

The Signal

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Symposium 2018: A Celebration of Academic Diversity



Participants at the 19th Annual Student and Postdoc Symposium of the W. M. Keck Center for Behavioral Biology

The 19th Annual Student and Postdoc Symposium of the W. M. Keck Center for Behavioral Biology showcased the Center's diversity covering behavioral genetics, neuroscience and ecology, and representing model systems as well as human studies from the cellular to the population level. Dr. John Vandenberg, founding member emeritus of the Keck Center, once proclaimed "Behavior is the window through which we can view all of biology." This was certainly true for this year's symposium. The 25 platform presentations and three posters ranged across such diverse topics as genetics of lifespan,

pesticide resistance in mosquitoes, Parkinson's disease, sexually dimorphic neural mechanisms in the brain, and behavioral sequelae of prenatal exposure to cadmium. Leslie Wilson received the award for best presentation and Katie Hudson received the runner-up award. Nagendran Muthusamy received the 2018 Robert and Margaret Grossfeld award for his 2017 publication in *Nature Neuroscience*, titled "Developmentally defined forebrain circuits regulate appetitive and aversive olfactory learning." A detailed account of the symposium is presented on pages 2-3 of this issue of *The Signal*

**The Signal will not be published during the summer recess.
Volume 20 of The Signal will be published in September, 2018.**

The 19th Annual Student and Postdoc Symposium of the W. M. Keck Center for Behavioral Biology

by Megan Thoemmes and Ahmed M Saveer

The W. M. Keck Center for Behavioral Biology hosted its 19th annual student and postdoc symposium on Friday, April 27, 2018. There were 25 presenters that covered a broad range of topics, highlighting the diversity of research among Keck Center-associated laboratories. It is worth noting that the great success of the Keck Center and its researchers is a testament to the dedication and strong leadership of our Keck Center director, Dr. Robert Anholt. We would like to recognize Dr. Anholt, as this was his last year organizing the symposium. He has accepted a position as the Director of Faculty Excellence for the College of Science at Clemson University. We wish him continued success in his new position.

Session 1 covered topics from behavioral adaptation to the genetics of disease. Brent Chen was the first symposium speaker. His research focuses on the relationship between histone modification, aging, and the increased risk of disease. Chen uses *Drosophila melanogaster* to track the expression of transposable elements with the loss of heterochromatin over time. His data support the heterochromatin loss model for aging, and he will use this system to elucidate the relationship between natural variation and loss of heterochromatin with age. Second was Dr. Amanda Krentzel. She studies how sex hormones act as neuromodulators and how this varies between the sexes. Krentzel quantified rapid estradiol signaling in the striatum of males and females, and her data suggest that increased movement in females is facilitated by changes in hormone production. Megan Serr then featured the work of her undergraduate researchers. Serr addressed behavioral differences between laboratory and wild mice. She compared nest structure and thermoregulation, as well as movement patterns. Her studies suggest that wild mice have increased nest complexity, better thermoregulation, and decreased movement compared to their domesticated counterparts. These behavioral changes should be considered when housing wild-derived strains in the laboratory. Next, Dr. Emily Moore characterized the genetic basis for how behavioral differences can lead to sympatric speciation in cichlid fish. Moore showed that gene expression determines the spatial use of microhabitats, and we can now more confidently use laboratory results as a proxy for the behavior of cichlid fish in the wild. Brandon Baker uses *Drosophila melanogaster* to characterize the role of genetic

variation in the increased consumption of psychostimulants (*i.e.*, cocaine and methamphetamine). He found significant phenotypic variation and identified thousands of SNPs across the genome. These genetic variants will be used to identify human orthologues and his studies might contribute to addressing the financial, psychological, and health issues related to drug abuse and addiction. Lastly, Leslie Wilson and Karen Butler, both members of the Sombers laboratory, spoke about the use of *l*-DOPA, a drug commonly used in the treatment of Parkinson's disease that induces abnormal body movements (dyskinesias). Wilson monitored real-time fluctuations of dopamine and hydrogen peroxide in the striatum after *l*-DOPA administration. Her data present a potential mechanism for oxidative stress in the brain, leading to the development of dyskinesias. Butler then discussed the use of pharmacology to regulate the dopamine and hydrogen peroxide system. She found that serotonin reuptake inhibitors can help to regulate dopamine production, thereby reducing dyskinetic behavior in Parkinson's patients.

Session 2 focused primarily on hormones. Jaime Willett spoke about how cyclical fluctuations of hormones affect the brain. She quantified changes in neuron firing and how much excitatory input a neuron needs to activate estrogen receptors. Willett found that these electrophysiological properties were modulated by the estrous cycle in females, which may influence sex-dependent behavioral differences. The second speaker was Stephanie Proaño. She measured the electrophysiological output of neurons in the striatum throughout the estrous cycle in adult rats. Proaño found that electrophysiological and excitatory synaptic input properties change significantly over the course of the female estrous cycle, as compared to males. These data also support the concept that hormone cycles may regulate sex differences in behavior, as well as the development of drug addiction. Next, James Withrow presented his study of honey bee behavior. To alleviate the burden of honey bee queen failure in apiculture, he switched honey bee systems from a single queen to multi-queen colonies. He is testing ways to manipulate fighting among queens and the acceptance of new queens by workers. Next, Withrow will determine whether multi-queen colonies grow faster and have better disease resistance. Dr. William Kenkel, representing Indiana University, discussed

physiological and social differences among prairie voles that were born by cesarean section, as compared to those born vaginally by induction with oxytocin. Voles born by c-section had a decreased ability to thermoregulate, huddled less, were less gregarious, were less alloparental, and were less likely to develop pair-bonds. Changes in thermoregulation and social behavior have also been observed in humans, so prairie voles may be a good model system for studying behavioral effects due to delivery method. The final presentation of this session was given by Dr. Nagendran Muthusamy, who was the recipient of the 2018 Robert and Margaret Grossfeld Award for his publication “Developmentally defined forebrain circuits regulate appetitive and aversive olfactory learning” in *Nature Neurobiology*. His paper identifies two sets of neurons that are generated at different stages within the olfactory region of the brain, where detection of adverse odors develops at birth and appetitive odor detection neurons develop later in life.

Section 3 focused on evolution of morphological adaptations, hybrid inviability and olfaction mediated behavior. The first presentation was by Aldo Carmona-Baez on the genetic basis of diet adaptation in Lake Malawi cichlid species. He performed QTL mapping for gut morphology (gut length) and compared carnivorous, herbivorous and omnivorous cichlid species. He identified candidate genes responsible for such variations. Next, Varpu Pärssinen from Lund University working together with Dr. Brian Langerhans talked about her ongoing project on the evolution of hybrid inviability in Bahamas mosquitofish, *Gambusia hubbsi*. Her talk focused on how natural selection acts against hybrids using eight populations of mosquitofish that have been geographically isolated for thousands of years. She created crosses between eight mosquito populations and collected information on their body shape, life history, genital morphology and behavior that contributes to the isolation of mosquitofish populations in the presence of several reproductive barriers such as predation, and resource competition. Jacob Deslauriers talked about the neurogenetic analysis of *cyfip2* in zebrafish startle behavior. Sensory gating is critical in an environment where an animal must respond to relevant stimuli while ignoring irrelevant ones. Larval zebrafish respond to acoustic stimuli with an escape response and several mutants that affect the threshold for auditory startle behavior have been identified. Katie Hudson gave a presentation about epigenetic responses to *in utero* cadmium exposure. Her study focused on the epigenetic mechanisms that regulate genes which are susceptible to environmental exposure during *in utero* development due to DNA methylation dynamics. To large extent insects rely on their sense of smell

(olfaction) to locate food, sex, and a suitable place to oviposit. Among several proteins involved in the olfactory transduction process, odorant binding proteins (OBP) have important roles in mating, development and taste. Sneha Mokashi presented her work on allelic variants of *Drosophila* odorant binding protein 56h associated with variation in mating behavior. Next, Johanna Elsensohn presented her work on potential host marking pheromones in the invasive frugivore, *Drosophila suzukii*. She performed two-choice assays to assess the egg laying behavior of female *Drosophila* in the presence of larvae and other associated semiochemicals. The outcome of her study will help in designing novel semiochemical/odorant-based traps for controlling this invasive pest insect.

In the final session Jennifer Baltzegar presented her work on evolution of insecticide resistance in the mosquito, *Aedes aegypti*, which transmits the pathogens responsible for deadly human diseases such as yellow fever, chikungunya, zika and dengue fever. She highlighted in her talk the evolution and development of insecticide resistance especially pyrethroids, a common class of insecticides used to control *Ae. aegypti* populations. Her study focuses on the development of *knockdown resistance (kdr)* and associated genetic loci especially two SNPs and explores the evolution of these SNPs across an 18-year period in Iquitos, Peru. Christie Lee presented her work on monitoring real-time opioid peptide fluctuations using multiple scan rate voltammetry in the rat striatum. She described methods for monitoring dynamic fluctuations of endogenous neuropeptides in the brain and used an electrochemical approach to monitor fluctuations of tyrosine-containing opioid peptides such as methionine-enkephalin in the rat brain. Grace Parker presented genomic regulation of limited lifespan and reproductive senescence. Her objective is to study the genetic basis of lifespan and senescence and identify the genes associated with such traits using *Drosophila* as a model system. Joel Johnston presented his work on *Drosophila* odorant binding proteins (OBPs) especially the *Obp50a-d* gene cluster. His study focuses on understanding the evolutionary and behavioral significance of this cluster using CRISPR/Cas9 for genome editing. Allison Schloop gave a talk on the genetic and genomic basis of species divergence, where she presented mating outcomes between panels of wild derived inbred lines of *Drosophila melanogaster* and *D. simulans*, species that diverged 5.4 million years ago. Finally, Erin Peterson and Melissa Lamm, both presented polygenic sex determination and genetic mechanisms controlling such polygenic sex determination in East African cichlid fishes.

The 2018 Robert and Margaret Grossfeld Award



From left: Keck Center director; Dr. Robert Anholt; doctoral advisor; Dr. Troy Ghasghaei; recipient of the Grossfeld Award, Dr. Nagendran Muthusamy; Dr. Robert Grossfeld; Chair of the selection committee, Dr. Brian Langerhans.

The Grossfeld Award was established through a generous gift from the Grossfeld family. This unique award is given each year to a student or postdoc who has published the most creative and impactful publication during the preceding year. Nominations are solicited annually and recipients are selected through a rigorous process by a committee of external reviewers. This year the selection committee received eight nominations.

The award comes with a plaque and gift certificate.

Muthusamy showed that postnatal and adult neurogenesis are region- and modality specific. He demonstrated that chemogenetic inactivation of a subset of forebrain and olfactory neurons generated at birth disrupts responses to an aversive odor. In contrast, novel appetitive odor learning is sensitive to inactivation of adult-born neurons, revealing that developmentally defined sets of neurons may differentially participate in hedonic aspects of sensory learning.

The Evolution of Smelly Science

by Joel Johnstun

The Keck Center co-hosted an enlightening seminar with the Department of Plant Pathology and Entomology featuring Dr. Tristram Wyatt, entitled “Success of the smelliest: how pheromone signals evolve (and do humans have them?).”

Wyatt began with a brief history of pheromones, noting that it was observed since at least the early 1600s that bees are more likely to attack when they sense that another bee has already stung. Charles Darwin further hypothesized that scents emitted by one sex of a species could induce behaviors in the other and be subject to sexual selection.

In 1959 Peter Karlson and Martin Lüscher coined the term “pheromone” and defined it as a chemical signal transmitted between individuals of the same species. A more modern definition has been proposed by Wyatt as “molecules that are evolved signals, in defined ratios in the case of multiple component pheromones, which are emitted by an individual and received by a second individual of the same species, in which they cause a specific reaction, for example, a stereotyped behavior or a developmental process.” The requirement that they be evolved signals distinguishes pheromones from cues, as in the case of the latter only the receiver (as opposed to both receiver and sender) has evolved to recognize the substance. Mosquitos have evolved to detect molecules that are emitted from their hosts, the hosts have certainly not evolved to emit them to that end.

There are two main routes for pheromone evolution, either through sender precursors or receiver sensory bias. An example of a pheromone evolving from sender precursors is ant alarm pheromones, as many are similar to poisons that the ants use to defend their nests (*e.g.* formic acid). It is likely that the ants which interpreted their nestmate’s release of poison as a call to arms had an evolutionary advantage. A likely example of receiver sensory bias is found in the oriental moth *Grapholitha molesta*, as females of the species prefer males which emit the highest amount of plant-derived pheromones which are similar to compounds found in their host food; the male sex pheromones likely exploited existing sensory preferences of the females.



Wyatt emphasized the complexity of identifying pheromones. The human armpit, for example, can be broken down into at least 700 compounds. Moreover, an organism’s chemical profile can stem from many sources, such as non-pheromonal secretions, the immune system, hormones, bacterial symbionts, diet, conspecifics, infections, or even collected from flowers.

To help distinguish between the plethora of other substances found on organisms and true pheromones, Wyatt proposed that five requirements must be

met in order to classify a substance as a pheromone: 1) a synthetic form of the proposed pheromone should elicit the same response as the naturally-occurring substance, 2) it should do so at the naturally-occurring concentration, 3) the substance (or each component of it, in the case of multi-component pheromones) should be both necessary and sufficient to elicit that response, 4) other similar molecules or blends that the organism normally encounters should not elicit that response, and 5) there should be a credible pathway for evolution to have evolved both its emission and detection by members of the same species.

Wyatt also addressed the literature on human pheromones. Although it is probable that humans have pheromones (we are mammals, after all, and our smells change at sexual maturity), most of the existing literature revolves around a single molecule, androstadienone, which was originally proposed as a human pheromone by a corporation-funded paper in 1991 which gave no evidence of the molecule’s biological function. Through “top-scientist” endorsement, the easy procurement of the substance (which removed the intense need for chemistry inherent in good pheromonal research), the lack of critical review of the literature, and all the other banes of science (*e.g.* publication bias, hypothesizing after results are known, *etc.*), the body of literature assuming the human pheromonal status of androstadienone exploded. However, today there are signs that the literature is changing course and is increasingly skeptical. As with biology, science also evolves.

The 2018 Indiana University Animal Behavior Conference

by Brandon Baker and Erin Peterson

The 25th annual Indiana University Animal Behavior Conference was held on April 13-14 in Bloomington, Indiana. This conference brings together behavioral biologists from across the country to present their findings from numerous model and non-model organisms. This year, the conference offered 23 oral presentations and 46 poster presentations on a wide variety of research topics such as aggression in males and females, hormonal studies, olfaction, and mating. The Center for the Integrative Study of Animal Behavior (CISAB) at Indiana University has a long-standing relationship with the Keck Center for behavioral biology at North Carolina State University where two students from each university are granted the opportunity to the other's establishment to showcase their findings and be immersed in their respective symposia. With the combined generosity of the CISAB and the Keck Center, Brandon Baker and Erin Peterson had the pleasure of attending this year's conference on behalf of NC State and the Keck Center for Behavioral Biology.

The conference began with talks revolving around sex differences in the brain and its relationship to behavior. The talks looked at pair-bond formation differences in prairie voles based on method of birth, social dynamics in toque macaques, modulation of microglial cell morphology in medial prefrontal cortex via gonadal hormones, and sex differences in oxytocin modulation of the rewarding properties of social interactions.

Particularly of note in this section was the talk from Indiana University's Justin Bollinger, whose talk focused on microglia cell morphology in stress responses in mice. Bollinger has previously shown that sex-specific differences occur in the morphology of pre-frontal cortex microglia in response to stress, with males having greater dendritic growth while females exhibit regression of dendrites. To understand the potential causes for these sex-specific differences, Bollinger looked to gonadal hormones and their potential role in these differences. Using gonadectomized individuals compared to those gonadectomized and given replacement hormones, Bollinger was able to show that testosterone regulates basal microglial state in males and that estrogen moderates effects of chronic stress on microglia morphology, respectively in males and females. This finding not only demonstrates a role of gonadal hormones on brain morphology, but opens up the possibility for more physiological effects relating to sex specific behaviors.

The second section of oral presentations focused on external factors, olfaction and feeding looking at topics such as responses to prey-associated volatile compounds in lizards, time of feeding determining offspring quality-quantity tradeoff in zebra finches, and how dim light at night impacts circadian behaviors in mice.

In this section, a postdoctoral researcher from Western Kentucky University, Ila Mishra, gave a fascinating talk on how timing of feeding determined quantity-quality trade-off in zebra finch reproduction. By comparing zebra finch who had food restricted to only 4 hours in the morning or in the evening to those with food presented freely, Mishra was able to observe effects not only on fertility and fecundity, but also offspring vitality and fitness. The morning restricted cohort produced offspring that had reduced activity and body size while the evening restricted cohort had a delay in the onset of breeding and reduced reproductive success. This sets up not only a paradigm for food restriction effects on quality versus quantity, but also many more potential studies about metabolic effects on reproduction in birds.

The final session of oral presentations was rounded out with a slew of talks revolving around social behavior. In particular, evolution of color as a model for social evolution, benefits of social play behavior and identification of a behaviorally significant compound in social odors of territorial animals.

The benefits of social behavior in hyenas were explained by Tracy Montgomery, a graduate student from Michigan State University. While the immediate cost, but long term adaptive effects of play have been substantiated, the immediate benefits are not well established. This theory posits that play provides benefits, including stronger social bonds, reduced aggression, and reduced stress. Using both behavioral observations and a non-invasive measure of endocrine sampling, Montgomery tested the "socialization hypothesis" of play in wild spotted hyenas by evaluating their social behaviors and relative amounts of stress hormones. Social relationships were evaluated alongside repeated endocrine sampling to test causal effects of play on stress hormones.

Day one was rounded out with a captivating plenary talk by the renowned Dr. Frans de Waal. Dr. de Waal spoke about the evolution of emotions and empathy in primates. Many impactful videos displayed the complexity of primate behavior, including the tendency of primates to show empathy just as humans do. One of

these videos, with over 13 million views on Youtube, looked at two monkeys being paid unequally for completing a task where one monkey furiously denies being paid less for completing the same task as its counterpart. The evening concluded with a tremendous poster presentation session with 46 posters, including the Keck Center's very own Erin Peterson who presented her work on polygenic sex determination in a cichlid fish.

Day 2 was structured very similar to the first day with three sessions of oral presentations, ending with a keynote seminar. The first session, titled "Mating, Reproduction, and Sexual Selection" contained interesting talks such as using robotic prairie-chickens to investigate species recognition, how parasitic exposure and infection induces behavioral alterations in a freshwater snail, and how male courtship preference is potentially adaptive in seasonally sympatric population divergence.

Jacqueline Augustine, a professor from Ohio State University at Lima, delighted us with videos from her field work looking at prairie-chicken behavior. Augustine uses a hybrid zone of greater and lesser prairie-chickens to study mate choice. Providing a great example of interdisciplinary collaboration, Augustine made robots with engineering students that allows for remote evaluation of male reproductive behavior. Through the use of dummy robots wrapped in taxidermy female prairie chickens, Augustine is able to observe the potentially costly behavioral aspects of male courtship and how it might differ between greater and lesser prairie-chickens.

The next section featured talks about physiological mechanisms of behavior. The section began with our own Brandon Baker speaking about the genetic underpinnings of cocaine and methamphetamine consumption in *Drosophila melanogaster*. Ensuing talks discussed the effect of extinction on the destabilization of an overtrained fear memory and the molecular mechanisms underlying expression of striatal oxytocin receptors in the prairie vole.

Byron Gardner, a graduate student from Emory University, gave a talk about the regulation of oxytocin receptor in the prairie vole, a well-established model for social behavior. Gardner uses prairie voles as a model for how variation in oxytocin receptor density affects brain and behavior phenotypes. Using both computational and molecular techniques, Gardner found a SNP in the regulatory region of the oxytocin receptor gene, in a genomic region marked with histone modifications that act in an enhancing manner. Following up with chromatin immunoprecipitation, he showed that this SNP was causative in producing greater

binding in the enhancer region. This study provides evidence of a molecular basis for differential behavior and social cognition.

The final session of oral presentations for the conference focused on social behavior, namely how early biparental care impacts epigenetic regulation of the oxytocin receptor gene in prairie voles; how fecal estradiol and progesterone can be used to determine the reproductive state of female red colobus monkeys; and how serotonin signaling differentially affects aggressiveness among two populations of the horn polyphenic beetle, *Onthophagus taurus*.

Keeley Newsom, a research assistant from Indiana University, spoke about an underlying pathway affecting aggressiveness in two beetle morphs of the same species. *Onthophagus taurus* either develop the large bodied horn morph, or the small bodied non-horned morph dependent on larval nutrition. These two morphs rely upon two different modes to attract mates, with differential levels of aggression. However, the molecular mechanisms that integrate behavior and morph are not known. Using a candidate approach, serotonin signaling was investigated by pharmacological manipulation. Though there were differences found by morph and population, the major trends showed that application of exogenous serotonin increased the frequency, intensity, and duration of aggressive behaviors.

The last presentation was a keynote seminar by Indiana University's Dr. Kimberly Rosvall. Rosvall presented tremendous information surrounding female competition and the challenge hypothesis in tree swallows. Much of the talk focused on female aggression and what costs there are to female competitive phenotypes such as reduction in quality of offspring. The conference ended with a wonderful reception at the home of the CISAB's director Dr. Cara Wellman with wonderful food, beer brewed by Wellman and her husband and plenty of discussions with those from around the country who attended the conference.



Publications

The following publications of the W. M. Keck Center for Behavioral Biology have appeared in print:

Cruise, A., Watson, D. W. and Schal, C. (2018) Ecological succession of adult necrophilous insects on neonate *Sus scrofa domesticus* in central North Carolina. *PLoS One* **13**: e0195785.

Funaro, C. F., Böröczky, K., Vargo, E. L. and Schal, C. (2018) Identification of a queen and king recognition pheromone in the subterranean termite *Reticulitermes flavipes*. *Proc. Natl. Acad. Sci. U. S. A.* **115**: 3888-3893.

Rock, K. D. and Patisaul, H. B. (2018) Environmental Mechanisms of Neurodevelopmental Toxicity. *Curr. Environ. Health. Rep.* **5**:145-157.

Gunning, C. E., Okamoto, K. W., Astete, H., Vasquez, G. M., Erhardt, E., Del Aguila, C., Pinedo, R., Cardenas, R., Pacheco, C., Chalco, E., Rodriguez-Ferruci, H., Scott, T. W., Lloyd, A. L., Gould, F. and Morrison, A. C. (2018) Efficacy of *Aedes aegypti* control by indoor Ultra Low Volume (ULV) insecticide spraying in Iquitos, Peru. *PLoS. Negl. Trop. Dis.* **12**: e0006378.

Of note...

Jennifer Baltzegar won 1st place in the BASF Minority Poster Session on April 20, 2018 at BASF in Research Triangle Park for her poster on evolution of insecticide resistance in *Aedes aegypti* from Iquitos, Peru.

Emily Moore has been selected as one of six finalists for the highly competitive Crow Award from the Genetics Society of America, for her dissertation project work on the evolutionary genetics of cichlid fish behavior.

Kasey Potts and **Catherine Mason**, undergraduate students in the Sombers laboratory presented posters at the NCSU Spring Undergraduate Research Symposium and received Sigma Xi awards for outstanding presentations.

Jaime Willett received the APS select designation for “distinction in scholarship in the Journal of Neurophysiology” for her publication, "Nucleus accumbens core medium spiny neuron electrophysiological properties and partner preference behavior in the adult male prairie vole, *Microtus ochrogaster*.”

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