Biomechanical risk factors in the development of osteochondrosis in Standardbred pacers and trotters

Samantha R. Carter, Christine T. Lopp, Annette M. McCoy
College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Osteochondrosis (OC) is a developmental orthopedic disease in which the process of endochondral ossification is disrupted. Although the etiology of OC is not clearly understood, there are genetic and environmental risk factors (i.e. diet, exercise) that can increase the likelihood of developing the disease. In previous work, it was found that pacers and trotters had a predilection to develop hock OC lesions at different locations. Trotters were more likely to develop medial malleolus lesions, whereas pacers developed distal intermediate ridge of the tibia lesions. The reason behind these differences is unknown, but biomechanical differences between the pace and the trot may play a role. We hypothesize that the amount of time young foals spend pacing or trotting in the field correlates with the subsequent lesions that develop, and with their severity. The aim of this study is to quantify spontaneous activity of Standardbred pacer and trotter foals and correlate these findings with differences in development and progression of OC lesions. Horses will be followed prospectively from 2 to 12 months of age. Field observations will be made weekly, and hock radiographs taken every two months. Observations of activity (sleeping, nursing, walking, trotting/pacing, cantering/galloping) will be recorded at 30 second time intervals for a minimum of 2 hours per foal per week, focusing on the duration of time spent at either the pace or trot. At each radiographic time point, 4 standard views of hock radiographs will be taken and evaluated for the presence or absence of OC lesions. We expect the lesions to be dynamic (i.e. capable of forming or healing) until 8 months of age and our work to contribute to knowledge of OC pathophysiology.

Research Grant: Morris Animal Foundation D16EQ-311 and USDA Hatch Fund
Student Support: Merial Veterinary Scholars Program

Do male sticklebacks use visual or olfactory cues to assess the history of a potential mate?

Marion Dellinger, Jennifer Hellmann, Alison M. Bell
Department of Animal Biology, School of Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, IL
Differential allocation occurs when individuals alter their reproductive investment based on their mate's traits. Previous studies have shown that male threespined sticklebacks (*Gasterosteus aculeatus*) alter their behavior toward a potential mate depending on her previous experience with predators: when a male encountered a female who had been exposed to predators while yolking her eggs, he showed fewer courtship displays as well as less care to her offspring relative to an encounter with an unexposed female. Although both chemical and visual cues mediate conspecific interactions and mate choice in this species, we do not know the mechanism males use to detect previous predator-exposure in mates. To test the extent to which those cues aid in male detection of female predation exposure, we compared male courtship behavior across four treatments in a 2×2 repeated measures design manipulating a potential mate's previous experience (predator-exposed or not) as well as the presence or absence of olfactory cues from predator-exposed females. We confined females to a transparent box providing visual cues only, while olfactory cues were added via water from the tanks of predator-exposed females (experimental) or predator-naïve ones (control). We predicted that if males use olfactory cues to assess female predation exposure, males exposed to experimental water would behave differently compared to males exposed to control water, regardless of female predation status. If males use visual cues, however, only the female status should alter male courtship. Determining the relative importance of visual versus chemical cues will help us better understand the mechanisms by which males adjust their behavior based on female phenotypes.

Research Grant: NSF 1121980, NIH GM082937
Student Support: Merial Veterinary Scholars Program

**Make food great again: effect of protein source and amount on whole-body metabolism and weight gain**

Danielle Engel, Kristy Du, Justin Rhodes
College of Veterinary Medicine (Engel), Department of Animal Sciences (Du) and Department of Psychology (Rhodes), University of Illinois at Urbana-Champaign, Urbana, IL

Recent studies have shown that over 66% of the U.S. adult population is overweight or obese. Approximately 30% of dogs and cats are overweight, indicating that obesity is a significant problem in the pet population as well. Protein is an important element of the daily diet. Previous studies indicated that certain types of protein, such as egg white (EW) and wheat gluten (WG), have satiating and anti-satiating effects on rats, respectively. The goal of this study was to determine if animal and plant-based protein sources affect
metabolism, leading to weight loss or slower weight gain in rats over a 30-day period. Higher metabolic rates and slower weight gain were expected in WG treatment groups due to a predicted increase in energy expenditure based on amino acid composition. After an overnight fast, adult Sprague-Dawley rats were provided 30 minutes of access to a meal of manipulated protein source and amount. These meals were either 20% or 35% WG or EW and the amount was calculated to be 10% of daily food consumption. Food intake and body weight were recorded daily. EchoMRI was used to measure body composition on days 1, 15, and 30. Columbus Lab Animal Monitoring System (CLAMS) cages were used to analyze respiratory exchange ratio (RER) and heat produced during the first and last 3 days of the study. No significant difference was found between treatment groups with regard to weight gain and metabolism. RER data suggested that as rats adapted to their new diet regimen, protein became a larger source of fuel. Although the results did not support the overall hypothesis, RER data suggest that long-term effects of high protein diets on protein digestion merit further study.

Research Grant: National Institute of Diabetes and Digestive and Kidney Diseases, NIH, DK082609
Student Support: Office of the Director, NIH, T35 OD011145

Phthalates and diet: effects on the developing cortex

Anthony Franceschelli, Daniel Kougias, Carly Drzewiecki, Steven Rhoads, Janice Juraska
College of Veterinary Medicine (Franceschelli), Neuroscience Program (Kougias, Drzewiecki, Juraska), Department of Psychology (Rhoads, Juraska), and Children’s Environmental Health and Disease Prevention Research Center, University of Illinois at Urbana-Champaign, Urbana, IL

Phthalates, a group of endocrine disruptors used widely in consumer products, disrupt thyroid function and are known to have estrogenic and anti-androgenic effects. Moreover, high-fat diets interfere with cognition in both rodents and human children. Phthalates and high-fat diets increase oxidative stress and inflammation, which negatively affect nervous system function and plasticity, including that of the cerebral cortex. Because infants and young children are exposed to higher levels of these chemicals than adults, this study tested the hypothesis that pre- and post-natal exposure to a phthalate mixture combined with a maternal high-fat diet will result in long-term alterations in cortical development and in cognitive behavior accompanied by indices on oxidative stress and inflammation as well as epigenetic alterations. Pregnant hooded Long-Evans rats were administered one of two doses of a phthalate mixture (based on the profile of urinary
phthalate metabolites in pregnant women in Champaign-Urbana, Illinois) or vehicle and placed on either a high-fat or control diet. Slices of the medial prefrontal cortex of offspring were mounted and stained for anatomical examination. Intra- and extra-dimensional shifts were examined as a test of cognition. We hypothesize that the combination of exposure to endocrine disruptors and a high-fat diet during development will be especially damaging for cognitive behavior and the neural regions of the brain that are important for this behavior. The results of this study will contribute to a better understanding of the effects of our abundant food and consumer products on cognition and the development of the nervous system.

Research Grant: Children’s Environmental Health and Disease Prevention Research Center, NIEHS, P01 ES002848 and USEPA, 83543401
Student Support: Office of the Director, NIH, T35 OD011145

Connectional modularity of the mouse inferior colliculus

Teodora Hristova, Alexandria Lesicko, Daniel Llano
College of Veterinary Medicine (Hristova), Neuroscience Program (Lesicko, Llano) and Department of Molecular and Integrative Physiology (Llano), University of Illinois at Urbana-Champaign, Urbana, IL

Auditory and somatosensory structures in the brain send information to the lateral cortex of the inferior colliculus, a structure that is believed to be important in multisensory integration. Previous studies have shown that this nucleus contains a network of modules, or compartmental zones which stain densely for certain neurochemical markers, such as glutamic acid decarboxylase-67 (GAD-67). Though the function of these modules remains unknown, the termination patterns of sensory inputs to the lateral cortex appear structurally similar to the distribution of neurochemical modules. The goal of the present study is to determine whether the auditory and somatosensory inputs to the lateral cortex project into the modules, interdigitate with the modules, or show no relationship with these neurochemical structures. The auditory and somatosensory projections to the lateral cortex were labeled with the anterograde tracer, biotinylated dextran amine (BDA) and GAD-67 immunohistochemistry was done to expose the modular network in the lateral cortex of the mouse brain. GAD-labeled modules and BDA-labeled input terminals within the inferior colliculus were visualized using confocal microscopy, then images were imported in Neurolucida®, a software program in which axonal reconstructions were performed. Analysis revealed that somatosensory inputs from the somatosensory cortex and dorsal column nuclei project into the modules, while auditory inputs from the auditory cortex and central nucleus of the
inferior colliculus interdigitate with the modules. These results suggest that the auditory and somatosensory inputs to the mouse lateral cortex form isolated processing pathways.

Research Grant: NIH R01 DC013073 and NSF IGERT Fellowship 0903622
Student Support: Office of the Director, NIH, T35 OD011145

Validation of putative chromosome 14 risk alleles for osteochondrosis in Standardbred horses

Eric J. Ledesma, Megan Darragh, Annette M. McCoy
College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Osteochondrosis (OC) is a developmental orthopedic disease characterized by focal failure of endochondral ossification. It is believed that vascular failure leading to ischemic chondronecrosis results in OC, although the etiology of this pathology is unknown. Both genetic and environmental factors are believed to play a role in the risk of developing OC. Previously, two regions of equine chromosome 14 were validated for association with risk of hock OC in Standardbreds. We hypothesize that specific variants within these regions have a moderate effect on hock OC risk. In this study, specific single nucleotide polymorphisms (SNPs) located within these regions were assessed for association with OC in an independently sampled Standardbred population. This population will be prospectively assessed radiographically for OC development, between 2 and 12 months of age, with a definitive OC diagnosis made at >8 months. Primers surrounding 14 SNPs of interest were designed using the assembly Equus caballus 2.0. Subsequently, NEBcutter v2.0 was used to design restriction enzymes for genotyping via restriction fragment length polymorphism (RFLP). SNPs for which RFLP could not be designed were genotyped by Sanger sequencing. Simultaneously, candidate genes in these regions were Sanger sequenced to discover novel SNPs. These will be tested by similar methods in the experimental population. Our long-term goal is to develop a diagnostic genetic panel to identify at-risk animals, which would allow clinicians and owners/trainers to make management changes to decrease environmental risk factors and overall risk of developing OC. The diagnostic genetic panel would also allow more-informed breeding decisions within high-risk pedigrees.

Research Grant: Morris Animal Foundation D16EQ-311 and USDA Hatch Funds
Student Support: Office of the Director, NIH, T35 OD011145
Morphology of peritoneal endometriotic lesions using an in-vivo mouse model of retrograde menstruation

Victoria McLean, Quanxi Li, Romana Nowak
Department of Animal Sciences (McLean, Nowak) and Department of Comparative Biosciences (Li), University of Illinois at Urbana-Champaign, Urbana, IL

Endometriosis is a painful, chronic disease in which endometrial tissue grows outside of the uterus in women of childbearing age. The condition commonly causes pelvic pain, dysmenorrhea, and dyspareunia, while also resulting in infertility in 30-50% of cases. The theory of retrograde menstruation is the most commonly accepted mechanism of endometriosis, stating that blood and endometrial fragments flow backward through the oviduct and exit through the ovarian bursa into the peritoneal cavity. The purpose of this preliminary experiment is to investigate the morphology of peritoneal lesions using a mouse model of endometriosis that mimics the process of retrograde menstruation found in humans. Endometrial tissues from female mice primed with pregnant mare serum gonadotropin (PMSG) were transplanted into the peritoneal cavities of ovariectomized, syngeneic mice treated with estradiol (E2) at regular intervals. Immunohistochemistry (IHC) was performed on lesion sections using antibodies against epithelial marker claudin-4, proliferation marker Ki67, endothelial marker CD31, smooth muscle cell marker ASMA, and mesothelial marker calretinin. Previous studies reveal an abundance of proliferating stromal cells, myofibroblasts, and mature blood vessels within these sections, which this study also confirms, but it is unknown what processes are occurring in the mesothelial cells that are in contact with the endometrial cells. If the results confirm the disappearance of mesothelial cells, further studies will determine whether these cells are undergoing a mesothelial-to-mesenchymal-transition (MMT), causing them to lose their adhesive properties and secrete factors that may aid in the establishment of endometriotic lesions.

Research Grant: NIH 1R21 ES026388
Student Support: Office of the Director, NIH, T35 OD011145

Licorice root: a phytoestrogen supplement with neuroprotective effects on cognition

Caitlin E. Ondera, Payel Kundu, William G. Helferich, Donna L. Korol, Ikhlas A. Khan, and Susan L. Schantz
Dept of Comp Biosci (Ondera, Schantz), Neurosci Prog (Kundu, Schantz), Beckman Inst (Schantz), and Dept of Food Sci & Human Nut (Helferich),
Licorice root (*Glycyrrhiza glabra*) is a dietary supplement that is widely used for the treatment of menopausal symptoms in women. It contains the phytoestrogens liquiritigenin and isoliquiritigenin that exhibit estrogenic activity. Phytoestrogens are a promising alternative to estrogen replacement therapy, which increases the risk of cancer, stroke, and cardiovascular disease, but their efficacy and safety are not well understood. Estradiol administration to ovariectomized rats improves performance on hippocampal-dependent tasks but causes deficits on prefrontal and striatum-dependent tasks. Conversely, our previous research showed isoliquiritigenin improves performance on a hippocampal task and has no effect on a prefrontal task. This study focused on the effects of licorice root on the striatum to determine if it causes deficits similar to estradiol. Licorice root powder, licorice root extract, and isoliquiritigenin were administered orally to ovariectomized Long Evans rats. Estradiol positive and negative control groups were also included. After 3 weeks of exposure, rats were subjected to a striatum-dependent object recognition task. Rats were placed in a testing chamber and allowed to habituate to two stimulus objects. The familiar objects were then replaced with two novel objects. Object exploration time was recorded, and a pattern separation index was calculated between habituation and test trials. Rats that recognize the second pair of objects as novel have a greater pattern separation index, indicating better performance. This work may lead to the identification of a dietary supplement for treatment of menopausal symptoms that confers neuroprotective effects without the negative effects of other estrogens.

Research Grant: NIH, 5 P50 AT006268-06
Student Support: Office of the Director, NIH, T35 OD011145

**The molecular basis of mate preference: the retinal mosaic of the Bluefin Kilifish (*Lucania goodei*)**

Zachary Osterholz, Alexa Sadier, Rebecca Fuller

Department of Animal Biology (Osterholz, Fuller) and Department of Cell and Developmental Biology (Sadier), University of Illinois at Urbana-Champaign, Urbana, IL

Discerning links between physiology and behavior is essential to a better understanding of the evolutionary forces acting to determine how an organism responds to particular stimuli. The visual system of *L. goodei* is an
effective model for understanding how evolution regulates the physiology of sensory systems. *L. goodei* is a freshwater fish that occurs in a range of lighting environments from tea-stained swamps (low transmission of UV/blue light) to clear springs (high transmission of UV/blue light). Selection appears to favor blue anal fins in tea-stained habitats while males with red anal fins are more abundant in clear springs. The reigning hypothesis is that visual contrast against the background water column is essential for mate recognition. These differences in male abundance also correlate with variation in cone cell abundance and opsin expression, which may be related to differences in mating preference. This study sought to further investigate the molecular basis of the differences underlying variation in mate preference. Ten fish from a swamp population and ten from a spring population were collected. Their retinas were dissected and the retinal pigment epithelium bleached. Retinas were then stained for opsins, which are light-sensitive proteins containing visually active pigments that receive particular wavelengths of light ranging from red to UV. By simultaneously staining six distinct opsins using multiplexed in-situ hybridization, a “mosaic” of the spatial distribution of opsins was created. The mosaics of clear and swamp populations were compared to determine if population-level molecular dissimilarities resulting from variable selection underlay the behavioral differences in these populations.

Research Grant: University of Illinois School of Integrative Biology
Student Support: Office of the Director, NIH, T35 OD011145

**Venturing into the unknown: characterization of the aerobic cultivable bacteria in wounds of wildlife species**

*Courtney Pike, Lois Hoyer, Carol Maddox, Matthew Allender, Julia Whittington*
*College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL*

Traumatic injuries, including blunt-force trauma and bites, are common in wildlife species. A lack of data detailing the microbiota of wildlife wounds, as well as unknown history and limited resources, hamper treatment of these injuries. The goal of this work was to characterize the aerobic cultivable bacteria from wounded animals brought to a wildlife hospital during summer 2016. We hypothesized that the bacterial populations in wounds and on the peripheral skin would be similar. Our secondary hypothesis was that wounds caused by contaminated sources would contain additional bacteria not found on the skin. Upon admission, open wounds and the peripheral skin of wildlife species were individually sampled with a double-tipped swab, taking care to
avoid cross-contamination. One swab from each site was used to plate the sample on blood, MacConkey, and Columbia Naladixic Acid agar, then incubated for 16 hours. The second swab was used for Gram staining. Pure cultures were derived for further identification. Isolates were analyzed using Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry, which is a newly emerging, cost-effective, and time-saving method in diagnostic microbiology. Genus-level identifications were accurately achieved and deemed clinically sufficient for this study. Preliminary data suggest that MALDI-TOF is an attractive and inexpensive option for the wildlife veterinarian. Understanding the normal bacterial populations on the skin of wildlife species provides insight regarding bacteria that may be present in a wound and facilitates judicious antimicrobial use.

Research Grant: University of Illinois College of Veterinary Medicine
Student Support: Office of the Director, NIH, T35 OD011145

**Anti-Toxoplasma efficacy of green algae extracts**

*Jonathan L. Powers, William H. Witola*

*Department of Pathobiology, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL*

*Toxoplasma gondii* is an obligate, intracellular, zoonotic, parasitic protozoa that infects about one third of the world population. There are no drugs nor effective vaccines to control *T. gondii* in livestock. In humans, current drugs used against *T. gondii* are limited by hypersensitivity and toxicity, and are only effective against the tachyzoite stage of *T. gondii*. Treatment is necessary in immunocompromised patients and infants infected congenitally who may experience severe symptoms such as eye damage, neurological pathologies, and difficulty breathing. The main goal of this study is to evaluate the efficacy of green algae extracts in killing *T. gondii* proliferative-tachyzoite stages in *vitro*. Algae has been shown to possess antibacterial and antifungal properties, however, no studies have been performed to test whether it has any anti/protozoal effects. We obtained a pure culture of green algae, Chlorophyceae, and an isolate of pond green algae. The samples were dried and treated with either methanol or hexane. The methanol and hexane extracts were dried by evaporation and reconstituted in DMSO. Confluent human foreskin fibroblasts were infected with *T. gondii* tachyzoites expressing YFP. Varying concentrations of the algae extracts were added and the cultures incubated for 48 h. Parasite growth was quantified by fluorescent microscopy. We found that both the methanol and hexane extracts from the pure culture and pond algae had concentration-dependent anti-Toxoplasma effect at low microgram concentrations. We will derive anti-
Toxoplasma IC50 values and determine the cytotoxic IC50 values in HFF cells. Possible implications of this work may include a new treatment for a T. gondii infection in humans and animals.

Research Grant: USDA Agriculture and Food Research Initiative
Student Support: Office of the Director, NIH, T35 OD011145

Evaluating body condition score with body weight on serum drug levels of phenobarbital and potassium bromide

Chelsea F. Randall, Devon W. Hague
Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Epilepsy is one of the most common neurological diseases affecting companion animals and seizures are managed through the use of anticonvulsants. Anticonvulsants require regular therapeutic drug monitoring and medication adjustment by the use of a first-order kinetics equation; however it is not always successful. The aim of this retrospective project was to assess whether a patient’s body condition score (BCS) affects the serum level of phenobarbital and potassium bromide and determine if inclusion of this factor would result in an improved approach for medication adjustments. Data were collected from a retrospective review of canine epilepsy patients at University of Illinois Veterinary Teaching Hospital from January 1, 2000 to June 1, 2016. Records that reported a phenobarbital and/or potassium bromide dosage (mg/kg), serum level, and BCS were included in the study. The data set currently includes 113 dogs on phenobarbital, 29 dogs on potassium bromide, and 27 dogs on both therapies. Once the data set is complete, statistical analysis will assess whether BCS affects serum drug levels. A novel drug adjustment approach using BCS could transform epilepsy treatment and increase the patient’s quality of life.

Research Grant: None
Student Support: Merial Veterinary Scholars Program

Demonstrating C. botulinum neurotoxin heavy chain cytosolic localization via modified Kirby-Bauer assays

Abby Reising, Mengfei Ho, Jamie Perry, Melissa Pires-Alves, Ema Khan, Brenda A. Wilson
College of Veterinary Medicine (Reising) and Department of Microbiology,
*School of Molecular and Cellular Biology (Ho, Perry, Pires-Alves, Khan, Wilson), University of Illinois at Urbana-Champaign, Urbana, IL*

*Clostridium botulinum* produces the most potent toxins known to man, which inhibit acetylcholine release from neuronal cells causing flaccid paralysis. The long half-life of toxins in cells paired with the lack of an available antitoxin that can act once the protein has entered the cell makes exposure untreatable and lethal after the critical prophylactic window. This notion and the possibility of toxin use in bioterrorism makes it crucial to find a novel antitoxin. This study harnesses the binding domain (BD) and the translocation domain (TD) of the heavy chain (HC) of botulism neurotoxin serotype A (BoNT/A) to bind to neuronal cells, transfer from vesicles into the cytosol, and deliver therapeutic machinery necessary to prevent further neurotransmitter inhibition. The heavy chain was linked to a prototype cargo, beta-lactamase (Bla), to exemplify a means by which the cargo was successfully delivered to the cytosol with enzymatic activity intact. To test for this delivery, modified Kirby-Bauer assays were developed. The positive control protein of Bla-LD-BD that utilized a modified translocation domain (LD) and negative control protein of Bla-BD that lacks a TD or LD have been expressed, purified, and assessed for their activity via Kirby-Bauer assays. Absence of a zone of clearing around an ampicillin-treated disk, indicating the localization of beta-lactamase in the cytosol and the maintenance of its ability to cleave the beta-lactam ring of ampicillin, reflects successful delivery of the Bla cargo. If efficacious, the HC of BoNT/A or the modified LD-BD could be used not only as a vehicle for delivery of antitoxin cargo, but also as a means to deliver other new therapeutics to neuronal cells.

Research Grant: National Institute of Allergy and Infectious Diseases, NIH, R33 AI101504
Student Support: Office of the Director, NIH, T35 OD011145