The Signal

Monthly newsletter of the W. M. Keck Center for Behavioral Biology
at North Carolina State University
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The Signal Wishes Its Readers a Happy Holiday Season!

Keck Center Scientist Links Ecology to Religious Beliefs

Religious beliefs are a unique feature of humans and influence human behavior profoundly. Keck Center scientist, Carlos Botero, a postdoctoral fellow in the Biological Complexity Initiative program investigated whether ecological forces influence the emergence of religion. Botero's study was motivated by comparative and experimental evidence that indicates that beliefs in moralizing high gods promote cooperation among humans, a behavioral attribute known to correlate with environmental harshness in animals. By bringing climatological information and advanced statistical tools to an interdisciplinary study that combined ecology and the social sciences, Botero and his collaborators were able to evaluate the potential effects of environmental forces, language history, and culture on the global distribution of belief in moralizing high gods in as many as 583 societies. After accounting for shared ancestry and cultural interactions among these societies, they discovered that religious beliefs are more prevalent among societies that inhabit poorer environments and experience harsher environmental fluctuations. Their approach predicts the global distribution of beliefs in moralizing high gods with an astonishing accuracy of 91%. The picture that emerges from their study is that human religious beliefs do not only result from cultural transmission, but also are shaped through a complex mixture of social, cultural, and environmental influences.

Carlos Botero

The study was published in a recent issue of the *Proceedings of the National Academy of Sciences of the USA* and has attracted worldwide attention. The article has been covered in main newspapers around the world, including the Washington Post and The Guardian, and weekly magazines (*e.g.*, Businessweek). Botero has also been interviewed by the BBC, and public radio stations in The Netherlands, Australia, and Germany.

This Issue of The Signal Contains the Announcement and Call for Abstracts for the Fifteenth Annual Student/Postdoc Symposium of the W. M. Keck Center for Behavioral Biology.

Symposium 2015

Announcement and Call for Abstracts

The Sixteenth Annual Student/Postdoc Symposium of the W. M. Keck Center for Behavioral Biology will be held on Friday, February 20, 2015, in the Stanley G. Stephens room, 3503 Thomas Hall, at North Carolina State University. Participation is open to all students, postdoctoral fellows and faculty, and is mandatory for students enrolled in the Concentration for Behavioral Biology.

Preliminary Program

8:30	Breakfast
9:15	Welcome by Dr. Robert Anholt, Center Director
9:30	Symposium
10:45	Coffee break and group photograph
11:15	Symposium
12:30	The Robert and Margaret Grossfeld Award Presentation
12:45	Lunch
2:00	Symposium
3:15	Break
3:45	Symposium
5:00	Reception and dinner

Presentations will be 12 minutes with 3 minutes for discussion. Participants should submit an abstract by e-mail to Caroline Leitschuh (caro.leit@gmail.com) or Megan Serr (meserr@ncsu.edu) no later than **February 6.** The abstract should contain no more than 300 words without figures or tables. It must provide a title and the name of the presenting author (without co-authors or affiliation).

Undergraduate students are invited to submit abstracts for poster presentations.

Trainees within their first year may present their future research objectives. Advanced trainees will present progress of their research. Computer-assisted projection and a PC-type laptop will be available for PowerPoint presentations. All presentations must be rehearsed with the mentor.

Breakfast, lunch and a reception and buffet-style dinner will be provided.

Announcing

The Robert and Margaret Grossfeld Award

Established through a generous gift by the Grossfeld family, the Robert and Margaret Grossfeld Award is presented each year to a student or postdoctoral fellow from a laboratory affiliated with the W. M. Keck Center for Behavioral Biology for best publication of the preceding year.

Eligibility requirements

- The nominee can be an M.S. or Ph.D. student or a postdoctoral fellow.
- The nominee must be the first author of the publication.
- The work reported in the publication must have been performed at North Carolina State University.
- The publication must report original research. Review articles or opinion papers are not eligible.
- The recipient must be able to present his/her work reported in the winning article at the annual student/postdoc symposium on February 20, 2015. If the nominee has recently moved to a position at another institution, the W. M. Keck Center will provide travel expenses for the successful nominee to attend the symposium and receive the award.

Nominations

The Principal Investigator must nominate the candidate, submit an electronic link to the publication, and explain in less than 250 words the significance of the contribution reported in the manuscript. Nominations must be submitted electronically before January 16 to the Director of the Keck Center.

Selection process

The successful candidate will be selected by the Keck Center's Executive Committee and two or more external judges either from within or outside the university. To avoid conflict of interest committee members will recuse themselves from the selection process if individuals from their laboratories have been nominated. The successful candidate will be notified prior to the annual student/postdoc symposium date.

Award

The recipient will receive a customized plaque indicating the recipient's name and accomplishment. The recipient will also have access to $1,000 of funds ($500 from the Grossfeld fund and $500 matching funds from the Keck Center operating fund) to be spent for research (e.g. supplies, computer, books) or career development (e.g. attendance at a conference), provided the funds can be encumbered through NC State University. For applicants who are no longer affiliated with NC State University, award money will be used to cover their travel expenses to receive the award.

Sex, Stress, and the Brain

by Meghan E. Rebuli

On Thursday, October 30th, 2014, the W. M. Keck Center for Behavioral Biology had the distinct pleasure of hosting Dr. Bruce S. McEwen, Alfred E. Mirsky Professor and Head of the Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology at the Rockefeller University. McEwen's seminar was titled, "Sex, stress, and the brain: hormone actions over the life course via novel mechanisms."

McEwen first discussed the discovery of the mechanisms of steroid hormone action in the 1960s. Estrogen receptors in the ventral medial nucleus and glucocorticoid receptors in the paraventricular nucleus of the hypothalamus are nuclear steroid receptors which function through action on the genome, producing transcripts within the nucleus that affect the function of the cell. Subsequent discoveries showed that there are glucocorticoid receptors in the hippocampus which are a target for adrenal steroids, enhancing memory at low doses and suppressing memory at high doses.

McEwen discussed the role of the hippocampus in spatial memory. Research on London cab drivers and food catching birds indicated that the hippocampus lights up when actively recovering a memory. This indicates, according to Dr. McEwen that "[the] hippocampus seems to be the canary in the coal mine for memory in the brain." During memory acquisition dendrites grow and shrink, synapses appear and reappear, and limited amounts of neurogenesis occur within the hippocampus.

He then went on to outline some of the extra-hypothalamic effects of estrogen on brain function, through both non-genomic and genomic action. Estrogens play a key role in synapse remodeling in the hippocampus. Research by Catherine Wooley and Elizabeth Gould from his lab indicates that dendritic spine density in the striatum radiatum of the CA1 region fluctuates during the estrus cycle. RU486 was used to confirm estrogen action by demonstrating that administration of this compound can block down-regulation of spine density. NMDA receptor blockade prevented synapse formation and estrogen treatment enhanced hippocampal memory. Estrogen also stimulates filopodial outgrowth from neurites to form synapses *in vivo* and *in vitro*.

Next McEwen addressed aging. He worked closely with Dr. John Morrison from Mt. Sinai to research the effects of sex hormones on the aging brain. Sex hormones play a role in the formation of sex dimorphisms in the brain at a young age, but are also

Dr. Bruce McEwen

important in the aging brain in learning as well protecting the brain in times of injury. McEwen asked whether loss of estrogen induced synapse formation in aging could be related to the loss of efficacy of hormone therapy 10 or more years after the onset of menopause.

Androgens, male hormones, are present as nuclear and non-nuclear receptors in the hippocampus. Testosterone is transformed into dihydrotestosterone (DHT) and estrogen by enzymes in the brain. Mild exercise increases DHT in the hippocampus, providing evidence for androgenic mediation of neurogenesis. This effect was blocked by flutamide, but gonadectomy does not affect the local increase of DHT, indicating it may be a locally produced product. This experiment also is evidence for a central theme in McEwen's research, that doing something to add purpose and meaning to your life can offset aging effects on memory. Moderate stress can act beneficially on the brain to promote learning and survival. An acute instance of stress and fairly low release of glucocorticoids can aid in synaptic functional enhancement, increasing synaptic transmission, long term potentiation, and learning for the purpose of self-preservation. Medium amounts of stress induce adaptive plasticity, suppression of neurogenesis, and mediate dendritic remodeling, for the purpose of protecting the brain from long term damage. Repeated or chronic stress and large amounts of glucocorticoid release can damage potentiation, induce a loss of plasticity, and lead to symptoms of depression and may be associated with post-traumatic stress disorder. The hippocampus is plastic and vulnerable to stress. CA3 neurons within the hippocampus are vulnerable to

damage and their dendrites shrink with exposure to stress. This shrinkage is reversible with a reduction of stress. In this experiment, stress was mimicked by chronic glucocorticoid treatment. Regular exercise counteracts the effects of stress, as it expands the hippocampus.

With chronic stress, chronic jet lag, lack of exercise, and chronic inflammation, the hippocampus can decrease in size. Counteracting chronic stress with regular exercise, intense learning, and even non-drug related anti-depression treatment, can reverse this process, as stress causes neurons to shrink (in the pre-frontal cortex and hippocampus) or grow (in the amygdala, an area associated with anxiety that can indicate onset of stress), but not necessarily to die.

McEwen discussed diverse mechanisms of adrenal steroid action. One such mechanism is the translocation of glucocorticoid receptors to mitochondria, which promotes calcium reuptake and reduces free radical formation; however, excessive translocation can cause increases in free radical formation. Acute stress up-regulates expression of four mitochondrial genes, while chronic stress up-regulates one gene (ND6), further indicating that the brain responds to stress differently when it is experienced in an acute *versus* chronic manner.

Finally, McEwen described differences in sex response to stress in the prefrontal cortex. Males undergo dendrite shrinkage, while females undergo no shrinkage, but in females the population of neurons in the prefrontal cortex that project to the amygdala expands. Further emphasizing a sex-specific response, females and males do equally well on empathy tests, but different brain regions are excited during fMRI recordings, highlighting differences in male and female neurochemistry.

Zelda, Key to Early Drosophila Development

by Megan E. Garlapow

On Monday, November 3, 2014, Dr. Michael Eisen from the University of California at Berkeley gave a compelling seminar titled "Activation of gene expression and the onset of gene regulation in early Drosophila development." He described the remarkable developmental story of how genomes encode the spatial patterns of gene expression.

Motivating the research was the question of how the early *Drosophila* embryo, which is entirely maternal, progresses from no transcription upon fertilization to a zygote transcribing thousands of genes. In fact, at approximately three hours post-fertilization, a massive transcription wave couples with degradation of maternal products such as organelles and RNAs. In this process, an embryo takes over its destiny and its spatial patterning. There are fourteen mitotic divisions in these first three hours, at which point cell membranes form and gastrulation occurs. By division 14, thousands of genes are transcribed, many of them in spatiotemporal patterns. These patterns are crucial for development, defining the subsequent structures.

Enhancers play an integral role in driving patterned transcription, integrating spatial information. Nonetheless, we do not understand enhancers sufficiently to find enhancers in newly sequenced genomes, match enhancers with their target genes, reliably relate the sequence to the transcriptional output of the enhancer, or predict consequences of poly-

Dr. Michael Eisen

morphisms in enhancers. Eisen wondered why only some sequences function as enhancers when potential transcription factor binding sites are everywhere. The Grammar Model posits that there is something special about how binding sites are organized, yet scrambled enhancers from distantly related flies drive identical expression patterns. In contrast, the Active Model states that enhancers function due to being packed in open, active chromatin.

Furthermore, enhancers are hot spots of transcription factor binding. For example, *evenskipped* patterns are expressed along the anterior-posterior axis, but dorso-ventral patterning transcription factors also bind to *eve*

enhancers. Eisen's group recognized a motif enriched in regions bound to transcription factors: CAGGTAG. In fact, CAGGTAG was enriched regardless of the gene. With knowledge of this motif, Zelda was found to be a protein that binds to the CAGGTAG motif. RNA sequencing from single *Drosophila* embryos allowed Eisen and his group to gain sex-specific, high-temporal resolution. The results of this RNA sequencing showed that Zelda is active by mitotic cycle eight, and in cycles eight to ten, Zelda binds at high levels to enhancers of genes that become active in cycle fourteen. In other words, Zelda binding predicts enhancer activity.

Indeed, Zelda binding predicts most enhancers, with 70% of enhancers bound by Zelda. Furthermore, Zelda is a pioneer transcription factor, meaning that it can open closed chromatin. However, early embryo chromatin is all open. In this case, chromatin will be packed except for where Zelda is bound early, with early Zelda binding contributing to keeping chromatin regions open at the cellular blastoderm, making it available for subsequent transcription factor binding activity. In fact, histones flanking Zelda-bound enhancers are acetylated by mitotic cycle eight causing the chromatin to remain open. Embryos lacking maternal Zelda do not have these patterns of Zelda recruiting acetylation, causing resistance to histone methylation.

Eisen's research on early Drosophila development has elucidated that enhancer specifications and enhancer identity are separable processes. It is of interest, however, that Zelda only exists in arthropods. Thus, it is likely that one or multiple proteins with similar functions, that yet remain to be discovered, may exist in vertebrate systems.

Of Flies, People and Slightly Better than Average Wine

by Sneha Mokashi

On November 20th, 2014, the W. M. Keck Center for Behavioral Biology held its first social evening discussion of the year at Chateau Mackanholt, led by Dr. Tatiana Morozova. It was an evening of stimulating conversation about topics ranging from the right method to diagnose alcoholism and the molecular etiology of alcoholism to the genetic basis of alcoholism among a diverse group of people from fields ranging from evolutionary thinking, quantitative genetics to neurotoxicology.

The discussion began with talking about why people drink in the first place and how social circumstances play a critical role. From there, the discussion moved on the very definition of alcoholism. Different psychiatric instruments use different and somewhat ambiguous definitions for this condition. Owing to different clinicians using different definitions and different diagnostic tools (primarily self-reported questionnaires), precisely diagnosing alcoholism is currently difficult. This ambiguity creates a need for a molecular marker which could make diagnosis more precise and reliable.

While discussing the definition and diagnosis of alcoholism, a point that came up was that individuals addicted to alcohol often show a propensity to be addicted to other substances. This makes considering the molecular etiology of alcoholism important. Do all addictive substances follow a common "addictive pathway" with individuals showing a common tendency towards impulsive behavior? Or do different

substances act through different mechanisms but achieve the same end result. Nicotine has been known to increase alcohol induced intoxication for years but the mechanism was not known until recently. They both activate the mesocorticolimbic dopamine system which acts by promoting the drug reinforcement process but via different receptors (nicotine via nicotinic acetylcholine receptors and alcohol via a variety of other receptors).

While the environmental component plays a significant role in whether an individual develops alcoholism, the 30-40% heritable component determined primarily through twin studies indicates a significant genetic component. In the pre-next generation sequencing and whole genome analysis

days, genes associated with alcoholism were identified by taking a candidate gene approach and using microarrays for transcriptomic profiling. Several groups looked for genes associated with alcoholism in different regions of the brain using postmortem human brain samples. They found several neurotransmitter receptors to be affected. However, nobody could pinpoint which specific variants in the genes result in a significantly increased risk for alcoholism. During that time, some groups also conducted genome-wide association studies on alcoholics to help identify these variants. However, as is often the issue with genome-wide association studies, the sample size for most of these studies was very small. There is also the issue of a clear definition of the alcoholic phenotype as mentioned earlier. Only associations for CDH13 and eight other genes were replicable across different studies.

Apart from these studies on human samples, several groups have been working on alcohol sensitivity, tolerance, intoxication and addiction in model organisms such as fruit flies and rodents. However, we do not have a model organism which can perfectly mimic the human condition of alcoholism with all its complex intricacies. For example, fruit flies can be used to study alcohol sensitivity, but not addiction, and rodents do not drink alcohol unless trained to do so. An intriguing point which came up was that prairie voles could be used to study the social aspect of drinking since they drink alcohol if their cage mates do. Even though hundreds of such studies (including several next generation sequencing experiments) in model organisms have been conducted, no alcoholism-associated polymorphisms have been shown to be conserved across species. Several genetic networks have been identified and the trend is now shifting from the single gene approach to the gene network approach because networks as a whole tend to be conserved more than individual genes.

This incongruity in data from different species makes translational research rather difficult and highlights the importance of takes a cross-species meta-analysis systems biology approach. Through this approach, the association of DNA sequence variants identified by genome-wide association studies in human samples with alcoholism can be genetically and functionally validated in model organisms. Such validated associations can then be used as targets for applications in diagnosis and/or treatment of alcoholism.

The evening was a refreshing blend of science and casual conversation complemented by scrumptious cookies and drinks courtesy of Robert Anholt and Trudy Mackay.

Publications

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

Botero, C. A., Gardner, B., Kirby, K. R., Bulbulia, J., Gavin, M. C. and Gray, R. D. (2014) The ecology of religious beliefs. *Proc. Natl. Acad. Sci. USA* **111:** 16784-16789.

Montgomery, S. L., Huang, W, Anholt, R. R. H., Mackay, T. F. C. and Rand, M. D. (2014) Genome-wide association analysis of tolerance to methylmercury toxicity in Drosophila implicates myogenic and neuromuscular developmental pathways. *PLoS On*e **9:** e110375.

Zhou, S., Mackay, T. F. C. and Anholt, R. R. H. (2014) Transcriptional and epigenetic responses to mating and aging in *Drosophila melanogaster*. *BMC Genomics* **15:** 927.

Albertson, R. C., Powder, K. E., Hu, Y., Coyle, K. P., Roberts, R. B. and Parsons, K. P.. Genetic basis of continuous variation in the levels and modular inheritance of pigmentation in cichlid fishes. *Mol. Ecol.* **23:** 5135-5150.

Gammerdinger, W. J., Conte, M. A., Aquah, E. A., Roberts, R. B. and Kocher, T. D. Structure and secay of a proto-Y region in tilapia, *Oreochromis niloticus*. *BMC Genomics* **15:** 975.

Okamoto, K. W., Robert, M. A., Gould, F. and Lloyd, A. L. (2014) Feasible introgression of an anti-pathogen transgene into an urban mosquito population without using gene-drive. *PLoS. Negl. Trop. Dis.* **8:** e2827.

Of note…

Robert Anholt presented a lecture titled “What animal models reveal about human disease” at the Osher Lifelong Learning Institute.

David Dorris and John **Meitzen** presented a poster at the annual Society for Neuroscience meeting in Wshington, DC, titled “Intrinsic excitability varies by sex in pre-pubertal striatal medium spiny neurons.”

Fred Gould gave the Keynote Address at the Entomological Society of America meeting and also did an outreach talk at that meeting on “Engineering pests to be nicer.”

Lisa McGraw attended the 2014 NAKFI conference on “Collective Behaviors: From Cells to Societies" in Irvine, CA.

The **NCSU iGEM team** won the "Best Policy and Practices Project" at the 2014 International Genetically Engineered Machine Competition in Boston. Our

project was entitled “Mapping Responsible Innovation: A First Principles Approach” and team members were **Jennifer Baltzegar** (Genetics), **Johanna Elsensohn** (Entomology), Sheron King (Public Administration), Tina Ndoh (Public Administration), Emily Nwakpuda (UNC - Public Policy), Barry Peddycord III (Computer Science), Elizabeth Pitts (Communication), Jayce Sudweeks (Public Administration), Sophia Webster (Entomology), and Rene Valdez (Fisheries, Wildlife, and Conservation Biology).

To contribute to The Signal, to be placed on our mailing list or for information about the W. M. Keck Center for Behavioral Biology, contact Dr. Robert Anholt, Department of Biological Sciences, Box 7614, North Carolina State University, Raleigh, NC 27695-7614, tel. (919) 515-1173, anholt@ncsu.edu.

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