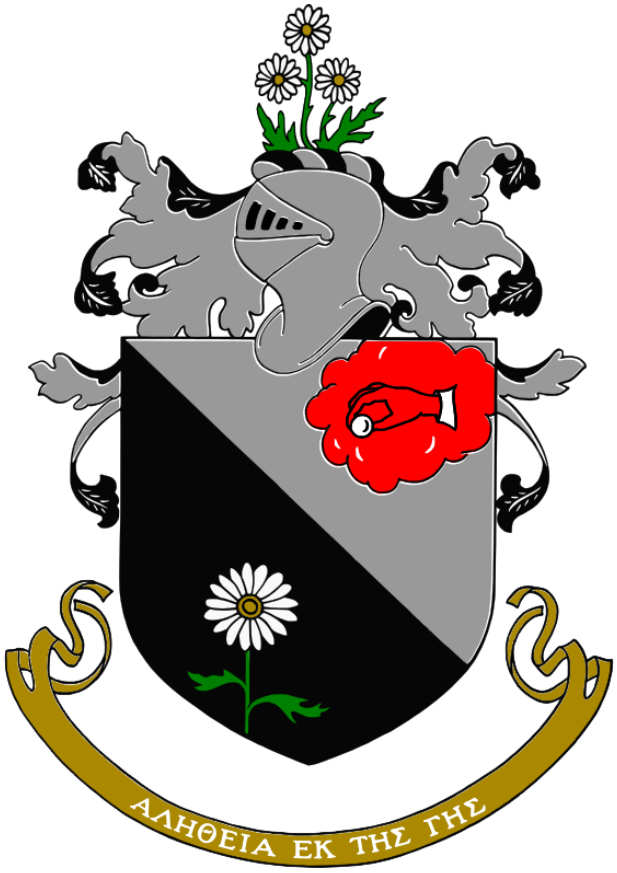
15th Annual Research Symposium
Phi Sigma Biological Society



27 February 2015
Illinois State University

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Moderator

Scott Sakaluk

- Schedule of Events -

8:00 – 8:45 AM **REGISTRATION & CONTINENTAL BREAKFAST**
Science Lab Building First-floor Atrium

8:45 – 9:00 AM **OPENING REMARKS**
Science Lab Building 121
C. Gatto, Director, School of Biological Sciences
S. Sakaluk, Oral Session Moderator

9:00 – 10:20 AM **ORAL SESSION 1**
Science Lab Building 121

- 9:00 Amanda Carter: Turtle hatchlings show behavioral types in righting response and exploration that are robust to environmental manipulations during development
- 9:20 Molly Schumacher: Terminal investment in *Aedes aegypti* males: potential to boost sterile-male-release control methods through increased mating competitiveness
- 9:40 Chris Loebach: Experimentally measuring seed dispersal distances of garlic mustard
- 10:00 Kassandra Lee: Visual Crowding: Contributions of Part-Based vs. Holistic Representations

10:20 AM – 12:00 PM **POSTER SESSION**
Science Lab Building Atrium

12:00 – 1:30 PM **LUNCH**
Science Lab Building 121

1:30 – 2:50 PM **ORAL SESSION 2**
Science Lab Building 121

- 1:30 Kristin Duffield: Terminal investment in gustatory appeal of male decorated cricket nuptial food gifts
- 1:50 Kris McIntire: Overcompensatory response to additional mortality as an effect of behavioral change
- 2:10 Cynthia Cass: Biotic and abiotic stress tolerance of phenylalanine ammonia lyase-suppressed *Brachypodium*
- 2:30 Phi Sigma board announcements

3:20 – 3:50 PM **PRE-SEMINAR REFRESHMENTS**
Felmley Science Annex, outside room 133

3:50 – 4:00 PM **TRAVEL AWARDS**
Moulton Hall 208

4:00 – 5:00 PM **KEYNOTE ADDRESS**
Moulton Hall 208

- Keynote Address -

Dr. Jaap de Roode

Emory University

Parasite infections, toxic milkweeds and medication behavior in monarch butterflies

Monarch butterflies (*Danaus plexippus*) are commonly infected with protozoan parasites that strongly reduce monarch survival, mating ability and fecundity. Monarchs are specialized on milkweeds as their larval food plants, and our experiments have shown that milkweed species with high concentrations of cardenolides (secondary toxic chemicals) reduce parasite infection and virulence. We investigated whether monarchs can use medicinal milkweeds to prevent or cure disease, and whether monarch medication behaviors depend on parasite risk in natural populations. Our experiments suggest that infected caterpillars cannot use milkweed as medicine, but that infected female butterflies may preferentially lay their eggs on medicinal milkweeds that make their offspring less sick. Moreover, the type of medication behavior appears to depend on parasite risk in natural populations. In eastern and western North America, where parasite risk is low, only infected monarchs had a preference for medicinal plants, indicating therapeutic medication. In contrast, in South Florida, where parasite risk is high, both infected and uninfected monarchs preferred to lay eggs on medicinal milkweed. Overall, our results suggest that monarchs can use medicinal milkweeds to mitigate the negative effects of their prevalent protozoan parasites, and that the risk of parasitism determines the type of medication that monarchs use.

Moulton Hall 208
4:00-5:00 PM

- ORAL PRESENTATION ABSTRACTS -

In order of presentation schedule
* = participating in travel award competition

Oral presentations should be 20 minutes in total
(15 minutes for presentation & 5 minutes for questions)

- Oral Session 1 -

9:00 AM **Carter (Graduate)***

Turtle hatchlings show behavioral types in righting response and exploration that are robust to environmental manipulations during development

Amanda W. Carter, Ryan T. Paitz, Katie E. McGhee, Rachel M. Bowden

Consistent individual differences in behavior appear to be widespread amongst taxa; however, the mechanisms underlying behavioral phenotypes are poorly understood. This study investigated the influence of environmental manipulations during development on righting response and latency to explore an environment in red-eared slider turtle hatchlings (*Trachemys scripta*). First, we examined the effects of three environmental manipulations (estrone sulfate exposure, corticosterone exposure, and thermal fluctuations) during incubation on hatchling behaviors. Second, we determined whether hatchlings were repeatable in their righting response and latency to explore, and whether these behaviors were correlated with one another. Finally, we examined whether righting response was predictive of ecologically relevant behaviors such as habitat choice and dispersal. We found that hatchling behavior was robust to our early manipulations; none of the pre-hatch treatments affected post-hatch behavior. We found significant clutch effects, which due to a split-clutch design, suggests genetic underpinnings and/or maternal effects. We found evidence for turtle behavioral types; both righting response and latency to explore were strongly repeatable and these behaviors were positively correlated. An individual's righting response was predictive of their tendency to make a choice amongst habitats in the laboratory, but was not predictive of their dispersal ability in the field, necessitating a revision in the use of righting response as a fitness proxy. We demonstrate repeatable individual behaviors that are robust to environmental manipulations and are linked to differences in habitat choice but not to dispersal, which collectively suggest a possible explanation for the maintenance of behavioral variation within turtle populations.

9:20 AM **Schumacher (Graduate)***

Terminal investment in *Aedes aegypti* males: potential to boost sterile-male-release control methods through increased mating competitiveness

Molly K. Schumacher, Ben M. Sadd, and Steven A. Juliano

Mosquito population control currently favors environmentally benign approaches such as the release of irradiated or genetically modified (GMO) sterile males. The success of these programs is contingent on the mating success of these males, which can be negatively affected if females prefer unmanipulated males, or if the released males are not competitive in mating. Artificially increasing male competitiveness prior to release may increase their probability of mating, thus increasing the success of these control programs. Theory suggests that manipulating investment into male life-history traits, such as reproduction and immunity, influence mating competitiveness. The *Reproductive Trade Off Hypothesis* states that immune investment following infection will compromise male reproductive effort, hence competitiveness. Alternatively, the *Terminal Investment Hypothesis* suggests an infected male may enhance current reproductive effort to compensate for future lost opportunities due to the consequences of infection. Reproductive behavioral assays and binary-choice mating trials were conducted on *Aedes aegypti* males that were either naïve or immune challenged (inoculated with live *Escherichia coli*, heat killed *E. coli*, or saline control). Males inoculated with a sham control and heat killed *E. coli* achieved a statistically similar number of matings relative to naïve males. However, when exposed to live *E. coli*, males achieved significantly more matings relative to naive males, consistent with terminal investment into reproduction. This study is the first to investigate and to demonstrate mosquito terminal investment in mosquitoes, a behavior that could enhance the success of these sterile-male-based control programs.

9:40 AM **Loebach (Graduate)***

Experimentally measuring seed dispersal distances of the invasive plant, garlic mustard (*Alliaria petiolata*).

Chris A. Loebach and Roger C. Anderson

Garlic mustard, an herbaceous plant, has aggressively invaded North American woodlands, where it displaces native ground layer vegetation and reduces native species diversity. It has been extensively studied to understand its impact on native communities and for developing control methods. However, seed dispersal distances are not well known and current dispersal model parameters are based on untested observations. Experimentally measuring dispersal distances is important for predicting rate of spread, understanding invasion ability, and for improving control strategies. To measure dispersal distances, seeds traps were placed in a sector design around three point seed sources. Point sources were 15 second-year plants within a 0.25m radius circle. Sectors were placed every 45 azimuth degrees beginning at zero degrees north. Traps were placed at intervals ranging from 0.25-3.25m. Trap number per interval increased with distance to maintain constant sampling effort. Traps were in the field prior to silique dehiscence and were collected after the majority of seeds were dispersed. Eight dispersal functions describing the probability of a seed landing into an area at a specific distance were fit to seed trap counts via maximum likelihood. The lognormal and 2Dt functions had the lowest AIC scores and were selected for further analyses. Seed density rapidly declined as distance increased. The lognormal and 2Dt functions predicted a mean dispersal distance of .56 and .52m with 95% of seeds dispersed within 1.23 and 1.14m respectively. These results fill a gap in our knowledge of garlic mustard biology, which will improve our understanding of its invasive ability.

10:00 AM **Lee (Graduate)***

Visual Crowding: Contributions of Part-Based vs. Holistic Representations

Kassandra Lee and Amrita Puri

Visual crowding is the phenomenon whereby an object or feature in the peripheral visual field is more difficult to identify when other objects are nearby (Whitney & Levi, 2011). Basic characteristics of crowding include an increased effect as targets and nearby objects (flankers) increase in similarity (Whitney & Levi, 2011), and that the severity of crowding is proportional to the distance between target and flankers relative to their eccentricity (Bouma, 1970). Farzin, Rivera, & Whitney (2010) and Louie, Bressler, & Whitney, (2007) found evidence that crowding occurs within and between high-level holistic face representations and not just at a part-based level, indicating that contributions to crowding must occur at multiple levels in the visual system. Here, we test whether holistic vs. low-level (feature, or part-based) representations produce crowding independently. We surround non-face objects (cars) by face flankers that are processed either holistically or in a part-by-part manner (upright or inverted, respectively), and asked participants to perform a discrimination task on the cars (targets) using their peripheral vision. Preliminary data show better target discrimination when face flankers are upright compared to inverted, suggesting less crowding when the target and flankers engage different types of processing (holistic vs. part-based). Follow-up experiments will determine whether this result is due to reduced crowding when target and flanker processing relies on different mechanisms, or to attentional enhancement related to the presence of upright faces.

- Oral session 2 -

1:30 pm **Duffield (Graduate)***

Terminal investment in gustatory appeal of male decorated cricket nuptial food gifts

Kristin R. Duffield, James Rapkin, John Hunt, Ben M. Sadd, and Scott K. Sakaluk

Investment in current versus future reproduction is a prominent trade-off in life-history theory, and is likely dependent on an individual's life expectancy. The terminal investment hypothesis posits that a reduction in residual reproductive value (i.e. potential for future offspring production) will result in increased investment in current reproduction. We tested the hypothesis that male decorated crickets (*Gryllodes sigillatus*), when cued to their impending mortality, increase current reproductive effort by shifting the composition of their nuptial food gifts, or spermatophylaxes, in a way that increases gustatory appeal to females. Using a repeated measures design, we analyzed the amino acid composition of spermatophylaxes derived from males both before and after injection of either a saline control or a solution of heat-killed bacteria, the latter of which, although non-pathogenic, may signal an impending threat to the survival of the cricket. One principal component explaining amino acid variation in spermatophylaxes (characterized by a high histidine loading) was significantly lowered in immune-challenged versus control males. The relevance of this difference for the gustatory appeal to females was assessed by mapping spermatophylax composition on a fitness surface derived in an earlier study identifying female preference for amino acid composition of nuptial food gifts. We found that immune-challenged males maintained the level of attractiveness post-treatment, while control males produced significantly less attractive gifts post-injection. These results are consistent with the hypothesis that cues of a survival-threatening infection stimulate terminal investment in male decorated crickets with respect to the quality of their nuptial food gifts.

1:50 pm **McIntire (Graduate)***

Overcompensatory response to additional mortality as an effect of behavioral change

Kris M. McIntire and Steven A. Juliano

The addition of mortality to a population has the potential to effect the population's rate of change in one of three ways. It can be additive, compensatory, or overcompensatory. Mortality that increases the rate of population change or population size is deemed "overcompensatory". This research focuses on a hypothesized mechanism for producing counter-intuitive overcompensatory mortality effects. The Prudent Resource Exploitation hypothesis (P. Abrams, 2009) postulates that the addition of mortality causes a change in individual behavior toward more limited resource exploitation, resulting in greater resource regeneration, and consequently greater growth and overcompensation of the consumer population. The hypothesis that the response of the mosquito, *Aedes triseriatus,* in the larval stage to cues from predation causes a change in individual larval behavior toward more prudent resource exploitation, causing overcompensation was tested. This predicted that the mere threat of mortality by predation would cause individuals to adopt less risky and less productive feeding behavior, allowing the amount of resource to increase. In the absence of a threat of predation, larvae were expected to forage more actively, depleting resources. Both survivorship and behavioral data are consistent with the prediction, indicating an overcompensatory response. An understanding of the mechanisms creating the counter-intuitive concept of population overcompensation in response to mortality could prove useful in management of this medically important disease vector.

2:10 pm **Cass (Post-doc)**

Biotic and abiotic stress tolerance of *phenylalanine ammonia lyase*-suppressed *Brachypodium*

Cynthia L. Cass, Antoine Peraldi, Patrick F. Dowd, Yaseen Mottiar, Nicholas Santoro, Steven D. Karlen, Yury Bukhman, Cliff E. Foster, Nick Thrower, Laura C. Bruno, Oleg V. Moskvin, Eric T. Johnson, Megan E. Willhoit, Megha Phutane, John Ralph, Shawn D. Mansfield, Paul Nicholson, and John C. Sedbrook

The phenylpropanoid pathway in plants generates a variety of structural and defense compounds and is an important target in efforts to reduce cell wall lignin for improved biomass conversion to biofuels. Pathway manipulations can have undesired effects on plant fitness and biomass yield. To gain new insights into the phenylpropanoid pathway and related processes, we targeted by RNA interference (RNAi) *PHENYLALANINE AMMONIA LYASE* (*PAL*) isoforms in the model grass *Brachypodium distachyon*. PAL enzymes catalyze the entry step into the phenypropanoid pathway, converting L-phenylalanine to *trans*-cinnamic acid and ammonia. *BdPAL* RNAi plants were found to have large reductions (up to 85%) in transcript abundance for two of the eight putative *BdPAL* genes. Along with a 43% reduction in lignin and a nearly two-fold increase in the amounts of polysaccharide-derived carbohydrates released by thermochemical and hydrolytic enzymic partial digestion, *BdPAL*-suppressed plants' cell walls had a 57% reduction in ferulate, suggesting a shared biosynthetic route for lignin-bound monolignols and hemicellulose-bound ferulate. *PAL*-suppressed plants exhibited increased susceptibilities to the fungal pathogen *Fusarium culmorum*, but surprisingly had wild-type (WT) resistances to caterpillar herbivory, drought, and ultraviolet light. Drug studies and RNA-seq data analyses revealed that the *PAL*-suppressed plants' reduced root growth and fungal susceptibility phenotypes were partially a consequence of increased ethylene production and subsequent signaling. These data reveal that trade-offs exist in how Brachypodium, and likely other grasses, respond to challenges involving the phenylpropanoid pathway, and provide targets for enhancing the fitness of economically important grasses including bioenergy-crop grasses having reduced cell wall lignin.

- POSTER PRESENTATION ABSTRACTS -

Presenters listed alphabetically [poster number]
* = participating in travel award competition

Dissecting the molecular basis for magnetic transduction in *C. elegans* [1]*

Chance Bainbridge, Trevor Rickard, and Andrés Vidal-Gadea

The number of animals identified as capable of detecting magnetic fields has steadily increased for decades. However, little is known about the neurogenetic basis for magnetotransduction in any animal. Two proposed mechanisms attempt to explain how organisms detect and orient to magnetic fields. One of these mechanisms is light-dependent (potentially used by many birds). The second mechanism relies on permanently magnetic particles (magnetite) to detect magnetic fields. Recently we discovered that *Caenorhabditis elegans* orients to magnetic fields in a light-independent manner, supporting the idea of a magnetite-dependent mechanism. *C. elegans* is a unique model system for studying transduction mechanisms as it is highly genetically tractable.

We isolated magnetic particles from the tissues of *C. elegans* and used mass spectrometry to identify proteins enriched in magnetic isolates. We hypothesize that some of these proteins will be part of the magnetic transduction pathway. Strains carrying knockout mutations in genes encoding these proteins were used to determine their necessity for magnetotaxis. We are using molecular tools to find the expression pattern of these proteins, and to test their ability to rescue magnetotaxis in knockout mutants. Identified proteins will likely be conserved across species and will contribute to the study of magnetic sensation across taxa.

Prevention of muscle decline in an animal model of Duchenne muscular dystrophy [2]

Celia Beron, Jesse Cohn, Jonathan T. Pierce-Shimomura, Andrés G. Vidal-Gadea

Duchenne muscular dystrophy (DMD) is a lethal neurodegenerative disorder that affects about 10,000 patients in the US alone. It is characterized by progressive muscular weakness, degeneration, and death. Despite its prominence, a cure has not yet been found for this disease. Previous studies have used animals to model the genetic background of the disease, but have failed to replicate the severity of muscle decline of DMD in humans. The nematode *Caenorhabditis elegans* has been similarly constrained by these limitations. We developed an assay capable of recapitulating the human phenotype of DMD in *C. elegans*. Modeling the genetics, locomotor decline, and muscular degeneration characteristic of the disease in *C. elegans*, we performed the first genetic screen to rescue muscle and motor degeneration in an animal without the aid of sensitizing mutations. This resulted in the isolation of several mutant lines capable of suppressing the locomotor and muscular decline characteristic of DMD. Further studies with RNA interference and gene overexpression will help us identify conserved pathways through which suppressor mutations accomplish their rescue. This will help us produce novel molecular approaches to combat the muscular and locomotor decline characteristic of humans with DMD.

Investigating the Mechanism of Modafinil Action [3]

Martin Bobak, Matthew Weber, and Paul A. Garris

Modafinil (Nuvigil©) is a mild psychomotor stimulant that exhibits therapeutic efficacy for treating narcolepsy, work-related fatigue, and attention deficit hyperactivity disorder (ADHD). While modafinil action is incompletely understood, there is evidence to suggest that its mechanism is distinct from that of amphetamine (Adderall®) and methylphenidate (Ritalin®). The mechanism of these two well-characterized psychomotor stimulants, also used for treating narcolepsy and ADHD, is thought to involve inhibition of the dopamine transporter. This protein clears released dopamine from extracellular fluid to terminate dopamine signaling. Modafinil poses limited risk for addiction, unlike the highly abused amphetamine or methylphenidate; therefore, there is great interest in establishing how this atypical psychomotor stimulant acts. While modafinil also interacts with the dopamine transporter, its affinity is considerably lower. Recent evidence has demonstrated that amphetamine and methylphenidate activate phasic dopamine signaling. This mode of communication used by dopamine neurons is critical to reward learning and may represent an important action by which amphetamine and methylphenidate treat ADHD. However, whether modafinil also activates phasic dopamine signaling is not known. We hypothesized that modafinil similarly activates phasic dopamine signaling. In this study we used fast scan cyclic voltammetry at a carbon-fiber microelectrode to measure electrically evoked phasic-like dopamine signals and pharmacologically evoked phasic dopamine signals in the striatum of anesthetized rats. To our surprise, we found modafinil appeared as potent as amphetamine at activating these phasic-like and phasic dopamine signals. Our findings thus suggest a novel therapeutic action of modafinil.

Investigating use of CRISPR in *Neurospora crassa* [4]*

Amy Boyd, Turner Reid, and Tom Hammond

The synthetic CRISPR system consists of a nuclease and an RNA. The nuclease component, Cas9, uses the RNA component (gRNA) to target and cleave DNA sequences in a genome. The CRISPR system has been successfully used to modify the genomes of diverse organisms. Thus far only one fungus, S. cerevisiae, has been investigated with respect to CRISPR functionality. We aim to establish CRISPR for use in the model filamentous fungus, N. crassa. Our approach uses a novel plasmid for targeting the Cas9 enzyme to the his-3 locus of N. crassa and allows for positive identification of the Cas9 allele by screening for nourseothricin resistance. We will insert the DNA sequence for the gRNA into the csr-1 gene, resulting in resistance to cyclosporin A. The gRNA will target al-1, mutation of which will produce a change in spore color of conidia. Once functionality of the CRISPR system has been confirmed in N. crassa, we will use the system to further study the genome, particularly elements involved in meiotic drive.

A test of soil nitrogen levels as sex specific selection on *Lobelia spicata* [5]

Mitchell Czerwinski and Diane L Byers

We are testing whether the differences in the concentration of soil nitrogen affects female frequency in populations of Lobelia spicata. Based on results from a previous field study, there is a positive correlation between nitrate and organic matter in the soil with female frequency.

Lobelia spicata, which is a native prairie plant, has a gynodioecious breeding system (plants have either female or hermaphrodite flowers). Lobelia spicata grows in different types of prairies including hill and tallgrass. The frequency of females in a population ranges from 2 to 85%. We propose that this difference in female frequency could be affected by the soil characteristics as well as genetic drift. Changes in female frequency matters as it alters seed and fruit production, which affects the species fitness.

To test if the variation of female frequency is due to the nutrients in the soil, we are starting a study that will experimentally alter the nitrogen in the soil. Pots will receive either low or high nitrogen levels, which are based on the ranges of nitrogen found in our sites. Each pot will receive the same set of offspring from several hermaphrodites and female plants and grown at a high density. We will be determining the sex ratio of the plants in each pot to assess the impact of nitrogen. If the correlation observed in the field contributes to the differences in female frequency, we expect to find that as the nitrogen levels increase then so will the frequency of female plants.

Behavioral plasticity in response to perceived predation risk in breeding house wrens (*Troglodytes aedon)* [6]

Erin Dorset, Scott Sakaluk, and Charles Thompson

Many recent studies suggest that birds show behavioral plasticity in response to perceived predation risk. In breeding birds an increase in perceived risk should lead to behaviors that reduce risk to the parents, to the offspring, or both. I tested the hypothesis that breeding house wrens (*Troglodytes aedon)* show behavioral plasticity in response to increased perceived-predation risk to reduce the threat to themselves and their offspring. After each pair had selected a nestbox, laid a clutch of eggs, and began incubation, pairs were randomly assigned to a control or experimental treatment. Pairs in the experimental treatment had their nestbox entrance enlarged from 3.2 cm to 5.0 cm. During the incubation stage, I measured female vigilance, nest attentiveness, and on-bout and off-bout duration; during the nestling stage, I measured female brooding and nestling provisioning. I predicted that females would increase vigilance, decrease nest attentiveness, decrease on-bout duration, and increase off-bout duration if acting to reduce risk to themselves, or that they would increase vigilance, increase nest attentiveness, increase on-bout duration, and decrease off-bout duration if acting to reduce risk to their offspring. Experimental females spent less time being vigilant and had shorter on-bouts and off-bouts compared with control females. Shorter on-bouts were expected if females reduced their own risk, and shorter off-bouts were expected if females reduced offspring risk. Thus, breeding house wrens show behavioral plasticity in response to perceived predation risk and this plasticity results in reduced risk to both themselves and their offspring.

Action potential collision without annihilation - were Hodgkin and Huxley wrong? [7]

Rosangela Follmann, Epaminondas Rosa, and Wolfgang Stein

Action potentials are electrical impulses generated in excitable cells including neurons, where they propagate along the axon. Action potentials typically only propagate in one direction, but in some cases can also travel against their preferred direction. It is generally understood and experimentally demonstrated that action potentials propagating in opposite directions collide and annihilate each other. This is believed to be so because the refractory period following either spike (Hodgkin & Huxley, J Physiol, 1952; 117(4): 500–544) prevents the generation of an impulse in either direction. However, recently Gonzalez-Perez et al. (Phys. Rev. X 4, 031047, 2014) pointed out two cases where the collision of action potentials did not result in annihilation of the signals (in the ventral cords of earthworms and lobsters), indicating that the effects of the refractory period as postulated by Hodgkin & Huxley may not apply to all axons. Here we investigate action potential collisions in the axons of the earthworm and of the stomatogastric nervous system of the crab to confirm (or refute) these findings. Our preliminary results indicate annihilation of the colliding action potentials in the crab. In the case of the earthworm our experiment shows that the signals do not annihilate after collision, suggesting that whether annihilation happens or not may depend upon the type of axon being investigated. We are currently testing what processes may be involved during the collision that would allow the signals to pass through in numerical simulations using a Hodgkin-Huxley model applied to a multicompartment axon.

Sensory-specific recruitment of multimodal neurons in motor pattern generation [8]*

Chris Goldsmith and Wolfgang Stein

Animals have to constantly adapt their motor behavior to perform adequately in their environment. A fundamental component of this process is a reconfiguration of the motor circuits to elicit distinct output activity for different sensory inputs. Little is known about the mechanisms mediating such motor program selections. We are investigating this problem using the well-characterized gastric mill central pattern generator in the crustacean stomatogastric ganglion. Descending modulatory projection neurons from the commissural ganglia (CoGs) control the gastric mill motor pattern and respond to different sensory stimuli. How their combined activity determines motor output is unknown. We hypothesize that a population coding mechanism exists such that different sensory inputs recruit overlapping but distinct pools of CoG neurons, which in turn elicit distinct versions of the same motor pattern.

We are testing this hypothesis with whole-ganglion voltage-sensitive dye imaging allowing us to monitor the activity of many CoG neurons simultaneously. Our initial experiments indicate that (1) CoG neurons are recruited by activating sensory pathways that elicit gastric mill motor activity, (2) the composition of the pool of recruited neurons varies over the duration of the gastric mill rhythm and (3) different pools of CoG neurons are active when comparing across sensory modalities. Our results thus indicate that CoG neuron activity not only differs between different sensory inputs, but that there is also variable neuronal participation in the control a stereotypic motor program. Using post-hoc retrograde axonal backfills, we are currently investigating which of these recruited neurons innervate the gastric mill motor circuit.

Tissue-specific expression of Ferulate Co-A Monolignol Transferase [9]

Cody Gorski, Cynthia L. Cass, and John C. Sedbrook

Project goal: Improve plant biomass digestibility by altering lignin quality using Ferulate Co-A Monolignol Transferase in *Brachypodium distachyon*.

A cascade of ten different enzymes generate the three monolignol subunits that are transported to the cell wall and polymerized into the aromatic cell wall polymer, lignin. Our lab has previously shown that ubiquitous expression of the enzyme Ferulate Co-A Monolignol Transferase (FMT) in *Brachypodium distachyon* resulted in the generation of specific monolignol conjugates that were transferred to the cell wall and ultimately polymerized into lignin. Incorporation of these conjugates into the growing lignin polymer introduces an ester linkage, or "Zip". Having the Zip linkage present in the cell wall lignin resulted in a small, but significant, increase in biomass digestibility.

We hypothesize that if *FMT* expression is driven in a temporally and spatially regulated manner, more Zip conjugates will be produced and incorporated into lignin, thereby further increasing the biomass digestibility. To test our hypothesis, a secondary cell wall promoter, *CESA,* is being used to drive the expression of a Yellow Fluorescent Protein (*YFP*)-*FMT* fusion protein. Fusion protein expression is being quantified by Western blot analysis and YFP visualization. To date, 27 independent lines have been tested; however, no consistent YFP fluoresce has been observed, nor has the fusion protein been detected by Western blot analysis. Troubleshooting strategies will be discussed.

The effect of ambient temperature on avian incubation behavior prior to clutch completion [10]

Jason T. Hanser & Joseph M. Casto

While most incubation behavior in birds occurs after the completion of egg laying, parents of several species often begin incubation before clutch completion. Consequently, eggs typically develop and hatch asynchronously, often resulting in reduced rates of growth and survival amongst later-hatched nestlings. In many species, incubation prior to clutch completion and hatching asynchrony are associated with high ambient temperatures. In some cases, prolonged exposure to high ambient temperatures can result in suboptimal development and negatively affect the viability of eggs. Thus, when ambient temperatures are high, incubation prior to clutch completion may serve to minimize reductions in the hatching success of eggs. Here, we used information collected by automated data-loggers (iButtons) to model the effect of ambient temperatures on the incubation behavior prior to clutch completion in a cavity nestling species, the European starling (*Sturnus vulgaris*). By placing data-loggers on the underside of each nest box and within the cup of each nest, we were able to record ambient and nest temperature for each nest over the course of the laying period. While nest cup temperature and the likelihood that eggs experience temperatures necessary for embryonic develop are strongly influenced by ambient temperature, nest attentiveness - inferred by comparing by ambient and nest cup data-loggers - seems to be unrelated to ambient temperature. These results suggest that while incubation prior to clutch completion and hatching asynchrony may be associated with high ambient temperatures, this relationship does not reflect a change in incubation behavior as a response to high ambient temperatures.

Factors constraining the reproductive output of *Baptisia alba macrophylla* [11]*

Ashley Hembrough and Victoria Borowicz

Baptisia alba macrophylla, a native, herbaceous perennial plant, produces inflorescences with a large number of flowers, yet produces a limited number of mature fruits. I hypothesize that (i) the number of pods initiated, (ii) the number of pods matured, and (iii) the number of seeds matured by *Baptisia alba macrophylla* is optimized based on resource availability at each stage of development, but this optimal number is further compromised by extrinsic factors such as pollen limitation and predispersal seed predation by the weevils *Apion rostrum* Say and *Tychius sordidus* LeConte. I tested this hypothesis by randomly assigning whole plants to one of two fertilizer treatments (no fertilizer, application of 70g of a 10-10-10 N-P-K fertilizer prior to first flowering and post flowering) and one of two seed predation treatments (no pesticide, application of Tanglefoot Insect Barrier on a straw placed around the base of the plant). Individual flowers were then assigned to one of two pollination treatments (natural pollination, natural pollination supplemented by hand-pollinated outcrossing). Preliminary analysis indicates that no treatment affected pod initiation, but pre-dispersal seed predation by *A. rostrum* significantly reduced pod maturation. Based on these data, it appears as though reproductive output of *B. alba macrophylla* is only significantly impacted by pre-dispersal seed predation which greatly limits overall reproductive output.

Molecular machinery responsible for C. *elegans* detection of earth's magnetic fields [12]*

Moe Khalil and Andrés Vidal-Gadea

Many animals use the earth's magnetic field for orienting in their environment. However, we have yet to figure out any magnetosensory cell or transduction mechanism responsible for this very unique and remarkable behavior. One mechanism proposed to be responsible for this distinctive behavior in many animals involves magnetic particles. Recently, we have discovered that the nematode C. elegans performs this remarkable behavior. We are investigating the molecular machinery responsible for worms detecting magnetic fields. We extracted magnetic particles from the tissues of the worms and have also used mass spec to identify the proteins that are associated with magnetic particles. To test the potential role of these proteins in magnetic transduction and orientation, we are testing the ability of mutants with deleterious mutations in these genes to orient to magnetic fields in order to identify the subset of these proteins that are candidates for forming part of the magnetic transduction machinery. Once we are able to understand the molecular basis of magnetic field detection, this will allow us to discover how other animals interact with magnetic fields in their particular environment.

Expression, localization, and characterization of CTP: phosphocholine cytidylyltransferase from *Leishmania* [13]

Justin D.T. Lange

The eukaryotic parasite *Leishmania* is the causative agent of visceral leishmaniasis. A common phospholipid found in the cell membranes of *Leishmania* is phosphatidylcholine. The enzyme CTP: phosphocholine cytidylyltransferase, or CCT, catalyzes the addition of cytidine triphosphate to phosphocholine, a critical step in the formation of phosphatidylcholine. Amino acid sequence analysis of CCT from *Leishmania infantum, Leishmania major, Caenorhabditis elegans, Plasmodium falciparum,* and rat shows a high level of homology in the CCT catalytic region but a lack of a known carboxy-terminal membrane-binding region in *L. infantum* and *L. major*. However, this also shows an exclusive amino-terminal region in *L. infantum* and *L. major* which has a high level of homology with the sequence for choline phosphotransferase, the enzyme which catalyzes the next and final step of phosphatidylcholine synthesis, in other organisms. Here we present progress toward our goal of cloning the gene encoding CCT with a red fluorescent protein marker, expressing the recombinant protein in non-pathogenic *Leishmania tarentolae* and *Escherichia coli*, characterizing possible multi-step catalysis by the enzyme in vitro, and observing cellular localization of the enzyme using confocal microscopy.

Characterization and the effect of season on gut-associated lymphoid tissues in red-eared slider (*Trachemys scripta*) hatchlings [14]*

Sarah M. Marrochello, Laura A. Vogel, and Rachel M. Bowden

As with the mammalian immune system, gut-associated lymphoid tissues (GALT) in reptiles play a vital role in immune defense throughout ontogeny. In mammals, GALT is responsible for recognizing parasites and food-borne antigens. Very little is known about GALT in reptiles and unlike mammals, reptiles do not have Peyer's patches. Previous studies in lizards and snakes have noted species specific and seasonal variation in the structure of GALT, but this has not been examined in turtles. The first goal of this study was to characterize GALT located in the small intestine of red-eared slider turtle (*Trachemys scripta*) hatchlings. We were also interested in looking for seasonal variation in hatchling GALT. *T. scripta* produces two clutches during the course of a single nesting season in our population. We hypothesized that differences in GALT development would be observed for hatchlings from first clutches relative to second clutches. To test our hypothesis, intestines were taken from both early and late season hatchlings. Immunohistochemistry was then used to identify B cells in the collected tissues. Staining confirmed the presence of distinct patches of B cells lining the small intestine of many hatchlings. B cell density was also related to nesting season. Early season hatchlings had significantly more B cell patches. Our study confirms GALT presence in young reptiles. Seasonal differences may have important implications for hatchling immunocompetence and survival.

What are the effects of hands-on learning experiences on career choice within the biological sciences? [15]*

Andrew L. McDevitt

Science, technology, engineering and mathematics (STEM) fields are known to be an important driver of technological advancements and economic success that creates an ever-increasing demand for qualified employees. However, there is a growing achievement gap in these fields which has left many individuals, especially from underrepresented groups, struggling to fulfill their potential in STEM disciplines. The purpose of this qualitative study was to explore the role that hands-on learning experiences play in motivating students towards a specific discipline or career choice. Five biology graduate students were selected to share their experiences with hands-on learning. Through the use of semi-structured interviews, they were asked to interpret how they believed their career aspirations developed and identify factors that influenced those decisions. Self-Determination Theory (SDT) was used as a lens to examine how basic psychological needs (i.e., competence, autonomy, and relatedness) were met and to evaluate the level in which internal and external pressures regulated their motivation toward STEM careers. This study highlights variability in career decision factors due to differing perceptions of specific disciplines, family and cultural support, professional mentorship, and non-professional aspirations. By gaining a better understanding of how novice scientists' hands-on experiences influenced career decisions, it is possible to hypothesize and broadly examine how different types of hands-on experiences support or undermine undergraduates' motivations toward STEM careers, particularly for historically under-represented groups, enabling all students to reach their potential in STEM disciplines.

Demonstrating *Agrobacterium*-mediated transformation of Pennycress (*Thlaspi arvense*) by expressing *EaDAcT* to alter seed oil composition [16]*

Michaela McGinn, Sunil Bansal, Cynthia Cass, Timothy Durrett, and John Sedbrook

Pennycress (*Thlapsi arvense*) is a Brassica species related to Arabidopsis and rapeseed that holds considerable agronomic and economic potential due to its unique combination of attributes including extreme cold tolerance, rapid growth, over-wintering growth habit, and natural ability to produce copious amounts of seeds high in oil and protein content. Pennycress could be grown on millions of hectares of farmland throughout temperate regions of the world including the U.S. Midwest Corn Belt, e.g. being planted in the fall near the time of corn harvest and harvested the following spring in time for planting soybeans. While pennycress could potentially generate billions of liters of oil annually without displacing food crops or requiring land use changes, work must be done to develop pennycress varieties having improved seed germination and in-field stand establishment as well as improved seed oil and meal quality. To facilitate research on pennycress, we have developed a pennycress genetic transformation protocol using an *Agrobacterium*-mediated floral dip method. We found that, as with camelina, pennycress transformation was enhanced by applying a vacuum to floral spikelets while submerged in a sucrose- and Silwet L77-containing Agrobacterium solution. Hygromycin and DsRED worked well for selection of/screening for transformants, whereas pennycress seedlings exhibited naturally high resistance to kanamycin. We will discuss targets and approaches to genetically improving pennycress and will present data showing that we have successfully generated pennycress plants stably transformed with the *Euonymus alatus* diacylglycerol acetyltransferase (*Ea*DAcT) gene; these plants produce seeds accumulating 3-acetyl-1,2-diacyl-*sn*-glycerols (acetyl-TAGs), a low-viscosity oil.

A gene important for cell elongation in *Arabidopsis thaliana* [17]

Christy Moore, Bangxia Suo, Stephanie Seifert, Mais Zahde, and Viktor Kirik

Arabidopsis hypocotyls contain long, narrow cells that elongate along the axis of plant growth, pushing the developing shoot through the soil. Certain cell wall mutants are deficient in the process of elongation, causing their hypocotyls to be swollen and shorter than those of wild type plants. This phenotype is most noticeable when these mutants are grown in darkness because hypocotyl cells will elongate more in an effort to break through the darkness and find sunlight.

My research focuses on the *short hypocotyl (sh)* mutant, which, when grown in darkness, exhibits a smaller and slightly swollen hypocotyl than those of wild type plants. *SH* was rough mapped to a 1199kb region on chromosome 5. We tested known mutants within our mapping region that have *sh*-like phenotypes (*cobra* and *procuste1/cesa6*) to verify that *sh* is novel. A conditional radial expansion test revealed that *sh* does not exhibit a *cobra* phenotype. A confocal microscopy analysis of progeny from a *sh* x YFP-tagged CESA6 cross indicated that *SH* is not *CESA6* and that CESA6 appears to be sequestered in Golgis of *sh* plants. In addition, we measured birefringence, an optical property of crystalline cellulose, in mutant and wild type trichomes, revealing a possible cellulose deficiency. We will perform cellulose quantifications for the hypocotyls, leaves, and trichomes of mutant and wild type plants to confirm this possibility. Through the identification and preliminary characterization of the *SH* gene, we hope to learn new information regarding the molecular processes important for cell wall building and cell elongation.

How mating experiences influence the expression of alternative mate-finding tactics in the burying beetle, *Nicrophorus orbicollis* [18]

Tess Piening Mulrey, Anne-Katrin Eggert, Scott K. Sakaluk

Male *Nicrophorus* burying beetles utilize two alternative mate-finding tactics. One tactic involves flying in search of a carcass on which to meet a mate and raise a brood. Although this tactic can result in high levels of paternity, it is risky in that carcasses are rare and competition for carcasses is fierce. The other tactic involves emitting pheromone in the absence of a carcass to attract a female. While this tactic is energetically less costly, pheromone-emitting males must mate with multiple females to achieve the same reproductive returns as a successful searcher male.

While most male burying beetles employ both tactics, the amount of time spent employing each tactic differs between individuals. Because all males are phenotypically plastic in the time invested in alternative behaviors, the expression of the tactics may be condition-dependent and influenced by cues of resource availability. I tested the hypothesis that the expression of alternative tactics is influenced by previous mating experience, and that alternative tactics are expressed based on the perceived availability of females versus carcasses. I tested this hypothesis using a repeated-measures design in which I observed mate-finding behavior of males before and after exposure to: 1) multiple females but no carcasses, 2) a single female and multiple carcasses, 3) multiple carcasses but no females or 4) no females or carcasses (control). I predicted that if males are able to gain information about resource availability based on previous mating experience, they would adjust the amount of time invested in each tactic accordingly.

Examining the cellular and motor effects of exercise in an animal model of Duchenne Muscular dystrophy [19]*

Daniel Nuccio, Lucas Barickman, and Andrés Vidal-Gadea

Duchenne Muscular dystrophy (DMD) is a degenerative muscular disease that affects 1 in 3,500 males. DMD occurs due to mutations in the dystrophin gene and is characterized by a loss of functional dystrophin in muscle tissue. This results in progressive muscle necrosis, loss of mobility, and death. There is evidence suggesting that severity of muscle deterioration is correlated with the amplitude of muscle contraction during exercise. However, it remains unclear whether there is a safe or even beneficial level of muscle activity for DMD patients. This question is of great importance to DMD patients and their caregivers. Yet, despite its importance it remains uncertain whether some level of exercise may slow the progression of muscle deterioration, or if even minimal levels of exercise will accelerate it. This uncertainty stems from the lack of an adequate organism for the study of DMD given that many fail to exhibit the phenotypic abnormalities associated with mutations of the dystrophin gene in humans. Through a novel assay developed by our lab to modulate the amplitude of muscle contraction, we have recreated the cellular and motor hallmarks of DMD in *C. elegans* dystrophin mutants. We used this assay to determine if there is a range in muscle exertion that could be used to slow down the progression of muscle deterioration characteristic of DMD patients.

Behavioral responses of *Aedes aegypti* larvae to water-borne predator cues from *Toxorhynchites rutilus* [20]*

Geoffrey D. Ower, Mitchell A. Czerwinski, Steven A. Juliano

Larvae of the mosquito, *Toxorhychites rutilus*, are generalist ambush predators on other treehole- and container-dwelling insects, particularly mosquitoes. Predation by *T. rutilus* has the direct effect of maiming and killing prey but predation also releases chemical cues from damaged victims into the water, and these can alter prey behavior. Prey can respond to predator cues by reducing their activity levels, lowering their predation risk but at a cost of missed foraging opportunities. This could be particularly costly to female mosquito larvae, which require more resources to pupate successfully. Little is known about the anti-predator responses of *Aedes aegypti*, a prominent vector of arboviruses infecting humans. Video assays were used to record activities and positions of *A. aegypti* larvae when exposed to cues contained in water that held: 10 conspecifics (**control**), 10 conspecifics consumed by a *T. rutilus* larva (**predator**), and 10 crushed conspecifics (**crushed**). The behavior of *A. aegypti* larvae differed significantly among treatments (Pillai's Trace=0.59, $P<0.0001$), but there were no significant behavioral differences between sexes, and no significant interaction. Treatments all differed significantly from one another ($P \leq 0.0008$) with *A. aegypti* larvae spending significantly more time browsing at the wall in the **control** treatment. Larvae in both the **predator** and **crushed** treatments reduced their predation risk by spending more time resting at the air-water interface with a significantly stronger response in the **crushed** treatment. This is the first empirical evidence of anti-predator responses by *A. aegypt* to *T. rutilus* predator cues.

Testing for Cyclospine-A sensitivity in *Fusarium verticillioides* [21]*

Tejas Patel, Jay W. Pyle, Thomas M. Hammond

In the laboratory setting one of the most useful tools for genetic analysis and molecular cloning is selectable markers. Selectable markers are typically segments of DNA that can be introduced into a system that effect a phenotypic change that is readily identifiable. This phenotypic modification allows researchers to identify putative transformants for further downstream analysis. In our lab one of the systems that we work in is the filamentous fungus *Fusarium verticillioides*. *F. verticillioides* is not a well-developed model organism and as such does not have a broad range of molecular tools available for analysis of the genome. The gene *Cyclosporine-resistance-1* (*CSR-1*) in the model fungus *Neurospora crassa* encodes a cyclophilin protein (Cyp20) which plays a critical role for sensitivity to cyclosporine A, an antifungal compound. Cyclosporine A, together with Cyp20, inhibits the ability for fungi to generate transcription factors that are essential for hyphal growth. Therefore, a targeted knockout to the CSR-1 locus would result in the negative effects on growth in fungal cultures grown in the presence of cyclosporine-A. The goal of this project is to determine if *Fusarium verticillioides* is sensitive to cyclosporine-A. The identification of a sensitivity to cyclosporine-A would allow for a specific approach for transforming *Fusarium* verticillioides that would also allow the selection for desensitization to cyclosporine-A. Here we present our progress in analyzing growth assays across a range of concentrations to look for changes in growth patterns that can be correlated to cyclosporine sensitivity.

Development of monocot transformation vectors housing *Brachypodium distachyon* (Brachypodium) gene promoters for specific expression during secondary cell wall formation. [22]

Debrah Petrik and John Sedbrook

In order to genetically modify characteristics of the secondary cell wall without unwanted plant health detriments, one must have the means to alter gene expression in a tissue and time specific manner. A good first attempt in this regard would be to isolate and utilize the promoters of genes which are known to be expressed during secondary cell wall biosynthesis. Namely, the promoters of enzymes involved in lignin monomer biosynthesis would provide a promising pool of candidates for a secondary cell wall "promoter toolbox". Alternatively, enzymes which have been shown to be involved in subsequent lignin polymerization or side-group modification could also be screened. By using these isolated regulatory sequences to drive expression of reporter genes, we plan to visually characterize their tissue and developmental specific patterns. The overall goal is to create plant transformation vectors which will express inserted genes of interest in a well characterized, reproducible manner in the secondary cell wall.

Differential selection for competitive ability in *Chamaecrista fasciculate* [23]

Robert Philips and Diane Byers

In the tallgrass prairie region which includes Illinois, *Chamaecrista. fasciculata* (partridge pea) is commonly found in two contrasting prairie ecosystems - sand and tallgrass. The sand prairies are vegetatively sparse and have a well-drained sandy soil. While the tallgrass prairies are vegetatively dense and have a very moist clay based soil. While most of the plants in both prairie types are perennials often growing for many years (and storing biomass below ground) before reproducing, *C. fasciculata* is an annual and thus needs to grow and reproduce during one summer. Thus in all locations *C. fasciculata* will be expected to have strong selection to quickly grow and reproduce. We proposed the differences in density and diversity of the plants in these contrasting prairie types will lead to differences in selection for competitive ability. We predict It is predicted that populations in the tallgrass prairie will need be to relatively stronger competitors compared to plants in the sand prairies. To test this prediction, first we have assessed the diversity and density of plants in eight *C. fasciculata* sites, four tallgrass prairies and four sand prairies. Soil was also sampled for nutrient and texture analysis for a greenhouse experiment to test if there is a variance in competitive ability in plants from sand versus tallgrass prairies. This experiment has two treatments, a non-competitor and competitor using *Schizachyrium scoparium* as the competitor. Data is still being gathered so this poster is an early report.

Mus-52 RIP mutants for targeted integration of transgenes in *Neurospora crassa* [24]

Nick Rhoades, Dilini Samarajeewa, and Thomas M. Hammond

In *Neurospora crassa,* efficient insertion of a transgene into a precise genomic location requires disruption of a biological process known as non-homologous end-joining (NHEJ). When NHEJ is functional, transgenes usually integrate into ectopic locations. Previous work has shown that deletion of a gene called *mus-52* disrupts NHEJ and allows for nearly 100% targeted integration of transgenes and most Neurospora laboratories use strains whose *mus-52* alleles have been replaced with a selectable marker known as *bar*. While this is useful, it prevents the use of *bar* as a selectable marker for integration of transgenes at other genomic locations. Hence, our goal is to create a null *mus-52* mutant strain via RIP (repeat-induced point mutation), which will allow us to use the *bar* gene at other locations in the genome.

Investigating the effects of amphetamine on Pavlovian learning [25]

Douglas R. Schuweiler and Paul A. Garris

Attention deficit hyperactivity disorder (ADHD) is most efficaciously treated with the stimulant amphetamine (AMPH, brand name Adderall©). Therapeutic doses of AMPH increase norepinephrine and dopamine (DA) in the prefrontal cortex. The resulting increase in neuronal activity is widely thought to be a key therapeutic mechanism of action. Higher doses of AMPH suppress prefrontal cortex activity and enhance DA transients in the nucleus accumbens (NAC), actions thought to contribute to their abuse potential. The effect of therapeutic doses of AMPH on DA transients has not been investigated.

Pavlovian learning depends on NAC DA transients which signal the delivery of unexpected rewards or reward-predictive cues but not predicted rewards. Thus, learning correlates with a 'transfer' of the DA transient from the reward to the cue. The DA transfer deficit theory (DTD) of ADHD proposes that symptoms are caused by insufficient 'transfer' of the DA transients. To test DTD predictions that AMPH will accelerate learning and enhance cue-evoked DA transients, I have begun investigating the effects of AMPH in rats during unexpected food reward and Pavlovian conditioning paradigms. I am using therapeutic doses as well as a high dose that rats find rewarding. Preliminary results from the unexpected food reward paradigm reveal that rats stop consuming the food reward following the high dose. Additionally, DA release is enhanced by a presynaptic mechanism which should also enhance transients. Surprisingly, the reward ceases evoking DA transients. Future work includes recording NAC DA transients during Pavlovian conditioning.

Growth medium-dependent plasticity of *Staphylococcus aureus* membrane fatty acid profile and carotenoid content [26]*

Suranjana Sen, Seth Johnson, Sirisha Sirobhushanam, Yang Song, Ryan Tefft, Craig Gatto, and Brian J. Wilkinson

The observation that growth of *Staphylococcus aureus* in Mueller-Hinton (MH) broth led to a high (85%) content of branched-chain fatty acids (BCFAs) promoted an investigation of the effects of different growth environments on membrane lipid composition. BCFA levels were found to be significantly high in cells grown in MH broth, Luria Broth, Tryptone Broth, and defined medium, but lower in the commonly used Brain Heart Infusion broth and Tryptic Soy Broth. Membrane fluidity and staphyloxanthin measurements in cells grown in different media indicated membrane fluidity was relatively well regulated. Growth in serum produced significant amounts of straight-chain saturated and unsaturated fatty acids in the lipids that were likely not biosynthesized *de novo*. The results revealed a hitherto unappreciated plasticity of *S. aureus* membrane composition with implications for physiology, antimicrobial susceptibility, virulence factor expression, and fatty acid biosynthesis as an antimicrobial target.

Purification and kinetic characterization of phosphotransbutyrylase from *Listeria monocytogenes.* [27]*

Sirisha Sirobhushanam, Charitha Galva, Suranjana Sen, Brian J. Wilkinson and Craig Gatto

Listeria monocytogenes, the causative organism of the serious food-borne disease listeriosis, has a membrane abundant in branched chain fatty acids (BCFAs). BCFAs are biosynthesized from branched-chain amino acids via the activity of branched chain α-keto acid dehydrogenase (Bkd), and disruption of this pathway results in reduced BCFA content in the membrane. Supplementation of growth media with branched-chain carboxylic acids (BCCAs) allows *bkd* mutants to restore growth and membrane BCFA content. The pathway by which BCCAs are converted to CoA derivatives for fatty acid biosynthesis has not been investigated thus far. We hypothesize that the enzymes phosphotransbutyrylase (*ptb*) and butyrate kinase (*buk*), which are the first two genes of the *bkd* operon, comprise an alternate pathway by which carboxylic acids are converted to their active acyl CoA forms. Recombinant Ptb was obtained as His-tagged protein by Ni^{2+} chelate affinity purification and assayed in the acyl phosphate forming direction by monitoring the release of CoA. Ptb demonstrated significant activity with a large number of substrates including straight chain substrates such as butyryl CoA and pentanoyl CoA as well as branched-chain substrates such as 2-methyl butyryl CoA and isovaleryl CoA. The Km values for Ptb revealed a strong preference for branched-chain substrates. Additionally Ptb was able to utilize unnatural branched-chain substrates such as 2-ethyl butyryl CoA, albeit with lower efficiency, indicating that this enzyme along with Buk are the most likely candidates for the conversion of the exogenous substrates into CoA derivatives for utilization by FabH in BCFA biosynthesis.

Sub-second monitoring of electrically, pharmacologically and acoustically evoked phasic dopamine signals in the striatum of an anesthetized songbird [28]

Amanda R. Smith, Paul A. Garris, and Joseph M. Casto

Extracellular dopamine levels increase in the vertebrate striatum in response to novel or otherwise salient sensory stimuli and are thought to encode incentive value. Social signals can possess both innate and acquired incentive value, and understanding how these signals are encoded is key to comprehending how social behavior is generated. Monitoring real-time changes in extracellular dopamine in response to social signals is thus critical to understanding the neurobiology of social behavior. Here we establish *in vivo* fast-scan cyclic voltammetry (FSCV) at a carbon-fiber microelectrode (CFM) in urethane-anesthetized European starlings (*Sturnus vulgaris*), a technique permitting real-time monitoring of extracellular dopamine dynamics in the avian brain, as a promising approach for investigating the neural underpinnings of social behavior. We successfully evoked dopamine signals in medial and lateral striata of anesthetized starlings by electrically stimulating the ventral tegmental area. As previously described in rats, we found that combined pharmacological inhibition of dopamine and norepinephrine transporters and blockade of D2 dopamine receptors via systemic administration of the drugs nomifensine and raclopride, respectively, elicited short-lived, phasic changes in extracellular dopamine concentration (dopamine transients) in the medial and lateral striata. Finally, we evoked dopamine signals in the medial striatum of anesthetized starlings via playback of species-specific acoustic social signals, including distress vocalizations and male song. In sum, the results suggest that *in vivo* FSCV at a CFM has the sensitivity and spatial and temporal resolution to monitor real-time responses of the avian mesolimbic dopamine system to a variety of stimuli including species-typical acoustic social signals.

A key MSUD Protein [29]*

Zach Smith, Jon Friesen, Erik Larson, and Tom Hammond

Meiotic silencing by unpaired DNA (MSUD) is a process observed in the model organism *Neurospora crassa*. During this process unpaired DNA between homologous chromosomes is detected and silenced, resulting in the suppression of unpaired genes. The effects of MSUD can be seen using phenotypic markers such as the *Roundspore* gene and evidence supports the existence of a physical search for unpaired DNA. However, the mechanism for detecting unpaired DNA remains uncertain. Previously, we have shown evidence that a Rad54-like protein (SAD-6) is required for the efficient completion of MSUD and may be necessary for the detection of unpaired DNA. Currently, we are working to determine the mechanism and proteins (along with SAD-6) that are involved in the homology search that detects unpaired DNA. SAD-6, and its homolog Rad54, share a conserved helicase domain and other similarities between their amino acid sequences suggesting they play a similar role within the nucleus. Rad54 is an essential protein throughout multiple stages of homologous recombination-based DNA repair. While it is uncertain whether homologous recombination is required for MSUD, we are investigating the possibility that SAD-6 shares similar biochemical activities with Rad54.

Modulation of axonal spiking by descending projection neurons [30]*

Carola Städele and Wolfgang Stein

All nervous systems are subject to neuromodulation, adding extraordinary richness to the dynamics neuronal networks can display. Our understanding how neuromodulators reconfigure circuit output is mostly based on the notion that only dendrites and synapses are modulated. However, there are numerous reports demonstrating that action potential propagation and initiation in axons is subject to neuromodulation. Very little is known about the origin of axonal modulators and the consequences of axonal modulation on the postsynaptic circuits. We hypothesize that modulation is under neuronal control and that it alters information encoding as action potentials are transferred towards the postsynaptic circuits, leading to a change in system output.

We study axonal modulation using the anterior gastric receptor neuron (AGR) in the crustacean stomatogastric nervous system. At rest, AGR produces tonic spike activity of 2-5 Hz, initiated at an ectopic spike-initiation zone in its axon. Here, we show that AGR's axon is directly targeted by neuromodulators released from modulatory projection neurons. Activation of olfactory projection neurons diminished AGR's ectopic firing frequency by $26.72 \pm 7.87\%$ ($N=8$). This diminished activity level was retained for several minutes beyond the stimulation. When chemical neurotransmitter release at the axon was blocked the diminishment was absent, indicating a direct chemical modulation by projection neurons. The drop in AGR firing frequency was mimicked by focal application of Histamine, one of the projection neuron transmitters ($29.53 \pm 8.89\%$, $N=10$). The drop was reduced when Histamine receptors were blocked ($N=5$). We are currently testing whether axonal modulation affects information encoding and circuit output.

Na^+/K^+ pump Ion binding site interactions regulate the proton leak pathway [31]*

Kevin S. Stanley, Craig Gatto and Pablo Artigas

The Na^+/K^+ pump (NKA) is a membrane bound transporter located in all cells which transports three Na+ ions out for two K^+ ions into the cell for every one ATP molecule hydrolyzed. Two out-of-three ion-binding sites within the protein reciprocally bind two K^+ or two Na^+ ions (shared sites), while the remaining site exclusively binds Na^+. Without these two ions present the NKA passively transports H^+. In order to understand the proton transport mechanism, voltage-clamp was used to study the effect of tetrapropylammonium (TPA) and Beryllium Fluoride (BeFx) on inward proton current (I_H) transport by NKA expressed in Xenopus oocytes. TPA (a shared site blocker) had a dual effect on I_H; between pH 7.6-6.0 TPA showed partial inhibition I_H, and at pH 5.0 TPA showed partial activation I_H. When the phosphate analogue BeFx was injected into oocytes, stabilizing an externally open conformation of the ion binding sites, K^+ still inhibited inward current at pH 7.6. At pH 5.0 K^+ activated I_H resembling the effect of TPA on fully functional NKA. Blocking access at the shared sites with TPA activated I_H by relieving the inhibition normally observed at high [H^+]. Partial inhibition by TPA at pH 5.0 may reflect an incomplete block of H^+. After BeFx inhibition NKA was still capable of interacting with K^+. The mixed occupancy of K^+ and H^+ at low pH activated the leak, likely relieving the H^+ inhibition due to K+ occlusion. Supported by NIH GM 061583 to CG and NSF MCB-1243842 to PA.

Students' ideas about randomness and variation: do modules with high-frequency data support conceptual development? [32]*

Janet Stomberg, Alicia O'Hare, and Rebekka Darner Gougis

Understanding concepts such as randomness and variation is necessary for effective learning of scientific principles, collecting data, and statistical analysis. Further, comprehending a concept terminologically determines how one may recognize and utilize it in discourse. For this study, I examined the effectiveness of specifically designed laboratory modules employing high-frequency authentic data sets, which have been developed by Project EDDIE (*Environmental Data-Driven Inquiry & Exploration*). I compared students' learning in two upper-level science courses employing Project EDDIE modules with that of students enrolled in a graduate-level science course and an introductory science course for majors. Students' understanding of variation and randomness were completed using pre- and post-assessments. Two-way analysis of variance (ANOVA) was performed to compare pre-post gains across treatments (i.e., module vs. no module). Results indicate that the use of high-frequency data modules is effective in developing students' conceptions of randomness and variation. These results also suggest areas of module refinement and contribute to how students' conceptual development of randomness and variation can be supported in the environmental sciences.

Mechanism of decreased autolysis of targocil-exposed methicillin-resistant *Staphylococcus aureus* MW2 [33]*

Kiran B. Tiwari, Craig Gatto, Suzanne Walker and Brian J. Wilkinson

The continued rise of multiply antibiotic-resistant *Staphylococcus aureus* including methicillin-resistant and vancomycin-intermediate strains has prompted the search for novel antistaphylococcal agents. Peptidoglycan and wall teichoic acid (WTA) are the major staphylococcal cell wall components, and biosynthesis of the latter polymer has been explored for drug development. Targocil is a novel agent that targets TarGH, which is responsible for translocating WTA across the membrane to the wall. Previously we showed that targocil treatment of *S. aureus* led to a rapid and significant shut down of cellular autolysis. Peptidoglycan hydrolase enzymes (autolysins) are required for proper cell separation during division, but their uncontrolled activity can result in cell lysis. Here we report an investigation of the mechanism of targocil-induced decreased autolysis. Targocil treatment led to decreased autolysis in methicillin-resistant and –susceptible strains, but not in an isogenic WTA-deficient mutant. This indicates that WTA is necessary to observe targocil induced decreased autolysis, and establish that the effect is specific to WTA. Crude cell walls retaining autolytic activity isolated from targocil-treated cells had vastly decreased autolytic activity compared to those from untreated cells. However, purified cell walls were similarly susceptible to lysozyme or lysostaphin indicating major cell wall composition were not responsible for the decreased autolysis. Zymographic analysis of peptidoglycan hydrolase profiles revealed a deficiency of cell surface autolysins in targocil-treated cells, but much higher activity in cell membrane fractions. We propose that WTA that cannot be translocated accumulates at the cytoplasmic membrane where it sequesters autolysins such as the major autolysin Atl.

Effects of season and incubation temperature fluctuation frequency on oxidative stress in hatchling red-eared sliders (*Trachemys scripta*) [34]

Lisa A. Treidel, Amanda W. Carter, and Rachel M. Bowden

Maternally derived yolk antioxidants promote cell differentiation and immune function, while protecting offspring from oxidative stress during embryonic and early postnatal periods in oviparous species. The role of yolk antioxidants in reptiles is presently unknown, but given their life-history, antioxidants may play crucial and unique roles in reptilian development. We performed two experiments to independently determine how season and temperature fluctuation frequency during incubation impact oxidative stress in the red eared slider turtle (*Trachemys scripta*). To investigate seasonal effects of egg production on oxidative stress, clutches collected either early or late in the 2013 nesting season were randomly and evenly assigned to a constant temperature (29.5°C) or daily sinusoidal fluctuating temperature incubation (28.7$\pm$3°C) treatment, which has a constant temperature equivalent of 29.4°C. To assess the effect of temperature fluctuation frequency on oxidative stress, eggs from early season clutches were incubated in one of three fluctuating incubation regimes; 28.7$\pm$3°C sinusoidal fluctuations every 12 (Hyper), 24 (Normal), or 48 hours (Hypo). After hatching all individuals from both experiments were sacrificed and liver and brain tissues were harvested. Lipid peroxidation and total antioxidant capacity (TAC) of tissues were then spectrophotometrically determined. We report that regardless of season and incubation conditions, both lipid peroxidation and TAC were significantly related to clutch identity. Furthermore, while antioxidant systems appear to sufficiently protect individuals from oxidative damage during embryonic development, TAC was negatively affected by season and low frequency (Hypo) temperature fluctuations.

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