending and those presenting. The visiting public eagerly engaged with students about their research and left with a greater appreciation and understanding of the brain.
Slightly Better than Average Cocaine

by Joel Johnstun

The Keck Center’s social evening discussion was a complete success made possible by the presenter, Leslie Sombers, the many attendees who shared their perspicuous comments and questions, and of course the gracious hosts, Robert Anholt and Trudy Mackay. The topic of the dialogue was “Your brain on drugs... is it really all about the dopamine?”

The answer in a nutshell is of course “No” – Mother Nature would never make it that easy. However, it is beyond question that dopamine plays a crucial role in motivated behavior. For example, all drugs of abuse increase dopamine in the nucleus accumbens. Dopamine release in the nucleus accumbens underlies reinforcement learning, and phasic dopamine signaling is sufficient to establish place preference in rats.

At the same time there are many reasons to believe that the function of dopamine is not straightforward: a variety of treatments (e.g. CCK) and behavioral paradigms (e.g. tail-pinchhing of rats) increase dopamine release without causing positive reinforcement; dopamine antagonists are not aversive and do not block cocaine reinforcement; the ventral tegmental area, implicated in the reward system and which contains dopaminergic neurons which project to the nucleus accumbens, also contains GABAergic and glutaminergic neurons; and dopaminergic neurons co-contain a host of other neurotransmitters.

Science has a way of multiplying the questions before the answers, which was the precise result of a recent experiment in Leslie’s lab. Using fast scan cyclic voltammetry the levels of dopamine in the ventral tegmental area were measured following infusions of DAMGO (a mu opioid receptor agonist), CTOP (an antagonist of the same) and a combination of the two together. Though dopamine levels rose significantly in the two individual treatments, the combination of DAMGO and CTOP caused the greatest dopamine increase of all. Furthermore, while DAMGO caused place preference and CTOP caused place avoidance, the highest level of dopamine elicited by the combination treatment had no effect on place preference at all. Clearly, dopamine’s story is just beginning to be told.

Chicago Flies

by Megan E. Garlapow

From March 4 to 8, members of the Drosophila research community descended on Chicago to share their research. Representing the W. M. Keck Center for Behavioral Biology were undergraduate students Yazmeen Hussain, Sarah McAdams, and Kairsten Fay; graduate students Megan Garlapow and Chad Hunter; postdoctoral researcher Logan Everett; and professor Nadia Singh. All students gave poster presentations, Garlapow contributed a talk to the “Feeding Behavior, Metabolism, and Nutrition” Workshop, and Singh exercised her mentorship and guidance prowess by contributing to the “Plenary Session and Workshop for Undergraduate Researchers.”

The conference commenced with the Larry Sandler Memorial award lecture delivered by Zhao Zhang. The award is presented to an individual who has accomplished outstanding dissertation work utilizing Drosophila and is in memory of Larry Sandler, who contributed significantly to Drosophila genetics research and to mentoring Drosophila biologists. Zhang worked with Bill Theurkauf and Phil Zamore at the University of Massachusetts Medical School, where he studied the regulation of transposons in germ cells. He concentrated on the interaction of transposons with Piwi-interacting RNAs (piRNAs) and transposon silencing en route to elucidating the role transposons play in the accumulation of heritable mutations and in genomic instability. His research revealed consequences of transposon activation and silencing and cellular regulation of piRNAs.

Zhang’s lecture was followed by the keynote address, delivered by Allan Spradling, HHMI and Carnegie Institution for Science, titled “Drosophila: Assuming the mantle of leadership in biological research.” Throughout his talk on Drosophila oocytes and ovulation, Spradling emphasized the importance of Drosophila basic research, imploring the audience to communicate broadly and frequently about their research discoveries, suggesting funding distributions for the National Institutes of Health, and emphasizing how evolutionary conservation of Drosophila is much greater than originally anticipated.

In the second “Evolution and Quantitative Genetics” platform session, Joyce Kao (University of Southern California and New York University) presented “African and European admixture in southeast US and Caribbean Islands populations of Drosophila melanogaster affect postmating reproductive phenotypes.” She began by describing the migratory and evolutionary history of fruit flies, which originated
in Africa and migrated into Europe. Subsequent fruit fly migratory patterns brought primarily European ancestry flies into the United States and primarily African ancestry fruit flies into the Caribbean. Consequently, the most severe post-mating reproductive phenotypes were observed along the hybridization border between primarily European and primarily African fruit flies in extreme southeast Florida and in Bermuda. Her talk was followed by John Pool’s (University of Wisconsin-Madison) “Natural selection shapes the mosaic ancestry of the *Drosophila melanogaster* Genetic Reference Panel and the *D. melanogaster* reference genome,” in which he described his use of the Ancestry Inference method to show strong effects of natural selection. He used “ancestry disequilibrium,” in which unlinked loci should show disequilibrium if the unlinked loci are incompatible; for example, if locus 1 is incompatible with locus 2 when locus 1 is African and locus 2 is European, then it is expected that locus 2 is African when locus 1 is African. He observed little African ancestry on the X chromosome and little correlation between low recombination rates and high African ancestry.

During the first “Neurophysiology and Behavior” platform session, Alyson Sujkowski’s talk, “Drosophila exercise-training requires octopaminergic neuron activity” described her use of an automated exercise machine, dubbed “the power tower,” as she exercised male and female flies. She demonstrated that feminized male flies failed to respond to endurance training, while masculinized female flies did respond to endurance training. She then focused on octopaminergic neurons, revealing that solely masculinizing octopaminergic neurons in females was sufficient to confer an endurance-trained-like effect without ever exercising the flies. From this, she demonstrated that octopamine is necessary for adaptation to exercise.

Michael Gordon (University of British Columbia) opened the “Feeding Behavior, Metabolism, and Nutrition” Workshop with a talk about pharyngeal sweet gustatory receptor neurons (GRNs), which cannot be characterized with electrophysiological measurements. Shown with *in vivo* calcium imaging, the pharyngeal sweet GRNs do respond to a panel of sweet compounds, and *pox-neuro* mutants that are unable to taste sweet with their leg or labial palp GRNs continue to taste sweet pharyngeally. Subsequent talks in the workshop explored the neural circuitry of aversive taste memory (Pavel Masek, University of Nevada Reno), the serotonergic mediation of hunger (Stephanie Albin, Janelia Research Campus), and the connection of diet-induced metabolic dysfunction and cancer (Susumu Hirabayashi, Imperial College London).

The conference culminated with a plenary talk from Ulrike Heberlein (Janelia Research Campus), “Flies and alcohol: Interplay of nature and nurture.” Heberlein described how humans have complex genetic risk factors that can couple with complex environmental risk factors to result in addiction to ethanol. Furthermore, we observe universal ethanol intoxication behaviors across the animal world as well as negative effects of ethanol on fitness and survival. Her work with Drosophila has explored a variety of genetic, environmental, and gene-by-environment factors affecting ethanol intake and preference. From the inebriometer to odorant conditioning, courtship conditioning to optogenetics, Heberlein and her group have used a wide variety of assays to explore the genetic and environmental “whens, hows, whys, and wheres” of ethanol intake, preference, and intoxication. Her lecture seemed to put an exclamation point behind Spradling’s emphasis of the “greater than expected” evolutionary conservation between Drosophila and the rest of the animal world.

**Seminars**

On April 16, 3:30 pm, Dr. Elizabeth Adkins-Regan from the Department of Psychology at Cornell University will present a seminar titled “Neuroendocrinology of Socially Monogamous Pairing: A Bird's-Eye View.” The seminar will be in 101 David Clark Laboratories.

**Publications**


Of note…

Robert Anholt joined the editorial board of *Scientific Reports*.

Fu-Chyun Clay Chu gave a presentation on development of transgenic helper/donor *Diabrotica virgifera virgifera* strains for use in genome-wide mutagenesis” at Monsanto’s Corn Academic Summit in St. Louis, MO.

Kairsten Fay, Megan Garlapow, Chad Hunter, Yasmeen Hussain, Sarah McAdam, and Nadia Singh gave presentations at the 56th Annual Drosophila Research Conference in Chicago, IL. Logan Everett also attended the meeting.

Megan Garlapow received an Outstanding Graduate Teaching Award.

Trudy Mackay was appointed as a member of the Advisory Committee to the NIH Director, Working Group on the National Library of Medicine (NLM). She also gave a seminar at the Lieber Institute for Brain Development in Baltimore, MD.

John Meitzen presented the Behavioral Neuroscience Seminar at Elon University, NC.

Heather Patisaul presented a poster at the Annual Meeting of the Society of Endocrinology in San Diego, CA., titled “Soy but not Bisphenol A (BPA) or the phytoestrogen genistin alters developmental weight gain and food intake in pregnant rats and their offspring.”

Reade Roberts was quoted in a Newsweek article regarding a cichlid behavior study. The website link is http://www.newsweek.com/why-some-fish-build-sand-castles-attract-mates-314947.


Visit our website: http://keck.sciences.ncsu.edu