the 12th annual Indiana University ANIMAL BEHAVIOR CONFERENCE

Friday April 22, 2005
IMU Frangipani Room, 9:00 A.M. to 5:00 P.M.
12th ANNUAL INDIANA UNIVERSITY
ANIMAL BEHAVIOR CONFERENCE
hosted by
The Center for the Integrative Study of Animal Behavior (CISAB)

Friday April 22, 2005
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IMU Frangipani Room
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9:00 A.M. to 5:00 P.M.
(light breakfast will be served from 8:30 A.M.)
Indiana University Animal Behavior Conference

8:00 – 8:30 set up posters, load talks etc.
8:30 – 8:50 light breakfast

8:50 – 9:00 Welcome – Dr Emilia Martins

Session 1 - Moderator: Melissa Scotti

9:00 – 9:15 Jennifer Cianciolo
Sperm limitation and female choice in oribatid mites

9:15 – 9:30 Elsa Youngsteadt
Neotropical ant-gardens: Chemical basis of an ant-plant mutualism

9:30 – 9:45 Dr. Tobias Riede
The relationship between acoustic structure and semantic information in Diana monkey alarm vocalization

9:45 – 10:00 Erin Kelso
Species discrimination by the sagebrush lizard (Sceloporus graciosus) based on two display types: Does the context of signals matter?

10:00 – 10:15 Muchu Zhou
Electrocommunication signals of Adontosternarchus devenanzii

10:15 – 10:30 Dr. Milos Novotny
Chemosignaling Structural Attributes of MHC Mating Preferences in Mice

10:30 – 10:45 COFFEE BREAK

Session 2 - Moderator: Rebecca Dagley

10:45 – 11:00 Ping Wang
Molecular Evolution of Odorant Binding Protein Genes in Drosophila melanogaster

11:00 – 11:15 Jill Villarreal
Effect of Environmental Temperature on Morphology and Behavior of Postnatal Rats

11:15 – 11:30 Richard W. Vogel
Effects of AMPA-kainate Receptor Blockade in Anterior Cerebellar Cortex on Eyeblink Classical Conditioning

11:30 – 11:45 Eddie Fernandez

11:45 – 12:00 Dr Colin Allen
From Neurons to Intentions: the case of macaque mirror neurons
LUNCH AND POSTER SESSION

Session 3 - Moderator: Richard Granquist

2:00 – 2:15   Angela L McDowell
Contextual Alterations Eliminate the Appetitive-to-Aversive Transfer Impairment in Phenytoin-treated Rats

2:15 – 2:30   Dr. Matthew Cooper
Antagonism of Corticotropin-Releasing Factor Type 2 Receptors (CRF-R2), but not CRF-R1, Reduces the Acquisition of Conditioned Defeat

2:30 – 2:45   Daniel Sanford
Anthropomorphism in Victorian Science

2:45 – 3:00   Kevin T Ball
Serotonin (5-HT)2A and 5-HT2C/B Receptors Differentially Regulate 3,4-methylenedioxymethamphetamine-Induced Changes in Behavior and Striatal Single-Unit Activity in Freely Moving Rats

3:00 - 3:15   Dr. Michael Price
Reciprocal altruism in an experimental collective action

3:15 – 3:35   COFFEE BREAK

3:35 – 4:00   Awards Ceremony - Dr. Emila Martins

Introduction of Keynote Speaker by Hanna Kolodziejski

4:00 – 5:00   Keynote Address – Dr. Carl D. Hopkins, Cornell University
Temporal Coding and Communication: a behavioral neurobiologist's perspective on electrical communication in fish

7:00 – 9:00   Potluck Reception at Rod Suthers' home (directions/map in back of program)
Nearly 10% of oribatid mites are asexual and appear to be evolutionarily successful. This propensity of asexual oribatid species to occur and persist suggests that sexual oribatid mites are particularly vulnerable to invasion by asexual mutants. A need for reproductive assurance may favor asexual reproduction, because males of sexual species deposit and abandon spermatophores, such that spermatophores may be limited. Because choice by females could increase sperm limitation, I am examining whether females of the sexual species Epidameus species exhibit choice of spermatophores either from males over a range of distances, used as a proxy for relatedness, or from ‘younger’ to ‘older’ spermatophores. I plan to test whether outbreeding increases offspring fitness by measuring reproductive rate over a series of fathers in order to verify the criterion of relatedness for choice. In addition, I will test whether the use of multiple spermatophores increases female reproductive success (i.e. whether or not one spermatophore is ‘enough’), because the need for multiple spermatophores could also cause sperm limitation.

Throughout lowland Amazonia, ants collect seeds of specific epiphytes and cultivate them in nutrient-rich arboreal nests, forming diverse but species-specific symbioses known as Neotropical ant-gardens (AGs). In this obligate mutualism, ants depend on their symbiotic plants for nest stability, and plants depend on the ant nests for substrate and nutrients. However, the identity and origin of the cues by which AG ants recognize, collect, and cultivate the specific AG seeds are unknown. The importance of chemical cues in the ant-seed interaction was investigated by applying solvent extracts of AG seeds to other seeds that ants typically ignore, and evaluating ant response to these treated seeds. The AG ant Camponotus femoratus carried extract-treated seeds significantly more often than solvent blanks, and preferences among extract-treated seeds depended on both the species of seed extracted and the solvent used to make the extract. These results demonstrate that extractable chemical cues are responsible for seed-carrying behavior in AG ants, and that ant behavior toward seeds can be quantified in a robust behavioral assay. Therefore, behaviorally guided fractionation and analysis will be used to isolate and identify the specific behaviorally active components of AG seeds.
9:30 – 9:45 Dr. Tobias Riede
Indiana University, School of Medicine

The relationship between acoustic structure and semantic information in Diana monkey alarm vocalization

It is still unclear to what degree mammals are capable of actively controlling vocal tract filtering, a defining feature of human speech production. To address this issue we conducted a detailed acoustic analysis on the alarm vocalization of free-ranging Diana monkeys. These vocalizations are especially interesting because they convey semantic information about two of the monkeys' natural predators, the leopard and the Crowned eagle. We found that a vocalization-initial formant downward transition distinguishes most reliably between eagle and leopard alarm vocalization. Morphological measurements of Diana monkey vocal tract's and a computational model help explain the underlying motor pattern.

9:45 – 10:00 Erin Kelso
Indiana University, Department of Biology

Species discrimination by the sagebrush lizard (Sceloporus graciosus) based on two display types: Does the context of signals matter?

The ability to quickly and accurately distinguish conspecifics from heterospecifics is an important aspect of choosing or attracting mates, as well as during aggressive encounters. However, the details of how and when species identity is conveyed will likely vary according to these different contexts and the costs associated with ‘mistakes’ in species recognition. Here, we test for a species recognition function of two visual signals that differ in their context of use. Lizards in the genus Sceloporus have evolved two distinctive types of headbob displays: the well-studied "push-up display", used mainly as a broadcast along territorial boundaries and also during aggressive encounters, and a "shudder-bob" display, exhibited primarily by males during courtship (Carpenter & Ferguson 1977; Martins 1993). The two displays involve similar motions (raising and lowering of the head and body), but vary in both structure (the timing of the motions that comprise the signal) and the context in which they are used. Using a mechanized lizard model painted to resemble a male S. graciosus, we presented animals in the field with two standardized push-up displays, conspecific S. graciosus display and a heterospecific S. occidentalis display, and two standardized shudder-bob displays, from S. graciosus and S. occidentalis. Overall, we observed more differences in the responses of S. graciosus to conspecific and heterospecific push-up displays, including increased chemosensory behavior and increased latency to flee during presentations of S. occidentalis push-ups. However, both male and female responses to conspecific and heterospecific shudder-bob displays were not significantly different. Our results suggest that the push-up displays of Sceloporus lizards contain species identifying information while shudder-bob displays, a courtship component, do not.
Electrocommunication signals of Adontosternarchus devenanzii

Apteronotid knifefish produce a weak electric organ discharge (EOD) for electrolocation and communication. Electrocommunication in most species studied to date is sexually dimorphic. In Apteronotus leptocephalus, the EOD frequency of males is higher than that of females, while the reverse is true in A. albifrons. In both species, there is a sex difference in the structure of modulations in the EOD, though A. leptocephalus also exhibits a sex difference in the rate of modulations while A. albifrons does not. In this study, we examined electrocommunication behavior of another apteronotid, Adontosternarchus devenanzii, by measuring the EOD frequency of fish of both sexes and recording both spontaneous and playback-evoked EOD modulations. A. devenanzii produced two distinct types of modulations: H-type modulations exhibited large frequency excursions, while L-type modulations possessed small frequency excursions. Although the two modulation types were distinct, significant variation in modulation parameters were observed within each category. The duration of both modulation types ranged from milliseconds to seconds. Unlike in previously studied Apteronotus species, EOD frequency does not differ significantly between the sexes in A. devenanzii. There was a sex difference in the proportion and duration of H-type modulations. Overall number of modulations was not sexually dimorphic. These results suggest that sex differences in EOD behavior have diversified within the Apteronotidae, while the production of different types of modulations is a more basic characteristic.

Chemosignaling Structural Attributes of MHC Mating Preferences in Mice

The genes of the major histocompatibility complex (MHC) are highly polymorphic loci that primarily control antigen presentation. However, there is a growing body of evidence that the MHC complex also influences mating preferences and kin recognition through olfactory communication. Here we report the results of chemical analysis of the odors from different strains of the house mouse (Mus musculus domesticus) which include MHC-congenics, mutants and transgenic male animals. Chromatographic profiles reveal that MHC influences quantitative changes in a number of volatile compounds, including testosterone-mediated pheromones. Identification of the chemical profile constituents through mass spectrometry also implicates certain products of sulfur metabolism and oxidative pathways as significant in distinction. We also find that some of these discriminating molecules occur in blood and their urinary excretion is not influenced by antibiotic administration. The findings thus do not support the role of bacterial microflora in this type of "genetic signaling."
**Molecular Evolution of Odorant Binding Protein Genes in Drosophila melanogaster**

Olfaction is crucial for survival and reproduction of most animals, including localization of food, selection of mates, and avoidance of noxious stimuli. Although odorant binding proteins (OBPs) have been implicated in insect olfaction, the precise functions of individual members of this multigene family are poorly understood. To assess whether different OBPs follow similar or distinct evolutionary trajectories and to evaluate to what extent polymorphisms in members of this family are associated with natural variation in olfactory behavior, we analyzed the gene sequences of two clusters of Obp genes, the Obp99 cluster and Obp56 cluster, in 50 inbred lines from a natural Raleigh population of Drosophila melanogaster, as well as Drosophila simulans and Drosophila yakuba as outgroups. A total of 213 single nucleotide polymorphisms (SNPs) in 4 Obp99 genes and 219 SNPs in 9 Obp56 genes were identified. Molecular evolutionary analyses using both Tajima’s D and Fu and Li’s D and F statistics and the McDonald and Kreitman test reveal a significant departure from neutrality in some, but not all Obps, suggesting that different OBPs have evolved along different evolutionary trajectories. Genetic variation in behavioral avoidance responses to the odorant, benzaldehyde, was assessed among these 50 lines and significant phenotypic variation was observed in both sexes. Preliminary analysis shows that 18 SNPs located in all Obp99 genes are associated with variation in olfactory behavior and no associated SNPs were found in Obp56 genes.

**Effect of Environmental Temperature on Morphology and Behavior of Postnatal Rats**

Villarreal, J. A., Schlegel, W. M., & Prange, H.D.

Norway rats are widely distributed throughout greatly diverse environments from the tropics to the arctic. Environmental air temperature experienced during postnatal life influences the development of body morphology and thermal preference. To quantify the relation, rats were housed from birth to adulthood in either cool (17 degrees Celsius), standard lab (25 degrees Celsius), or warm (33 degrees Celsius) environments. Body morphology measures were recorded weekly and thermal preferences were measured every 3 weeks using a 2-choice test (cool 17 degrees Celsius air vs. warm 33 degrees Celsius air). The results from these experiments indicated that the thermal environment effected the development of body morphology measures, including body mass, tail length, and ear length, p < .01. In addition, rats housed in different thermal environments displayed differences in the establishment and maintenance of air temperature preferences, p < .01. These results quantify how the thermal environment shapes morphology and behavior of developing rats.
Effects of AMPA-kainate Receptor Blockade in Anterior Cerebellar Cortex on Eyeblink Classical Conditioning

Eyeblink classical conditioning is a simple form of motor learning for which the cerebellum is critical. The cortex and interpositus nucleus of the cerebellum interact with each other and form a network upon which acquisition and performance of the conditioned eyeblink response is dependent. A multitude of studies, using a variety of techniques, have helped to reveal specific critical contributions made by the interpositus nucleus. Although several areas of cortex are known to be involved in learning the conditioned response, the extent to which these areas are involved, and their specific contributions to learning remain largely unclear. One area of cerebellar cortex, the anterior lobe, has been implicated in response timing and Purkinje cells in this area respond preferentially to short and long inter-stimulus intervals. It is hypothesized that AMPA receptor mediated LTD at the Granule Cell/Purkinje Cell synapse is the mechanism of action underlying cortical contributions to response characteristics such as timing. Here we reversibly inactivate the anterior lobe with the AMPA antagonist CNQX, and employ an inter-stimulus interval shift procedure to investigate the role that the cortical excitatory system plays in various aspects of response acquisition, including timing and time shifting. Data suggests that the AMPA system of the anterior cortex may not be responsible for response timing or time shifting. However, the system appears necessary for normal acquisition.


Over the past several decades, a number of captive animal settings have begun to focus more on the use of environmental enrichment to promote the “well-being” of their animals, (Markowitz and Aday, 1998). One of the purposes of environmental enrichment is to produce more naturalistic behaviors on the part of the captive animal(s), (Mellen & MacPhee, 2001). The purpose of the current study was to examine the use of a preference assessment in determining potential items to be used as food enrichment. 8 food items were initially run in a paired-choice preference assessment, similar to the procedures used by Fisher et al. (1992). The preference assessment was conducted with 4 species of lemur, and then tested as enrichment for 2 of the 4 species: Ring-tailed and collared lemurs. During the enrichment condition, items were split into low- (LP) and high-preferred (HP) items, and tested in bamboo feeding devices. In general, both LP and HP produced greater activity and area use in both species compared to baseline, and HP produced greater effects than LP. The results suggest that paired-choice preference assessments could be used to systematically evaluate potential environmental enrichment, thereby increasing the likelihood of discovering successful enrichment strategies.
From Neurons to Intentions: the case of macaque mirror neurons

Recent studies of so-called mirror neurons raise puzzling questions about the function of these neurons for macaque monkeys. Neuroscientists and philosophers have suggested that these neurons are very important for social cognition or "theory of mind" in humans and monkeys. But monkeys have generally failed on behavioral tasks related to "theory of mind", raising a difficulty for our understanding of the functions of monkeys' mirror neurons. I will briefly describe some of the latest findings and discuss their implications for the evolution of social cognition in primates.

Contextual Alterations Eliminate the Appetitive-to-Aversive Transfer Impairment in Phenytoin-treated Rats

We have shown previously that the antiepileptic phenytoin impairs transfer in an instrumental learning task (Banks et al., 1999). The present study examined the effects of relatively minor contextual alterations on appetitive-to-aversive transfer performance of rats treated with either phenytoin or tang. Adult rats were tested in tone-signaled appetitive and aversive instrumental tasks, where the animal bar-pressed to obtain a food reward (sugar pellet) or to avoid shock. Rats were trained on the appetitive task for 31 days. Beginning on the 21st day, rats were gavaged with either phenytoin or tang twice daily. Animals were then transferred to aversive training, with the phenytoin or tang treatment continuing throughout the 25 testing days. For some animals, contextual changes were introduced as they shifted from appetitive to aversive training, while for other animals these changes were not made. Phenytoin-treated rats that were presented with changes in context as they transferred from the appetitive to the aversive task learned the avoidance response to levels substantially higher than drug-treated rats not presented with the contextual changes. These results indicate that phenytoin impairs avoidance learning following transfer from the appetitive task, and that this impairment can be eliminated by introducing changes in context at the point of transfer. In the tang-treated control subjects, on the other hand, there was no improvement in transfer learning performance associated with the changes in contextual cues. This pattern of results suggests that contextual encoding processes in rats being trained in an instrumental appetitive-to-aversive paradigm are dramatically affected by phenytoin.
Antagonism of Corticotropin-Releasing Factor Type 2 Receptors (CRF-R2), but not CRF-R1, Reduces the Acquisition of Conditioned Defeat

In male Syrian hamsters (Mesocricetus auratus), social defeat produces a striking change in agonistic behavior that we have termed conditioned defeat. This change is characterized by marked increases in submissive/defensive behavior and a complete loss of territorial aggression. Previous research has shown that corticotropin-releasing factor (CRF) and the urocortins modulate the learning and memory of stressful events. In this study, we tested the hypothesis that a non-selective CRF receptor antagonist (D-Phe CRF(12-41)), a selective CRF-R2 antagonist (antisauvagine-30), and a selective CRF-R1 antagonist (CP-154,526) would reduce the acquisition of conditioned defeat. In Experiment 1 we infused D-Phe CRF(12-41) (0 µg or 25 µg in 3 µl vehicle), in Experiment 2 we infused antisauvagine-30 (0 µg or 10 µg in 3 µl vehicle), and in Experiment 3 we infused CP-154,526 (0 µg, 10 µg, or 20 µg in 3 µl vehicle). All drugs were infused into the lateral ventricle 30 min prior to the initial social defeat. In all experiments, animals were tested for conditioned defeat 24 h later in their own home cage with a non-aggressive intruder. Infusions of D-Phe CRF(12-41) and antisauvagine-30 significantly reduced conditioned defeat, however infusion of CP-154,526 did not. Our results suggest that CRF-R2 receptors modulate the acquisition of conditioned defeat and are consistent with the view that CRF receptors are involved in the learning and memory of stressful events. This study was supported by NIH F32 MH072085 to MAC, NIH MH62044 to KLH, and in part by the NSF under agreement #IBN-9876754.

Anthropomorphism in Victorian Science

Social and cultural forces are instrumental in shaping the framework in which science takes place. A proper understanding of those forces is vital to fully comprehending scientific research in a given period. The Victorian Era in England was a unique melding point of religious and scientific ideologies. The blend of scientific and religious ideas had a key impact on the direction of Victorian science. This makes discerning the social forces at work in Victorian science a prerequisite to understanding the goals and means of scientific research during this time. Research dealing with animal cognition and evolution was especially influenced by Victorian ideas about the relationship between animal and man. Looking at anthropomorphism and related concepts in Victorian England allows us to increase our understanding of the research at the time.
Serotonin (5-HT)2A and 5-HT2C/B Receptors Differentially Regulate 3,4-methylenedioxymethamphetamine-Induced Changes in Behavior and Striatal Single-Unit Activity in Freely Moving Rats

Like amphetamine, a locomotor-activating dose of 3,4-methylenedioxymethamphetamine (MDMA) predominantly excites striatal single-unit activity in freely moving rats. Although both D1- and D2-like dopamine (DA) receptors play important roles in this effect, MDMA, unlike amphetamine, strongly increases both DA and serotonin (5-HT) transmission. To investigate the 5-HT receptor mechanisms underlying the striatal effects of MDMA, we recorded the activity of >200 single units in the striatum of awake, unrestrained rats in response to acute MDMA administration (5 mg/kg) combined with the selective blockade of either 5-HT2A or 5-HT2C/B receptors. Prior administration of SR-46349B (a 5-HT2A antagonist; 0.5 mg/kg) blocked nearly all MDMA-induced striatal excitations, which paralleled its significant attenuation of MDMA-induced locomotor activation. Conversely, prior administration of SB-206553 (a 5-HT2C/B antagonist; 2.0 mg/kg) had no effect on the amount of MDMA-induced locomotor activation or the distribution of single-unit responses to MDMA. However, a coefficient-of-variation analysis indicated significantly less variability in the magnitude of both MDMA-induced neuronal excitations and inhibitions in rats that were pre-treated with SB-206553 compared to vehicle. Analysis of concurrent single-unit activity and behavior confirmed that MDMA-induced striatal activation was not merely due to behavioral feedback, indicating a primary action of MDMA. These results support and extend our previous findings by showing that 5-HT2A and 5-HT2C/B receptors differentially regulate the expression of MDMA-induced behavioral and striatal neuronal responses, either directly or through the modulation of DA transmission.

Reciprocal altruism in an experimental collective action

Subjects participated in a one-shot, double-blind experimental economic collective action (public good game), in order to determine if their cooperative behavior conformed to the predictions of Trivers’ theory of reciprocal altruism. Subjects were given a private endowment of tokens (redeemable for real cash), and chose how to allocate these tokens: they could produce resources for their group by allocating to a public fund (allocations were doubled and shared equally by all members); however, they could benefit more individually by “freeriding,” i.e., by keeping their own tokens while consuming the resources produced by co-member allocations. In one treatment, subjects also had the opportunity to allocate to a punishment fund, in order to impose fines on co-members who were low contributors to the public fund. Such punishment is altruistic because while it is individually costly to administer, it produces resources (the equally-shared contributions of would-be freeriders) for all group members. Subjects could thus cooperate (1) by allocating to the public fund, and (2) by allocating to the punishment fund. Before subjects made their decisions about how much to allocate to each fund, they guessed the amounts of the maximum, minimum, and average co-member allocations to each fund. The prediction from reciprocal altruism is that when subjects expect higher allocations from co-members, they will themselves be willing to allocate highly. This prediction was supported: allocation amounts were highly positively correlated with expected co-member allocation amounts. In deciding how much to allocate to the public and punishment funds, subjects appeared to follow a decision rule that was approximately, “cooperate to the expected extent of the average co-member.”
3:15 – 3:35  COFFEE BREAK

3:35 – 4:00  Awards Ceremony - Dr. Emila Martins

Introduction of Keynote Speaker by Hanna Kolodziejski

4:00 – 5:00  Keynote Address
Dr. Carl D. Hopkins
Cornell University, Department of Neurobiology and Behavior

Temporal Coding and Communication: a behavioral neurobiologist's perspective on electrical communication in fish
Indiana University, Department of Psychology, Program in Neural Science

*Neuronal Activity in Nucleus Accumbens Core and Shell of High Alcohol Drinking Rats During Operant Responding for Sucrose, Ethanol, or a Mixed Solution*

Increasing evidence indicates that the nucleus accumbens (NAcc) is part of a neural circuit that is important for behaviors associated with both natural and drug rewards. Additionally, the mesolimbic dopamine system, which innervates the NAcc, appears to contribute to the reinforcing effects of ethanol (EtOH) (Samson et al., Ann. NY Acad. Sci., 654 (1992) 242-53). Furthermore, a majority of NAcc neurons show changes in firing rate in relation to the operant response, tone stimulus, and EtOH delivery in a self-administration paradigm (Janak et al., Brain Res. 817 (1998) 172-184). The NAcc is a heterogeneous structure that can be divided into distinct subregions, the core and shell. These subregions differ in density of cortical afferents and, more importantly, differ in efferents (Zahm & Brog, Neurosci, 50 (1992) 751-767). To assess the neural substrates of responding for natural and EtOH reward, we recorded core and shell neuronal activity, using microwire bundles, in high alcohol drinking (HAD) rats responding for 10% solutions of sucrose and EtOH and 5% sucrose - 5% EtOH solution. Each rat began 10 days of recording with 2 days of responding for 10% sucrose solution and ended with 2 days of responding for 10% EtOH with six intermediate days of responding for a sucrose-EtOH mixture. Our data indicate that core and shell units are more responsive to the nosepoke cue in the 10% EtOH and 5% sucrose - 5% EtOH paradigm than in the 10% sucrose paradigm. Both core and shell units are highly responsive to reward onset in all paradigms. Interestingly, although the proportion of excitations and inhibitions differs between core and shell and between paradigms, responses were predominantly inhibitions to EtOH in both subregions. Collectively, our results suggest that NAcc neurons are involved in the seeking of both natural and drug reward. This work was supported by NIAAA (U01AA-13517).

2. John V.K. Pulliam, Paul M. Plotsky  
Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta GA.

*The Expression of Cocaine and Amphetamine Regulated Transcript Peptide (CART) in the Socially Defeated Male Long Evans Rat*

Exposure to extreme stressors may either enhance or reduce the hypothalamic-pituitary-adrenal and behavioral responses to subsequent stressors. These effects can also be observed at the central level via alterations in the number of neurons positive for the immediate early gene, cFos-ir. Recently, CART peptide has been implicated to have a role in anxiogenic behavior in rodents. The effects of an ethologically relevant psychological stressor can be assessed in rats using social defeat. The aim of this study was to examine the expression pattern of CART peptide in the brain of socially defeated male Long Evans rats. In our study, 3 month-old male resident rats were paired with virgin females for one week to establish territory of the resident. Following this week, a 2 month old novel intruder male rat was exposed to the male resident in his home cage for 10 minutes. Immediately following this defeat, the intruder was separated from the resident by a partition for an additional 10 minutes. During this period of separation, the intruder experienced psychosocial stress via exposure to visual, olfactory and vocal cues from the resident. The control group consisted of rats experiencing cage transfer. One hour after the separation, rats were euthanized, perfused and the brains removed. Immunohistochemical analysis for c-Fos and CART peptides in brain regions of animal is currently underway. (Supported by NIH Conte Center MH58922, American Psychological Association Minority Fellowship Program NIH # 5,T32, MH18882 & The Center for Behavioral Neuroscience (NSF agreement #IBN-9876754), Atlanta GA
3. Ian Hall
Indiana University, Department of Biology

**The Serotonin Releaser Fenfluramine Alters Auditory Responses in the Inferior Colliculus**

As in other sensory systems, the neuromodulator serotonin influences neural processing in the auditory system. Previous experiments establishing the effects of serotonin on auditory midbrain neurons have used iontophoresed serotonin, bypassing the endogenous network of serotonergic fibers. Here we explore the contribution of endogenous sources of serotonin in the inferior colliculus (IC) of the Mexican free-tailed bat by iontophoretically applying fenfluramine, a releaser of serotonin. Fenfluramine had effects that were broadly similar to those previously reported for iontophoresed serotonin. Fenfluramine changed the spike counts of 27 out of a sample of 71 IC neurons, usually decreasing (24 neurons) but occasionally increasing (3 neurons) spike counts in response to tone bursts or linear FM sweeps. For 20 neurons to which both fenfluramine and serotonin were applied, 12 showed similar responses to both, 5 responded more to serotonin, and 3 responded more to fenfluramine. In addition, fenfluramine had effects on response latency similar to those of serotonin. Fenfluramine changed the first response latency in 17 out of 71 IC neurons, usually increasing (11 neurons) and occasionally decreasing (6 neurons) the time to first response to auditory stimuli. One difference in the effects of fenfluramine and serotonin was in the timecourse of the response. Seven neurons showed a peak and subsequent decline in the effects of fenfluramine, something not seen with serotonin iontophoresis. This suggests that fenfluramine depletes serotonin in some cases, a hypothesis supported by the fact that, after responding to initial application of fenfluramine, several neurons became unresponsive to subsequent applications. These results are consistent with the hypothesis that locally iontophoresed fenfluramine releases serotonin within the IC, facilitating a comparison of the dynamics of endogenous and exogenous sources of serotonin.

4. Rebecca Dagley and Roderick A. Suthers
Indiana University, Department of Biology

**Song Production in Northern Cardinals**

The syrinx is a bipartite vocal organ that is unique to birds. Each half of the syrinx is able to produce sound independently and the two sides can be used either simultaneously or sequentially by a singing bird. Northern Cardinals use the two syringeal halves sequentially to produce broadband frequency modulated sweeps, which can span a large range of frequencies. The left half of the syrinx produces frequencies below 3.5 kHz and the right produces frequencies above 3.5 kHz. It has not previously been determined if the syrinx halves are restricted to phonating in these frequency ranges or if the entire frequency range can be produced on a single side. We impaired one side of the syrinx (either right or left) throughout the first year of development to determine if individuals can produce the entire frequency range using only the intact half of the syrinx.
Few field studies have investigated how circulating levels of antibodies vary throughout the year. Of particular interest is how immunity may vary across the breeding season, and its potential relationship to energetic trade-offs among immune function, reproductive effort (e.g., incubation and nestling feeding) and survival. Also of interest is the role of testosterone (T) in mediating these potential trade-offs. The Dark-eyed junco (Junco hyemalis) displays seasonal variation in T levels, with the highest levels during the pre-breeding period (territorial establishment and pair-formation), and the lowest levels during nestling rearing. In our current investigation, we tested the hypothesis that trade-offs between reproduction and immune function occur in juncos and are mediated by T. Specifically, we sampled male and female juncos during different stages of the breeding season (pre-breeding, nestling feeding, and post-breeding), and determined circulating levels of Immunoglobulin G (IgG) (the primary circulating antibody) and T. We found that IgG levels were lowest during the pre-breeding season and high during nestling feeding, with the highest levels occurring in the rearing of a second brood (after successfully raising one brood). Interestingly, this pattern is opposite of what has been previously reported for circulating T levels, which are typically high during pre-breeding. These results support the notion of a trade-off between reproduction and immune function and may indicate a possible mediating role for T, in the re-allocation of finite energy reserves away from competition and reproduction and back to individual survival during the latter portion of the breeding season. The relationship between T and IgG levels and the possible role of T in mediating changes in immune investment across the breeding season will be discussed.
Variation in territory quality can have important fitness consequences. Although this has been well established at the population level, we currently have little information about the underlying physiological mechanisms that mediate the effects of habitat heterogeneity on reproductive success and survival. In this study, we investigated several physiological mechanisms that may be important in regulating variable reproductive success during the nestling phase of Florida Scrub-Jays, a territorial resident songbird that inhabits fire-adapted xeric oak scrub mosaics. We measured growth rates, multiple indices of body condition, hematocrit, and immune responsiveness (via phytohemagglutinin [PHA] challenge) in nestlings inhabiting “too short,” optimal, and “too tall” territories, as assessed by multiple-scale habitat measures and previous demographic studies. Controlling for nest/family effects, chick order, and body condition, we found that Florida Scrub-Jay nestlings inhabiting optimal territories had significantly higher immune responses than nestlings in “too short” or “too tall” territories. Though there were significant effects of territory quality on body condition, post-hoc differences were non-significant, and body condition was not significantly correlated with PHA response. These results suggest that nestling immune function may be an important mechanism underlying differences in reproductive output among Florida Scrub-Jay territories. Several hypotheses may explain the observed variation in immune function among territory types including variable antibody/pathogen exposure of adults or nestlings and/or variation in energetic constraints (e.g. food, microclimate) not captured by traditional body condition indices.

Characteristics of social defeat associated with immune response in Siberian hamsters (Phodopus sungorus)

Previous human and animal research has linked physical and psychological stress with changes in immune function. Animals treated with keyhole limpet hemocyanin (KLH) have displayed significantly less serum antibodies to KLH and an elevation of serum cortisol concentrations when the animals have experienced a social stressor. The present study sought to determine which characteristics of the social stressor, defeat associated with territorial defense, were contributing to the fluctuation of immune response in male Siberian hamsters (Phodopus sungorus). Experimental animals were characterized as KLH-immunized hamsters which acted as intruders in one of four conditions: 1.) a clean cage, 2.) a dirty cage without the resident present, 3.) a partitioned cage prohibiting direct contact of the intruder and resident, or 4.) direct contact with the resident. Each session lasted 15 minutes for five consecutive days. It was predicted that a greater severity of social defeat/stress (i.e. the most direct interaction) would have the greatest effect on immune response. Blood samples were analyzed using an ELISA procedure, and the results suggest that serum cortisol concentrations and KLH antibody levels are most significantly affected in animals experiencing the most direct contact and the greatest level of defeat and stress. These data establish the level of contact/interaction as a key characteristic of social defeat in affecting immune response.
Indiana University, Department of Biology, Program in Neural Science and Center for the Integrative Study of Animal Behavior

**Role of Dehydroepiandrosterone (DHEA) in Mediating Photoperiodic Changes in Aggression in Male Siberian Hamsters**

Among the suite of seasonal adaptations displayed by non-tropical rodents, some species demonstrate reproductive regression and increased territorial aggression in short compared with long day lengths. The finding of decreased aggression is particularly intriguing given that circulating levels of testosterone (T) are basal in short days. Although short-day increases in aggression can be mimicked by administration of short-day-like patterns of melatonin, the precise physiological mechanisms mediating short-day or melatonin-induced increases in aggression remain largely unknown. Recent evidence has suggested that adrenocortical rather than gonadal hormones mediate aggression during the non-breeding season (i.e., winter or short days). For example, both short-day and melatonin-induced increases in aggression can be attenuated by bilateral adrenalectomy. Furthermore, circulating concentrations of the adrenal androgen dehydroepiandrosterone (DHEA) are correlated with territorial aggression in some species. The goal of the present study was to examine the role of DHEA in mediating photoperiodic changes in resident-intruder aggression in male Siberian hamsters (Phodopus sungorus). Specifically, hamsters were housed in long “summer-like” days (LD 16:8) or short “winter-like” days (LD 8:16). Half of the hamsters from each group received Silastic capsules filled with DHEA whereas the remaining animals received empty capsules. Four weeks later, hamsters were tested using a resident-intruder model of aggression and the number and duration of attacks, and latency to initial attack were recorded. Results from these behavioral tests, as well as DHEA concentrations, will be presented. Collectively, the results of the present study should shed light on the potential role of adrenal androgens in mediating seasonal changes in aggression in this species.

10. Sayuri Kojima  
Indiana University, Department of Psychology

**A Sensitive Period for Forming Odor-Based Huddling Preferences in Rat Pups**

Rat pups huddle vigorously with sources of heat, whether animate or inanimate. By Day 15, however, pups demonstrate social bias by huddling preferentially with conspecifics. Previous studies from our laboratory demonstrated that pups learn to huddle preferentially with an object bearing an odor previously paired with a thermotactile stimulus. Here we hypothesized the existence of a sensitive period for the development of odor preference. We examined two, 10-day-long periods of odor conditioning: Days 1-10 and Days 5-14. Each day, groups of 4 pups received either of two types of odor exposure: odor paired with dam on odd days and mere exposure on even days (or vice versa). Huddling preference tests (paired odor vs. odor of mere exposure) were conducted on Day 15. Pups in both groups developed a bias for the odor paired with a foster dam. Contact time to the paired odor was statistically equivalent in the two conditioning groups. However, physical contact time by pup(s) in the Day 5-14 group to the odor of mere exposure was significantly greater than that in the Day 1-10 group. These results suggest that early exposure to an odor paired with a dam is not critical for odor preference on Day 15. Instead, pups may retain memory of an odor preference developed during the thermally-vulnerable period and develop another odor preference, possibly directed by non-thermal stimuli as they mature.
Ethological assessment of a murine knock-in model of Huntington’s disease

Huntington’s disease (HD), which results in degeneration of the striatum and corticostriatal pathway, is an inherited, autosomal dominant condition caused by an unstable, expanded trinucleotide (CAG) repeat (HD Collaborative Research Group, Cell. 72 (1993) 971-983). Symptoms include personality change followed by gradual onset of adventitious movements. Development and assessment of behavior and neural function in transgenic and knock-in models of HD provide a way to understand the neural mechanisms of HD. More importantly, these models enable pre-clinical evaluation of possible treatments. Currently, the most characterized HD model is the R6/2 line expressing a transgene that includes the huntingtin promoter region and ~150 CAG repeats. At six weeks of age, R6/2 mice develop a progressive neurological phenotype similar to that observed in humans. Mice expressing the HD gene also develop learning deficits and are particularly impaired on tasks assessing spatial learning. A newly developed knock-in model with 140 CAG repeats develops the disease later and with a longer progressive course than the R6/2 line. Although the phenotype observed in the 140 model may be more typical of adult HD, this model has not been well characterized. In this study we evaluate male and female 140 CAG mice and litter mate controls using rotarod and open-field assessments of behavior. Initial results show lower scores on the rotarod test in the knock-in animals compared to controls from 13 to 30 weeks of age. Knock-in mice assessed between 16 to 19 weeks of age demonstrate a lower percentage of time spent rearing in the open-field. Other assessed behaviors (jumping, locomotion, digging, climbing, resting, grooming, sniffing, and scratching) were not significantly different between knock-ins and controls. Future research will help determine age of onset and progression in the 140 CAG model. Supported By: NINDS R01 NS35663 (GVR); NSF Graduate Research Fellowship (JLD)

Chemotactic response of mouse sperm toward extracts from female reproductive organs

Rodents use chemical signals as one of their primary means of communication, and chemical substances from an individual are known to influence both the behavior and physiological conditions of other individuals. Examples of physiological influences include male odors eliciting estrus in females and female odors suppressing estrus in other females. Chemicals from an individual can also have a direct influence on gametes of the opposite sex. For example, chemicals released from the female reproductive organs, e.g., the uterus and ovary, can enhance, suppress, and determine the direction a sperm cell should swim. This latter category of response by sperm to chemical substances from the female reproductive organs is called sperm chemotaxis. In this study involving sperm chemotaxis, extracts from the uterus and ovary of superovulated females were prepared and used as an attractant for sperm. On microfluidic devices, sperm were exposed to the extracts and exhibited a strong chemotactic response in certain concentration ranges. In addition, an aliquot from each extract was analyzed by gas chromatography-mass spectrometry (GC-MS) to determine the chemical composition of the extracts. These early results are very promising and could yield valuable information of the role of chemotaxis in the reproductive biology of animals.
Comparative Investigation of the Volatile Urinary Profiles in Different Phodopus Hamster Species

Stir bar sorptive extraction method, combined with gas-chromatography/element-specific detection gas chromatography/mass spectrometry, was used for investigation of the urinary volatile profiles in male and female Phodopus campbelli and Phodopus sungorus hamsters. Additionally, Phodopus roborowsky urinary profiles of females were briefly characterized. A quantitative analytical approach allowed a comparison of 17 selected compounds. The results indicated that campbelli and sungorus species show qualitatively similar urinary volatile profiles for males and females. However, statistically significant quantitative differences were observed. Several unique compounds, such as pyrazine derivatives, were found to be gender- and age-specific. Methylaniline and phenylacetonitrile were age-specific in both male hamster species. The individual compound variations within the P. sungorus were clearly larger than in the P. campbelli species. The urinary profiles of roborowsky female hamsters were dominated by ketones, alcohols and esters. Similar pyrazine arrays, as seen in campbelli and sungorus, were clearly not observed. Instead, less volatile alkyl quinoxalines were detected.

Determination of Putative Chemosignals of the Ferret (Mustela furo) by Stir Bar Extraction and Gas-Phase Analytical Techniques

The domestic ferret (Mustela furo) belongs to genus Mustela which exhibits a predatory behavior. Along with the ferret, other Mustela species such as mink (M. vision) and European polecat (M. putorius) are known for their strong odors emitting through the anal glands. The purpose of such odors can be chemical communication within the species (pheromones) and with other species (e.g., defense chemical signals). Urine is yet another common source of chemosignals, especially in carnivores. Some Mustela species appear to use urine scent marking for sex recognition. The chemical composition of ferret urine has been relatively unknown. Understanding predator chemical signals is potentially important in utilization of the innate chemical signaling compounds for predator population control in Nature and control of populations of their prey (e.g., rodents). In this study we have compared female and male ferret urine and anal gland volatile compound profiles by gas chromatography (GC) combined with mass spectrometry (MS) and element specific detection. A novel approach, stir bar extraction in the aqueous and headspace mode, has been used in conjunction with gas-phase analytical methods. The stir bar methodology provided additional information on the volatile profiles in ferret urine and gland secretion: ten new glandular volatile compounds were detected, including aldehydes, ketones, 2-methylquinoline and 4-methylquinazoline. Statistically significant differences were found in the volatiles between male and female urine, suggesting that urine chemical signals are facilitating gender recognition. Male- and female-specific compounds may possess pheromone activities inducing behavioral and physiological changes in ferrets.
Stir Bar Sorptive Extraction: a New Quantitative and Comprehensive Sampling Technique for Determination of Chemical Signal Profiles from Biological Media

Various applications of a novel sampling procedure using sorptive stir bar extraction (SBSE) in chemical ecology are demonstrated. This methodology has been demonstrated through the examples of small-volume mouse urine samples, rat glandular tissue volatiles and the air blown through animal cages. Its analytical merits are compared with those of the previously established purge-and-trap (dynamic headspace) technique.

The novel sorptive extraction methodology with a polydimethylsiloxane (PDMS) coated stir bar has been proven to be highly reproducible and linear in the aqueous sampling of volatiles and semivolatiles from mammalian urine and tissue. This technique allows the use of relatively small sample volumes (100 l). In addition, the method was found suitable for the extraction of small organic animal odor-related compounds in air samples in the dynamic sampling mode. Equilibrium properties of the PDMS material facilitated multiple sample extractions simultaneously and significantly increased the analysis throughput. Efficient extraction and thermal desorption properties of the PDMS phase also produced previously unseen semivolatile compound patterns in the sample profiles, as demonstrated with the mouse and hamster urine samples.

Does tetrodotoxin act as a chemical defense against predation in newt (Taricha granulosa) eggs?

Antipredator defenses include behaviors to evade capture, physical structures to deter predation, and noxious or toxic chemicals aimed at predators. While adults are often well protected, early life history stages (e.g., eggs) may be more vulnerable. In these cases, parents may provide defenses either through their behavior or through chemicals provided to the embryo. Nest defense behaviors have been well studied, but relatively little is known about chemical defenses in eggs. Studying the defenses of early life history stages is particularly interesting because of the influence early survivorship can have on population growth rates. Rough-skinned newts, Taricha granulosa, possess tetrodotoxin (TTX), a neurotoxin that inhibits the propagation of nerve signals, leading to paralysis and potentially death in most organisms that ingest it. TTX acts as a chemical defense against predatory garter snakes in adults, and is also present in newt eggs. My work has shown that caddisfly larvae prey upon newt eggs, despite the presence of TTX. Because adults do not defend eggs, TTX may be eggs' only defense against predation. It was previously unknown to what degree caddisfly larvae consume eggs of varying toxicity and how TTX affects them. Here I report the results of two studies examining caddisfly predation on newt eggs, one performed in the field and one performed in the laboratory. These studies suggest that predation by caddisfly larvae can be an important factor in the survival of newt eggs.
Frog declines: exploring connections among climate change, behavior, development, immunity and disease.

For the past 16 years, marked declines have been recorded for 40% of the frog and toad species in the Monteverde Cloud Forest Preserve (MCFP), Costa Rica. Climate change and disease serve as two strong hypotheses to explain these declines. To explore possible connections among climate change, immunity and disease susceptibility, our research examines how tadpole perception of climate-induced changes in pond water levels may influence metamorphic rate and immune system development. We examined immune development and function in meadow treefrogs (*Hyla pseudopuma*), a metamorphically-plastic and declining species in the MCFP, when tadpoles developed under various water regimens. During the wet seasons of 2000-2004, laboratory experiments were conducted in the MCFP by subjecting tadpoles to a constant (8cm) or sharp decline in water level (2cm). Field studies took place in man-made and natural forest ponds that experienced different rainfall and pond water retention patterns. Our studies suggest that *Hyla pseudopuma* tadpoles perceived variation in pond water level as they developed. In amphibian species that breed in ephemeral ponds, this neuronal perception has been shown to ultimately lead to increased levels in corticosterone and thyroid hormones, synergizing to cause precocious metamorphosis. In addition to hastened developmental rates, both *Hyla pseudopuma* tadpoles and 1 month-old froglets had a blunted ability to reject foreign tissue, suggesting a negative impact not only on the function but also the development of their immune system. Collectively, these results may have important implications concerning climate change effects on amphibian populations, as well as the possible role of immunosuppression and increased disease susceptibility in species declines.

Mobility costs of secondary sexual traits in the horned beetle *Onthophagus nigriventris*

Aggressive fighter and non-aggressive sneaker morphs are common among species with alternative male phenotypes in a population with intense sexual selection. In the beetle genus *Onthophagus* there is a size-dependent expression of horns in males, resulting in two distinct male morphs: large horned and small hornless male phenotypes. Large horned males use fighting behavior to gain access to females while small hornless males rely on a high degree of mobility to sneak copulations inside breeding tunnels. Previous research has shown that horn possession improves male fighting success. However, it remained unclear why small males develop only rudimentary horns. Here we test the hypothesis that lack of horns improves male maneuvreability inside tunnels. We use a straightforward behavioral assay to quantify male maneuvreability as a function of male size and presence/absence of horns. We show that hornlessness significantly increases male maneuvreability in small males and large male whose horns had been removed. In contrast, large horned males, and males whose horns had been removed and then re-attached exhibited a significant mobility handicap. We observed no such differences when comparing small and large females who are hornless regardless of size. Our results suggest that hornlessness, rather than just small body size, significantly improves male maneuvreability inside tunnels, which in turn may enhance male sneaking performance. We discuss our results in the context of the behavioral ecology of horn-polyphenic beetles.
19. Stephen Crowley  
Indiana University, History and Philosophy of Science

**How do you know you don't know: What Uncertainty Monitoring can teach Epistemology**

Recent work (See Smith et al. 2003 for survey and discussion) has established uncertainty monitoring in dolphins, macaques and humans. This work has important implications for epistemology. Smith et al. (1997) trained humans and macaques on a visual discrimination task with 3 choices. The subject could identify the visual field as either possessing or lacking a certain property *or* the subject could decline the trial. Given that the macaques' performance on the task very closely matched human performance and that humans accounted for their performance in terms of an awareness of uncertainty, the experimenters take this to be good evidence that the macaques' responses were also indications of uncertainty. What are the implications of this result for philosophic inquiry into the nature of knowledge? A major issue in epistemology concerns what is added to true belief to convert it into knowledge. Two major lines of investigation have been pursued. On the one hand, there is the idea that the crucial ingredient of knowledge is the reliability of the mechanisms that match the organism's beliefs to the external world. On the other hand, there is the idea that some degree of internal cognitive access to a process of justification is crucial. Typically those who think this require that the agent have access to their reasons for believing the item of knowledge being considered. This position requires intensional ascent, that is, the ability to represent mental representations. Uncertainty monitoring however suggests the existence of epistemically relevant meta-cognition without intensional ascent. This work therefore highlights the possibility of a new philosophic perspective on knowledge, a modest internalism, and provides unusually clear evidence for both the nature of such a capacity and its epistemic value. In summary, integrating work in philosophy and animal cognition generates valuable insights into the epistemic value of meta-cognition.

**Works Cited:**


20. Tim Linksvayer  
Indiana University, Department of Biology

**Direct and indirect genetic effects on mass and caste in an ant**

The phenotype of an individual is determined by its genes (direct genetic effects) and its environment. When the environment is influenced by social partners, genes expressed in these social partners also influence an individual's phenotype (indirect genetic effects). In this case, the environment has a genetic basis and can evolve. In social insects, the social environment is determined by interactions with parents, sibling adults, and sibling brood. For example, maternal care, care by sibling adults, and competition with sibling larvae, all affect the social environment of a developing larva. Thus, a variety of indirect genetic effects, as well as direct genetic effects, and interactions among all of these effects, influence traits expressed by social insects and are likely to impact the evolutionary dynamics of these traits.

I report the preliminary results of a study designed to determine the relative importance of these varied genetic effects on gyne, worker, and male mass, and female caste in the acorn ant *Temnothorax curvispinosus*. Significant proportions of among-colony variance (i.e., heritability) were found for all treatments for mass and caste. Estimates of the variance in sib-social effects and maternal effects were similar to estimates of the variance in direct effects. This study demonstrates the potential evolutionary importance of both direct and indirect effects on individual and colony traits in social insects.
Reception 7:00 – 9:00 at the Suthers home.

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Turn right on Bender Road (4 miles past College Mal Road, and 2 miles past SR-446)

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