Oklahoma State University
Center for Veterinary Health Sciences

Summer Research Training Program

2014
The Summer Research Training Program at Oklahoma State University is designed to identify talented and highly motivated veterinary students interested in exploring a career in veterinary research and then provide those students with an outstanding biomedical research summer training experience. The overarching objective of this program is to persuade outstanding veterinary students to pursue biomedical research careers. The program is structured to achieve this objective both explicitly, through formal training in the process involved in becoming a research scientist, and implicitly, by providing professional support and encouragement through informal interactions with successful veterinary research scientists who are excited about the personal and professional satisfaction gained from their careers.

The 12-week program experience gives first and second year veterinary students the opportunity to conduct a mentored summer research project. Students are assigned to a basic or clinical research faculty mentor for the summer. The mentors guide the students through all aspects of a research project including experimental design, methodology, data collection and analysis, and drawing conclusions. Students also receive specific instruction on a number of research-related topics, tour specialized research facilities in the region, present the results of their research at our annual College Research Day, and have the opportunity to travel to present their research at a national research meeting.

The combination of professional research training and personal exposure to positive role models who can convey a sense of excitement and career satisfaction has the greatest likelihood of success in our efforts to recruit the next generation of veterinary research scientists. Students who have participated in the program in the past have given it high marks for being fun, exciting and beneficial to their development.
Oklahoma State University’s Veterinary Research Scholars Program supported by competitive grants from National Institutes of Health

Merial Veterinary Scholars Program

Center for Veterinary Health Sciences
Healthy Animals – Healthy People
### Schedule

**May 12 – August 3, 2014**

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Symposium
Monday, July 28, 2014

Students and Mentors

Ashley Chandler .................. Dr. Brenda Smith
Peter Czajkowski ................ Dr. Ashish Ranjan
John Evans .......................... Dr. Carey Pope
Taylor Farris ..................... Dr. Jennifer Grindstaff
Kate Gault .......................... Dr. Susan Little
Mary Girton ................... Dr. Sai Tummala (OMRF)
Derek Hagan ...................... Dr. Lin Liu
Alia Houser .................. Dr. Junpeng Deng
Ellen Jackson .............. Dr. Véronique Lacombe
Casey Landis .................. Dr. Robert Fulton
Delaina Skinner .............. Dr. Bruce Noden
Stasia Sullivan ................. Dr. Lyndi Gilliam
Effects of Phenolic Compounds in Berry Extracts on the gut mucosal immunity

Ashley Chandler, Jennifer L. Graef, Vinh Nguyen, Erica Crockett, Brenda J. Smith

OSU Department of Nutritional Sciences

Foods rich in phenolic compounds, such as berries, have health benefits associated with their anti-inflammatory properties. Due to the phenolic compounds relatively low bioavailability, the mechanism through which the immune response is altered remains unclear. This study was designed to investigate the effects of phenolic compounds on the gut mucosal immune response utilizing an animal model of diet-induced obesity and a cell culture system. For the in vivo study, 6-wk-old male C57BL/6N mice (Charles River) were randomly assigned to four groups: Con=AIN-93 control diet (10 % fat kcals), high fat diet (HF 45% fat kcal), or HF diet supplemented with 25% dried berry or HF + antibiotics. Throughout the 8 wk treatment period, food intake and body weight were recorded. At the end of the study, body composition was determined using dual-energy x-ray absorptiometry (LunarPIXI, GE Medical Systems) and Peyers’ Patches from the ileum and jejunum were dissected. Alterations in cytokine gene within the Peyers Patches will be evaluated using qRT-PCR (7900HT Fast Real-Time, Applied Biosystems). In vitro, co-cultures of human intestinal epithelial cells (CACO-2, ATCC) and T-Lymphocytes (Jurkat, ATCC) will be used to further explore the direct effects of phenolic compounds from berries on the gut mucosal immune response under normal and inflammatory conditions. Understanding the extent to which phenolic compounds alter the immune response will provide useful data related to the prevention and treatment of many health concerns.

Acknowledgement- This research was supported with a grant from the National Institute of Health, grant number T35 OD011186-19, the Oklahoma Agriculture Experiment Station, and the OSU Department of Nutritional Sciences laboratory.
High Intensity Focused Ultrasound Mediated Thrombolysis in combination with echogenic Liposomes

P. Czajkowski, D. Maples, K. Ektate, A. Ranjan
Center for Veterinary Health Sciences, Stillwater, Oklahoma

Introduction - Plasminogen activator is currently the standard of care, first line treatment for thrombosis despite its clinically significant toxicity for the patient. There is a critical need to develop novel methods that can limit side effects and enhance thrombolysis. Objectives of this study were: 1) formulate gas-filled echogenic liposomes (EL) for enhanced thrombolysis, and 2.) characterize in vitro clot lysis of EL in combination with High intensity focused ultrasound (HIFU)

Materials and Methods - Caprine blood clots were formed by placing 2 mL of blood in dialysis tubing. EL was prepared by thin film dispersion method, and loaded with an echogenic agent (Perfluropentane, or PFP). Three test groups were assessed: EL, PBS (negative control), and free PFP (positive control). Each of the test groups were treated with a 1 MHz HIFU transducer for 50 seconds with duty cycle 25% and power 100W, generating a peak negative pressure of -9 MPa. Results were recorded as measurements of the clot using an 8 MHz ultrasound pre and post treatments. Treatment groups were compared for differences in mean percent volume reduction.

Results - EL treatment in combination with HIFU resulted in a significantly greater reduction in clot size compared to the controls. Quantitatively, average reduction in clot volume achieved using EL plus HIFU was approximately 18% greater than corresponding PBS. Percent clot reduction achieved by free PFP plus HIFU was not significantly different than EL, as was expected.

Conclusion and future works - Preliminary results suggest that the combination of EL and HIFU can achieve significant clot lysis. Studies are currently underway to determine the thrombolytic activity of tPA loaded EL to determine the targeting profile of ELs plus HIFU vs tPA alone. This technology can ease clinical therapy of intravascular blood thrombi with fewer side effects than thrombolytics.

Acknowledgement - This research was supported with a grant from the National Institute of Health, grant number T35 OD011186-19, and the CVHS laboratory of nanomedicine and targeted therapy seed support.
Effects of the Cannabinoid Receptor Antagonist AM251 on Chlorpyrifos Toxicity in Mice.

J. Evans, J. Liu and C. Pope, Center for Veterinary Health Sciences (CVHS), Oklahoma State University, Stillwater, OK.

Organophosphates (e.g., chlorpyrifos, CPF) elicit toxicity by inhibiting acetylcholinesterase (AChE), leading to accumulation of the transmitter acetylcholine. Cholinergic toxicity may be modulated by endocannabinoids (eCBs) by reducing acetylcholine release at presynaptic terminals. Some organophosphates can increase eCB levels by inhibiting enzymes (e.g., monoacylglycerol lipase, MAGL) that degrade them. We hypothesized that blocking eCB signaling with the cannabinoid receptor antagonist AM251 would increase the toxicity of chlorpyrifos. Young adult, male C57 mice were treated with CPF (0, 150, 200 or 250 mg/kg), and then given vehicle or AM251 daily for 3 days (n=4/group). Body weights and signs of toxicity were evaluated daily for 7 days and then tissues were collected for biochemical assays. CPF reduced body weight (about 10-15% from 24-72 hr). Functional signs of toxicity were noted from 24-72 hr in some treated animals, but by 96 hr no signs were observed. Lethality (38-50%) was noted in all CPF treatment groups from 48-96 hr after dosing. There was a trend towards lesser functional signs and decreased lethality in AM251 post-treated mice. Forebrain and hindbrain AChE activity was significantly inhibited (45-68%) in all three CPF treatment groups, while AChE activity in liver was inhibited >90%. MAGL activity was lesser but similarly inhibited (30-50%) in all three tissues. The results suggest that CPF elicits classical signs of cholinergic toxicity in C57 mice, associated with prolonged AChE and MAGL inhibition. Blocking cannabinoid receptors may modify the expression of CPF toxicity.

Supported by T35 OD011186-19, CVHS Research/Graduate Education and Administrative Affairs Offices, R01 ES009119, and the OSU Board of Regents.
Effects of 17α-ethinylestradiol on Mating Behavior of Zebra Finches (Taeniopygia guttata)

Taylor Farris¹, Madeleine Naylor², Jennifer Grindstaff²

1-College of Veterinary Medicine, Oklahoma State University, 2-Department of Zoology, Oklahoma State University

The synthetic estrogen 17α-ethinylestradiol (EE2), used in oral contraceptive pills, is an endocrine disrupting chemical frequently found in ecosystems receiving untreated waste-water. EE2 has negative organizational effects on a diversity of organisms at environmentally relevant levels, but few studies have considered the potential activational effects of EE2. We studied the activational effects of EE2 on both mate choice and courtship behavior in a sexually dimorphic bird species; the Zebra Finch (Taeniopygia guttata).

Courtship behaviors are crucial to pair bonding. We hypothesized that females would prefer control males to males dosed with EE2 in mate choice trials and that males dosed with EE2, particularly high levels, would exhibit abnormal courtship behavior. Males and females were divided evenly amongst four treatment groups: control, 0.03, 4.0, and 100.0 ng EE2. The 0.03 ng EE2 dose serves to mimic low environmental contamination levels while the 4.0 dosage mimics high levels of contamination. After orally dosing birds every other day for 3 weeks, we quantified mate choice preferences of females shown two differently dosed males simultaneously. We assessed mate choice behavior by recording the time females spent with each male, as well as the number of beak wipes, tail flutters, hops, and turns. Next we housed females with their preferred male to conduct courtship trials. Male behavior, including mounting attempts and beak wipes, was compared to determine if EE2 inhibits courtship behavior. Preliminary results suggest EE2 exposure does not affect mate choice preference of females or the attractiveness of males. However, males dosed with higher levels of EE2 (4.0 or 100.0 ng) were less likely to attempt mounting. We conclude that EE2 may have negative activational effects on males at high environmentally relevant doses because it decreases their propensity to mount a female; with potential direct negative effects on fitness.

Survival and elimination of ticks in home environments

Kate Gault¹, Anne Barrett¹, Mason Reichard¹, Susan Little¹

¹Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, Oklahoma, USA

The brown dog tick, *Rhipicephalus sanguineus*, commonly infests homes and kennels; other species of ticks are not thought to survive indoors long term. Currently, acaricidal treatment is recommended for environmental brown dog tick infestations, but thermal treatment is commercially available for home infestations with other arthropods such as bed bugs (*Cimex lectularis*). To evaluate thermal treatment as an alternate method of eliminating environmental stages of *R. sanguineus*, we placed adult, nymph, and larval brown dog ticks at 47.3 – 56.1°C and 5.0% +/– 3.0% RH for 1-3 hours and evaluated for viability immediately and at 24 and 48 hours after thermal treatment. To compare survival duration of different tick species after introduction to a climate-controlled building, we placed adults and nymphs of *R. sanguineus*, *Dermacentor variabilis*, *Ixodes scapularis*, and *Amblyomma americanum* in air-permeable, sealed containers at four different indoor locations (two homes, one office, one laboratory) and monitored viability of each cohort daily. To determine if standard laundering would kill ticks in bedding and garments, adults and nymphs of each species were placed inside fabric pouches and run through a hot-wash/ high-heat dryer, or dryer alone, with temperature monitoring. Minimum environmental temperature and duration that resulted in ≥95% mortality of adults, nymphs, and larvae of *R. sanguineus* was 51.2°C for two hours, one hour, and one hour, respectively. All species of ticks survived washing alone but were killed in the dryer (45 minutes, average 53.1°C), supporting standard laundering as an effective means of eliminating environmental ticks. Final data on survival longevity of ticks indoors are pending, but thermal treatment appears to be an effective alternative to acaricides for environmental *R. sanguineus* infestations if adequate temperature and duration is reached.

Funding provided by the Merial Veterinary Scholars Program and the Krull-Ewing Endowment, Oklahoma State University
Glioblastoma multiforme (GBM) is an aggressive high-grade glioma type commonly diagnosed each year. Despite current available treatments, GBM maintains a poor prognosis with few patients reaching the 3 year survival mark. A new biomarker, Spondin1, has been recently found to be overexpressed in various cancers, and was previously discovered using GAMMA (Global Microarray Meta-Analysis) to be an extracellular matrix protein associated with cell adhesion, angiogenesis, cell migration, and cell growth. These developments have indicated Spondin1 as a possible target for antibody therapy against glioma progression. This hypothesis was tested using a GL261 mouse glioma model in male C57BL6 mice treated with an anti-Spondin1 antibody agent (n=7), and compared them to those with untreated tumors (n=7). Tumor volumes were monitored every 2-3 days after day 12 of intracerebral post implantation using morphological magnetic resonance imaging (MRI), and angiogenesis was measured at the final time point using magnetic resonance angiography (MRA). Cell migration was measured via western blot analysis of c-Met expression. Preliminary data in GL261 glioma-bearing mice shows there to be a significant decrease in tumor volume and angiogenic activity in mice receiving the anti-Spondin1 antibody treatment compared to those receiving no treatment. Survival time was significantly increased in those mice receiving anti-spondin1 antibody treatment compared to those not receiving treatment. There was a reduction in c-Met protein expression in treated mice compared to the non-treated mice. These results indicate the possibility of utilizing anti-Spondin1 antibody as an additional treatment option against high-grade gliomas resulting in an improved prognosis for patients.

Funding was provided by the National Institutes of Health (T35 OD011186-19)
Novel function of DVL2 during influenza A virus infection

Derek Hagan, Sunil More and Lin Liu
Oklahoma State CVM, Stillwater, OK

The Influenza A virus (IAV) is a leading cause of deaths due to pneumonia and secondary bacterial infection in the world. The virus targets alveolar and bronchial epithelial cells in the lungs. Influenza virus has a small genome so it is dependent on host factors for carrying out various stages of its life cycle. Also influenza virus replication mechanism is error prone which enable it to become resistant to current antiviral drugs. So there is a need to find host factors which virus uses for its replication and develop drugs against those host factors. A drug targeted against host factor is less likely to develop antiviral drug resistance. Wnt signaling, a host signaling pathway has been implicated during the influenza virus infection. Various components of this pathway have been reported to have interaction with influenza virus proteins. In our study of the influenza virus we have noticed down-regulation of DVL2 (Dishevelled 2) protein during viral replication in human lung epithelial (A549) cells and human kidney epithelial (HEK293) cells. DVL2 plays a role in both canonical and non-canonical Wnt signaling; specifically it binds to the cytoplasmic C-terminus of frizzled trans-membrane protein and transducers the Wnt signal to down-stream effectors. We hypothesize that gene over expression for the DVL2 protein during IAV infection will cause a significant decrease in virus replication. We are going to employ strategies of over expression as well as silencing of DVL2 to in 293T cells with plasmid vectors. We expect that silencing DVL2 will further increase virus replication, while over expression will reduce the virus replication. Furthermore if we see DVL2 over expression reduces virus replication we would like to define the mechanism of antiviral properties of DVL2 by looking at the interacting partners either in host or in virus genome.

Financial support provided by National Institutes of Health (T35OD011186-19)
Camelid Single Domain Antibody facilitated X-ray Crystallography: Towards structural determination of Poxvirus Protein A6

Alia Houser, Bing Zhang, Junpeng Deng, and Yue Han, Oklahoma State University, Stillwater, OK

Poxvirus is unique in that it infects a wide variety of vertebrates and replicates entirely within a cell’s cytoplasm. The vaccinia virus A6 protein has been discovered to be a key player in poxvirus morphogenesis. It is required for the recruitment of membranes and other important proteins from the secretory compartments to the viral factories. Although the sequence of A6 is highly conserved through all vertebrate poxvirus, there are no known homologs outside the poxvirus family. To date, the mechanism by which A6 functions remains unknown, therefore we aim to reveal the structure of A6 by x-ray crystallography to obtain insight into its role. However, the A6 protein has been recalcitrant to crystallization, necessitating a new approach we employ here using camelid single domain antibody (Sd/Ab) as a chaperone in improving crystal lattice formation. Sd/Ab lowers the surface entropy by stabilizing the flexible regions of the target protein, while creating crystal lattices through antibody-mediated interactions. A6 was constructed as a SUMO fusion protein bearing a N-terminal His6-tag. The protein was over-expressed in E.coli BL21gold DE3 and purified by affinity method using Ni-NTA resin followed by size exclusion chromatography. Individually purified A6 and Sd/Ab were mixed at a 1:1 ratio and the complex was purified on a size exclusion chromatography column. The A6:Sd complex was concentrated to 16 mg/mL for crystallization. A Gryphon Robot from Art Robbins was used for setting up crystallization screening experiments using more than 1000 conditions at two temperatures. We will be collecting x-ray diffracting data from A6 crystals using synchrotron radiations for structure determination. The discovery of the A6 structure will lead to a new understanding of poxvirus membrane formation and possible clues for development of novel pharmaceuticals in the future.

Student funding provided by NIH T35 OD011186-19 and Lab funding provided by NIAID R01AI081928.
The Novel Role of Toll-like Receptor 4 during Peripheral and Cardiac Insulin Resistance

Ellen Jackson, Elizabeth Rendina-Ruedy, Matt Priest, Brenda Smith, and Veronique Lacombe, Oklahoma State University, Stillwater, OK

Diabetes is an epidemic disease characterized by alterations in glucose transport, which is tightly regulated by a family of specialized proteins called the glucose transporters (GLUTs). Diabetic humans are at a high risk to develop cardiovascular disease. Toll-like receptor (TLR) 4, a central part of the innate immune system, may play a critical role in linking inflammation and metabolic disease. We hypothesized that TLR4 activation triggers cardiac insulin resistance. We used mice with a loss-of-function mutation in TLR4 (C3H/HeJ) and age-matched wild-type (WT, C57BL/6) mice (n=8/group) to investigate how feeding a high-fat diet (HFD, 60% kcal from fat) for 16 weeks affected whole-body and cardiac glucose metabolism. After 16 weeks, WT mice fed a HFD were obese and developed hyperglycemia and peripheral insulin resistance compared to WT mice on a control diet (10% kcal from fat). The C3H/HeJ mice were partially protected against these detrimental effects. WT mice fed a HFD had a 30% decrease (P=0.03) in GLUT4 protein content as measured by Western Blot of cardiac crude membrane protein extracts. In contrast, C3H/HeJ mice had partially rescued cardiac GLUT4 content in the face of a HFD. Interestingly, there was a 40% increase (P=0.015) in the novel GLUT isoform, GLUT8, in the heart when mice of either genotype were fed a HFD. Further, GLUT4 protein content was negatively correlated with GLUT8 in the heart, suggesting that these two transporters may be linked mechanistically. While no significant difference in SOCS3 expression was noted between groups, enhanced SOCS3 expression paralleled with increased abundance of GLUT8 content in the heart. Data collection for IL-6 is ongoing. In conclusion, these data suggest that activation of TLR4 during diabetes and obesity alters glucose transport. Additionally, the positive correlation between GLUT8 and some inflammatory markers supports the concept that GLUT8 may mediate the deleterious metabolic effects of long-term HFD.

Student support: AVMF/AVMA 2nd Opportunity Summer Scholars Grant
Research support: Oklahoma State University Center for Veterinary Health Sciences funds
Identification and Study of Viruses Contributing to Bovine Respiratory Disease

Casey Landis, Oklahoma State University, Stillwater OK.

Bovine respiratory disease (BRD) is one of the most damaging diseases to affect the beef cattle industry through the cost of treatment and the losses from morbidity and mortality. BRD affects both the upper and lower respiratory tracts and has symptoms including fever, coughing, eye and nasal discharge, and loss of appetite. Different viruses can contribute to BRD including bovine viral diarrhea virus (BVD), bovine respiratory syncytial virus (BRSV), bovine coronavirus (BCV), and parainfluenza 3 virus (PI3V). The experimental approach includes performing RNA extraction, reverse transcriptase enzyme reaction, and polymerase chain reaction. The amplified nucleic acid segments were then visualized with agarose gel electrophoresis and compared to positive viral controls. This process was executed using samples from cattle with BRD symptoms, or that had been exposed to BRD. Thus far five positive results for BVD and ten positive results for PI3 have been acquired from serum samples and nasal swabs. The samples tested for BVD were taken from six calves which had prior positive immunohistochemistry results. Five tested positive for BVD type 1, while the sixth was ruled a false positive. Samples that tested positive for PI3 were collected from calves in an experimental feedlot. Four of the positive samples had already tested positive for PI3 through cell culture testing. The other four positive samples were taken from three sick calves and were previously tested and reported as unidentified. Two other positive PI3 samples came from a research feedlot containing calves persistently infected with BVD. All of these results prove that molecular testing is an effective method for identifying viruses present in a sample, and yields results more quickly and accurately than other methods.

Student Financial Support: Niblack Research Scholars Program
Prevalence of tick infestation and *Ehrlichia* spp. in Oklahoma black bears (*Ursus americanus*)

Delaina Skinner¹, Jessica Mitcham², Bruce Noden², Lindsay Starkey¹, Eileen Johnson¹, Sue Fairbanks³, Susan Little¹

¹Department of Veterinary Pathobiology, ²Department of Entomology and Plant Pathology, and ³Department of Natural Resources and Environmental Management. Oklahoma State University, Stillwater, Oklahoma, USA

Black bears (*Ursus americanus*) are native to Oklahoma but were largely absent from the early 1900s until the 1980s due to unregulated hunting and habitat destruction. Successful reintroduction of black bears in the Ouachita and Ozark mountains in Arkansas resulted in a substantial increase in their numbers in eastern Oklahoma in recent years, but little information is available about ticks and tick-borne infections in this population. To evaluate tick infestations and tick-borne infections of this expanding bear population, we collected ticks and blood samples from live bears trapped in two different sites using barrel and snare traps. Ticks were identified by standard keys and *Ehrlichia* spp. infection status of up to 10 ticks per bear determined by PCR targeting a 16S rDNA fragment. Whole blood and serum samples were evaluated using PCR, commercial ELISA (SNAP® 4Dx® Plus), and/or IFA. To date, samples have been collected from a total of 31 bears. Ticks were present on every bear examined (31/31).

*Amblyomma americanum* was the most common tick found, accounting for 89.5% (367/410) of all ticks identified, and was present on every bear examined; *Dermacentor variabilis* were also present on 9/31 (29%) bears. *Ehrlichia chaffeensis* DNA was detected in 2/46 (4.3%) adult *A. americanum* ticks removed from bears, but has not been identified in blood samples tested to date (0/23). Antibodies to *Ehrlichia* spp. were identified by commercial ELISA in every bear tested (27/27); IFA results are in process. The study is ongoing, but based on these initial results, bears in eastern Oklahoma are commonly infested with *A. americanum* and frequently infected with *Ehrlichia* spp., although PCR has not revealed evidence of active infection to date. Further data collection will enhance understanding of ticks and tick-borne pathogens of black bears and zoonotic potential as the population continues to expand.

Funding provided by the Merial Veterinary Scholars Program and the Krull-Ewing Endowment, Oklahoma State University.
In vitro echinocytosis development secondary to rattlesnake and copperhead venom in the dog and the horse.

Stasia Sullivan, Lyndi Gilliam, Robin Allison, Jared Taylor, Jonathan Bagwell, Pi Jie Yang

Oklahoma State University Center for Veterinary Health Sciences, Stillwater, OK

Oklahoma is home to several species of venomous snakes, presenting a significant risk of envenomation to dogs and horses. The type of snake that bit the animal is often unknown. Animals bitten by rattlesnakes are more likely to need antivenom than those bitten by other venomous snakes; determining the type of snake that caused the bite is beneficial in directing treatment. It has been suggested that echinocytosis in snake bitten dogs indicates rattlesnake envenomation. However, echinocytes have been seen in known cases of copperhead envenomation (unpublished data). The objectives of this study were to document echinocytosis secondary to copperhead venom in dogs and horses using an in vitro model and to determine if echinocytosis occurs in a dose-dependent manner as previously demonstrated with rattlesnake venom. Fresh blood samples from healthy adult dogs and horses were exposed to six different concentrations of reconstituted venom from the western diamondback rattlesnake (*Crotalus atrox*) and the broad-banded copperhead (*Agkistrodon contortrix lacticinctus*). Samples were also mixed with sterile distilled water as a control. Blood smears were made from each sample at 5 and 10 minutes after adding the venom, air-dried and stained by Romanowsky procedure. Each smear was evaluated by light microscopy and 500 cells were counted to determine the percentage of Type I, Type II and Type III echinocytes. To date, echinocytes have been noted after mixing blood from one horse and four dogs with copperhead venom. The type and number of echinocytes appears to be time and concentration dependent. These results question the validity of using the presence of echinocytes to confirm rattlesnake envenomation.
National Institutes of Health
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and the
Office of Research and Graduate Education

Summer Research Training Program
2014