

LSU

School of Veterinary Medicine

Department of Comparative Biomedical Sciences



Annual Retreat 2025

**30 October
2025**

Room #2150

LSU School of Veterinary Medicine, Skip Bertman Drive, LA 70803



Graduate Student Retreat

Department of Comparative Biomedical Sciences

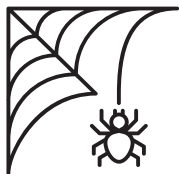
Thursday, October 30, 2025

LSU Vet School - Room 2150: Skip Bertman Drive, Baton Rouge, LA, 70803

This retreat was supported by the Michael Graziano CBS Excellence Fund. The awards and lunch were made possible by a generous donation from Avantor, Oxford Instruments, Nikon, and Thermo Fisher.

Organizing Committee: Anthony Ayanshina, Steven Bradley, Sharon Brown, Hannah Holmberg, Tanya Kramer, Alexander Lee, Ignitius Lim, Jimmy Lu, Demetrius McAtee, Alexander Murashov, Kimia Nourozi, Michael Ogundele, Sadia Sinza, and Ajn Vats





CBS Graduate Student Retreat

 **Thursday, October 30, 2025 | Room VMED 2150**

Join us for a spooky day of science, costumes, posters, and prizes!

Morning Session

- 8:00–8:45 – Breakfast, Poster Setup, and Presentation Loading
- 8:45–9:00 – Welcome Remarks: Dr. A.K. Murashov & Dean Garden
- **9:00–10:24 – Student Oral Presentations 1**
 - 9:00 Melanie Wilson
 - 9:12 Demetrius McAtee
 - 9:24 Angel Casillo
 - 9:36 Cheng Lu
 - 9:48 Hannah Holmberg
 - 10:00 Biplov Sapkota
 - 10:12 Abhishek Pandit

• **10:24–11:10 – Vendor Show / Poster Session 1**



- 11:10–11:15 – Introduction: Dr. Shisheng Li
- **11:15–12:15 – Keynote Speaker: Dr. Wentao Li**

Midday

- 12:15–1:00 – Networking Lunch with Faculty, Guests, and Alumni
- **1:00–1:55 – Vendor Show / Poster Session 2**

-  • **1:55–2:10 –  Halloween Fashion Show (Costume Competition)**

Afternoon Session

- **2:10–3:22 – Student Oral Presentations 2**
 - 2:10 Ajn Vats
 - 2:22 Ignitius Lim
 - 2:34 Sadia Sinza
 - 2:46 Rana Fallahsafa
 - 2:58 John Kara
 - 3:10 Kimia Nourozi
 - 3:22 Anthony Ayanshina

- 3:34–3:44 – Judging + Break

Finale

- **3:44–4:00 – Student Awards, Guest Recognition, and Closing Remarks**
- 4:00–4:05 – Group Photo



Guest of Honor



A veterinarian, clinical pharmacologist, teacher, researcher, and academic leader, Dr. Cyril R. Clarke became the Executive Vice President and Provost of Virginia Tech in January 2019, after serving in an interim capacity since November 2017.

A native of Johannesburg, South Africa, Clarke earned his professional veterinary degree from the University of Pretoria, South Africa, a Ph.D. in veterinary pharmacology from Louisiana State University, and a master's degree in higher education from Oklahoma State University. He is certified as a Diplomate of the American College of Veterinary Clinical Pharmacology.

Clarke's initial faculty appointment in 1987 was at Oklahoma State University, where he also served as an academic department head and associate dean for academic affairs in the Center for Veterinary Health Sciences. Funded by corporate, state, and federal agencies, including the U.S. Department of Agriculture and National Institutes of Health, Clarke's research focused on the interactions between antibacterial agents, animal patients, and infectious microbes. He is a recipient of the Pfizer Award for Research Excellence.

In 2007, Clarke was appointed to the position of Lois Bates Acheson Dean of the College of Veterinary Medicine at Oregon State University. During his time as dean, Clarke continued to teach pharmacology to veterinary students. In addition to receiving a Certificate of Excellence in Teaching, Clarke was honored with the Oregon Veterinary Medical Association's President's Award. He subsequently joined Virginia Tech in October, 2013, as Dean of the Virginia-Maryland College of Veterinary Medicine.

Clarke has held leadership positions in several professional organizations, including the board of directors for the Association of American Veterinary Medical Colleges and past president of the American College of Veterinary Clinical Pharmacology. He is also a past member of the National Agricultural Research, Extension, Education, and Economics Advisory Board and the AVMA Council on Education, the accrediting agency for veterinary medical education in North America.

Guest of Honor



Dr. Michael Graziano received a Bachelor of Science degree in Animal Science at Rutgers University (1979), a Master of Science degree in Veterinary Toxicology at Louisiana State University (1981), a PhD in Toxicology at the University of Kentucky (1985), and was a post-graduate Research Toxicologist in Pesticid Chemistry and Toxicology at the University of California-Berkeley (1985-1987).

After his post-doc, Dr. Graziano started his pharmaceutical career with Parke-Davis/Warner-Lambert (1987-2001) and then at Pfizer (2001-2003) which acquired Parke-Davis/Warner-Lambert in 2001. While at Parke-Davis and Pfizer, Dr. Graziano became Toxicology Director for the Anticancer and Antibacterial portfolio and conducted toxicology studies to support

the development of Lipitor, Gabapentin, Lyrica, and Palbociclib.

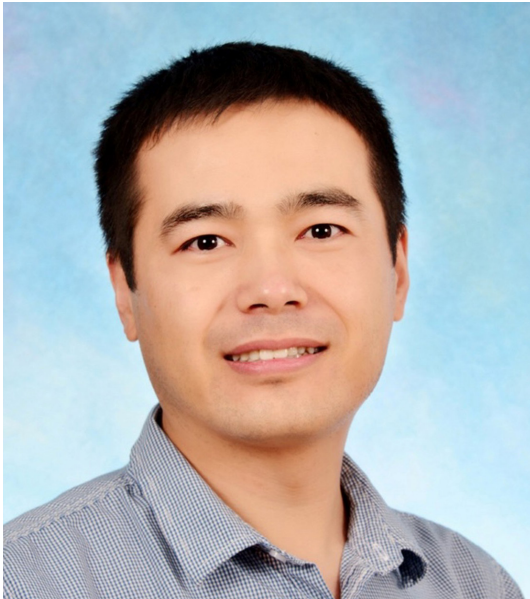
He left Pfizer in 2003 to join Bristol-Myers Squibb as an Executive Director and then was named Vice-President of Drug Safety Evaluation in 2006. In this role, Dr. Graziano was responsible for overseeing the GLP nonclinical safety testing and regulatory filings for all drugs within the BMS portfolio. He was also responsible for the Veterinary Sciences organization which provided animal care and husbandry for all BMS research facilities. Dr. Graziano was also a member of various senior governance and leadership committees. He led a department of more than 250 people that filed more than 100 INDs and supported the development and registration of many notable drugs including Eliquis, Opdivo, Yervoy, Farxiga, and Sotykto among others.

Dr. Graziano retired from BMS in 2021 but didn't like retirement, so he became Associate Vice-President of Discovery and Nonclinical Development at Organon (2021-2024), a new Women's Health company that was spun off from Merck. In this role, Dr. Graziano was responsible for building a new preclinical development team and for overseeing the integration and strategic direction of Organon's discovery organization in Turku, Finland. He was also heavily involved in various Business Development projects which helped to create Organon's early development pipeline.

Dr. Graziano retired a second time after 3 years at Organon and started his consulting business. One of his clients, QuantX Biosciences asked him to join full-time last year, where he is currently a Distinguished Fellow in Toxicology. He is also a consultant for Maip Therapeutics and Beech Biotech. These are small biotech start-ups where Dr. Graziano provides nonclinical expertise for lead compound characterization, GLP safety testing, and filing INDs.

Over his career, Dr. Graziano has authored/co-authored 1 book and more than 100 abstracts and publications, mainly dealing with the nonclinical safety of new therapeutics. He also served on various professional and industry consortiums throughout his career, many in a leadership role, including PhRMA Deputy Topic Leader for the new ICH S1 guidance on carcinogenicity testing. He was also a diplomate of the American College of Toxicology for 30 years.

Guest Speaker



Dr. Wentao Li is an Assistant Professor in the Department of Environmental Health Science at the University of Georgia College of Public Health. He earned his Ph.D. in Biomedical and Veterinary Medical Sciences from Louisiana State University, where he conducted his doctoral research in the laboratory of Dr. Shisheng Li in the CBS department.

Dr. Li completed postdoctoral training with Dr. Aziz Sancar at the University of North Carolina at Chapel Hill. In 2020, he established his independent research program at the University of Georgia. His work examines DNA damage and repair induced by environmental carcinogens, with a focus on nucleotide excision repair and the role of 3D nuclear architecture in maintaining genomic integrity.

Cancer Biology, Oncology, and Mutagenesis



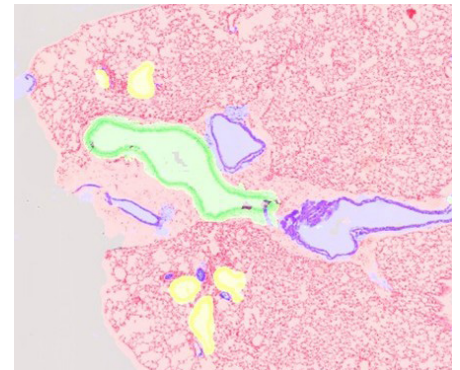
Joseph Francis, DVM, Ph.D.

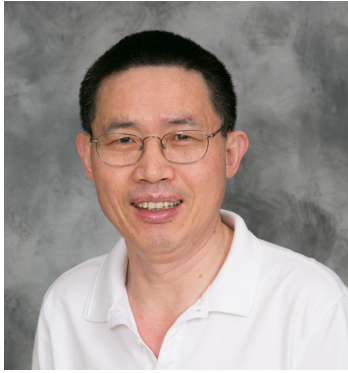
My research focuses on elucidating the role of cytokines and inflammatory pathways in the pathophysiology of chronic diseases, including heart failure, hypertension, metabolic syndrome, post-traumatic stress disorder (PTSD), and cancer. Over the past decade, I have concentrated on identifying the contribution of inflammatory molecules within the brain in PTSD and heart disease, exploring their impact on neuroimmune communication and stress-related pathology. More recently, my work has expanded to investigate plant-based therapeutic strategies, with a particular emphasis on cancer and PTSD, using both pharmacological and non-pharmacological interventions. This includes studying the bioactive potential of dietary agents, such as blueberries, in modulating inflammation and disease progression. In parallel, I am advancing research in multicellular organoid development to establish models for cancer precision therapy, enabling patient-tailored approaches and bridging experimental findings with translational applications.



Tomislav Jelesijevic, DVM, Ph.D.

Dr. Tomislav Jelesijevic is an assistant professor of histology at the CBS Department. He is a broadly trained, research-oriented, board-certified veterinary anatomic pathologist with an MSc degree in oncology (canine mammary gland tumors) and expertise in high-containment (BSL-3) work, acquired during his PhD in virology (SARS-CoV) and postdoctoral training in vaccine development for glanders and melioidosis. He is interested in studying gonadotropins roles in tumor development and discovery of antiviral treatments. Recently, he developed an interest in research related to machine learning (AI) applications in histology and pathology. After 60+ hours of training Olympus Deep Learning AI correctly identified background (light gray), nearly perfectly outlined the lungs (pink), and recognized blood vessels (blue), bronchi (green), and bronchioles (yellow).



**Shisheng Li, Ph.D.**

Living organisms are constantly exposed to a wide array of DNA-damaging agents. To safeguard genomic integrity, they rely on robust DNA repair and damage-bypass mechanisms that either remove or tolerate these lesions, thereby ensuring survival. Defects in these processes can lead to genomic instability, which is associated with cancer, neurodegeneration, premature aging, and various other human diseases. Our research focuses on elucidating the molecular mechanisms of DNA damage, repair, and mutagenesis.

**Shang Su, Ph.D.**

The Su Lab endeavors to decipher the interactions between the tumor micro-environment and cancer cells during the establishment and development of tumor metastases, with emphases on prostate cancer bone metastases. We are using in vitro, in vivo, and in silico multi-disciplinary approaches to explore the underlying molecular mechanisms that drive the key events in metastases and develop novel targeted strategies against the key drivers identified for the purposes of delaying or stopping metastases.

Oral Presentations

Oncolytic virotherapy and chemotherapy exhibit synergistic antitumor effects in murine models of pancreatic cancer

Biplov Sapkota¹, Naveen Chintala¹, Shreya Pokharel¹, Poorna Sai Vaddi¹, Mohammed Tanveer Hussain², Brent Stanfield², Konstantin Gus Kousoulas², Joseph Francis¹

¹Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA

²Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA

Pancreatic ductal adenocarcinoma (PDAC) accounts for nearly 90% of all pancreatic cancer cases. The 5-year survival rate of just 11% in PDAC is linked to late diagnosis, rapid recurrence, and an immunologically “cold” tumor microenvironment (TME). Therapies that convert this immune-scarce TME into an immune-active state may significantly improve patient outcomes. Oncolytic virotherapy can be a promising strategy in this context.

We hypothesize that combining oncolytic virotherapy with standard chemotherapy will synergistically reduce PDAC growth and metastasis. The research questions we are interested in are: (a) Can OV therapy alone reduce metastatic burden? (b) Does combining OV with gemcitabine improve control of primary tumors and metastasis compared to monotherapies? (c) Which pathways are differentially modulated under mono- and combination therapies?

We are evaluating HSV-1(VC2) engineered to express murine GM-CSF in immunocompetent orthotopic KPC mouse models of PDAC. Key techniques used in the study include intratumoral OV administration, gemcitabine chemotherapy, Nanostring RNA profiling, in vitro synergy assays using Bliss independence models, and organoid-based mechanistic studies. Preliminary findings show that OV monotherapy reduces liver metastases without affecting primary tumor volume. Transcriptomic profiling indicates suppression of metastasis-associated chemokines (CCL2, CXCR4), relief of immune checkpoints (CTLA-4, PD-L1), and downregulation of proliferation markers (Ki-67, PLK1). In optimization trials, low-dose OV (~10⁵ PFU) combined with gemcitabine (100 mg/kg weekly) reduced primary tumor burden compared to either treatment alone.

Ongoing studies are testing whether OV plus gemcitabine produces durable synergy in reducing metastasis and improving survival. These results may highlight a clinically translatable strategy to convert PDAC from an immune “cold” to “hot” tumor.

Funding: This work was supported by funding support from Cancer COBRE No. IP20 GM135000.

Keywords: Pancreatic ductal adenocarcinoma, Oncolytic virotherapy, Gemcitabine, Synergy, Tumor microenvironment

Poster Presentations

Exploration of a Peptide PROTAC Degradar Targeting FOXA2 as a Potential Strategy for Lethal Prostate Cancer

Yuxiu Ao M.E., Lin Liu M.D. & Shang Su Ph.D.

Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803

Prostate Cancer (PCa) is the second cause of cancer-related mortality in US men, with 1 man dying of PCa in every 16 minutes. Neuroendocrine prostate cancer (NEPC) is a lethal subtype of PCa that usually emerges upon castration therapy and has no approved therapies. Forkhead box protein A2 (FOXA2) activation is a known driver for NEPC progression but there are no FOXA2-targeting drugs available. Therefore, we aim to design peptide proteolysis-targeting chimeras (PROTAC) hijacking the cellular ubiquitin proteasome system to degrade FOXA2 for potential NEPC treatment.

Our PROTAC contains three components, FOXA2 binding peptide (FBP), a linker and an E3 ubiquitin ligase binder. The resulting PROTACs can therefore recruit E3 ligases to FOXA2 proximity to induce its polyubiquitination and subsequent proteasomal degradation. We designed FBPs via in silico exploration documented FOXA2-binding proteins, then we ranked the FBP by the docking scores of FBP::FOXA2 complexes predicted from AlphaFold and Boltz-2. We are currently performing in vitro binding assays between the top FBPs (biotinylated) and tagged FOXA2 to validate the predicted binding property. Once we validate the FBP binding, we'll generate the PROTAC library with linker and E3 ligase binders commonly used in PROTAC design, and screen the generated library via docking onto FOXA2 and E3 ligase. The FOXA2-targeting PROTAC candidates with superior binding affinities will be synthesized and validated for FOXA2 degradation efficacy both in NEPC cell lines and NEPC mouse xenografts.

Successful design and validation of FOXA2-targeting PROTAC will yield the first-in-class FOXA2 degrader for treating FOXA2-driven tumors including NEPC, and our established, transferable in-silico workflow will accelerate the discovery of other peptide PROTACs against virtually all undruggable proteins.

This work is supported by Department of Defense Exploration-hypothesis development award (EHDA #PC230068) and LSU/SVM Startup funds granted to Shang Su.

Deciphering tumor dormancy drivers in prostate cancer bone metastases

Lin Liu M.D.¹, Yuxiu Ao M.E.¹, Xiaohong Li Ph.D.², Shang Su Ph.D.¹

¹Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803

²Department of Cell and Cancer Biology, College of Medicine and Life Sciences, University of Toledo, Toledo, OH 43614

Metastases are the leading cause of prostate cancer (PCa) patient death, and more than 90% of PCa patients die with bone metastases. Metastases develop from the disseminated tumor cells (DTCs), which initially stay dormant, but can reactivate upon permissive conditions to proliferate into metastases. However, the cellular drivers for PCa dormancy entrance and exit remain unclear. We hypothesize distinct transcriptional programs, governed by specific transcription factors (TFs), dictate whether DTCs remain dormant or reawaken. Targeting these TFs will lead to potential novel treatment for lethal PCa metastases.

We previously developed an in vitro co-culture model of PCa cells and osteoblasts to induce PCa cells into dormancy in the bone context. Using this model, we defined a transcriptomic PCa dormancy signature with several TFs highlighted as upstream regulators. In parallel, we found that different PCa cell lines displayed two distinct patterns upon osteoblast co-culture: dormancy-responsive vs dormancy-nonresponsive. We found that dormancy signature scored higher in responsive cells compared to non-responsive cells and the highlighted TFs also have higher expressions in responsive cells. These data indicate that responsive PCa cells require such driver TF presence to trigger the dormancy-related transcriptomic responses upon osteoblast co-culture. We are now performing functional validation of these TFs by overexpressing them in nonresponsive cells and knocking down them in responsive cells to see if the response patterns get shifted. We will also perform ChIP-Seq analyses and locus-specific proteomes to investigate how these TFs drive dormancy and how they get activated in bone context.

The successful validation of PCa dormancy driver TFs will lay the foundation of future therapeutic development on manipulating the TF functions to eliminate DTCs or forcing them in long-lasting dormancy to delay the metastatic onset.

This work is supported by the NIH/NIGMS COBRE (5P20GM135000; subproject 8133) and LSU/SVM Startup funds granted to Shang Su.

USING IMAGEJ TO QUANTIFY COLLAGEN IN PICROSIRIUS RED AND MASSON TRICHROME STAINED HISTOLOGICAL SECTIONS

Ugochi J Emelogu¹, Kim Pedersen², Martin Ronis², Elizabeth Zunica³, Christopher Axelrod³, Alexandra Noel¹, and Tomislav Jelesijevic¹

¹Department of Comparative Biomedical Sciences, LSU, Baton Rouge

²Department of Pharmacology and Experimental Therapeutics, LSUHSC, New Orleans

³Pennington Biomedical Research Center, Baton Rouge

There are 28 different types of collagen in the body, and their function is to provide structural support for tissues and organs. Fibrosis is a pathological process characterized by the replacement of normal tissues with primarily type I collagen. Developing an accurate method to quantify the amount of fibrosis/collagen in tissues is crucial for evaluating the outcomes of pathological processes, as well as the efficacy of antifibrotic therapies. Traditionally, pathologists have relied on visual scoring of histological slides to assess the amount of collagen/fibrosis, on the scale from 0 (no fibrosis), 1 (minimal), 2 (mild), 3 (moderate), to 4 (severe). While this subjective approach has been widely used, it is limited by the observer's variability and, therefore, has low reproducibility. To overcome these challenges, we developed a less subjective approach for quantifying fibrosis using the ImageJ software. Tissue sections were stained with Picrosirius Red (PSR) and Masson Trichrome (MT), which are capable of detecting collagen type I fibers. Images of the PSR/MT stained tissues were captured using brightfield microscopy and analyzed using ImageJ software. In ImageJ, the images were split into red, green, and blue channels, and the channel with the best visual contrast was selected for the threshold adjustments. Threshold adjustments were applied to reduce background interference, followed by the image inversion to enable collagen quantification. The total amount of collagen in the tissues was determined using a 0-255 intensity scale, where the white signal represented tissue collagen and the black signal represented the background. In conclusion, ImageJ can be effectively applied as a semiquantitative method to compare the amount of collagen in PSR and MT stained samples, providing a broader range and distinguishing minuscule differences in collagen amounts across different samples than traditional visual scoring methods.

Caveolin-1 modulates Stemness, Chemosensitivity, metastasis in triple negative breast cancer

Shreya Pokharel¹, Naveen Chintalaramulu¹, Dhirendra Pratap Singh², Abhishek Pandit¹, Biplov Sapkota¹, Shilpa Thota¹, Rizwana Begum¹, Joseph Francis¹

¹Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA

²Krannert Institute- Cardiology, Indiana University Health Methodist Hospital, Indianapolis, IN 46202, USA

Caveolin-1 (Cav-1) is a scaffolding protein that has contrasting role in promoting and suppressing tumors based on the subtype and their stage of progression. Triple-negative breast cancer (TNBC) is the most aggressive subtype characterized by greater propensity to metastasize. Evidence suggests that TNBC has high expression of cancer stem-like cells (CSCs) leading to a poor clinical outcome. We have previously shown that Cav-1 knockout (Cav-1 KO) mitigated breast cancer metastasis to the lungs in a 4T1 syngeneic mouse model of TNBC. We hypothesized that CAV-1 promotes chemoresistance in TNBC by maintaining ABC transporter function and CSC traits.

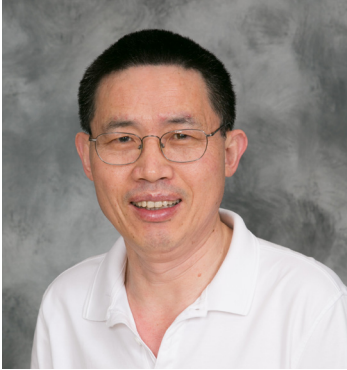
We compared 4T1 WT and Cav-1 KO cells in vivo (BALB/c) and invitro (drug response and cell viability assays) which displayed enhanced chemosensitivity of CAV-1 KO cells to Doxorubicin (Dox) and Paclitaxel (PTX) as compared to the corresponding 4T1.WT control. Also, there was a downregulation of MRP1/ABCC1 and ABCB1 drug efflux transporters in protein and mRNA levels. In agreement with our previous study, Dox treatment further inhibited lung metastasis in BALB/c mice injected with Cav-1 KO cells as compared to the 4T1.WT group. In 4T1 cells, CAV1 knockout decreased CSC markers (SOX2/OCT4/NANOG) and CAV1 silencing similarly attenuated stemness traits in SUM159PT cells assessed in both proteomic and transcriptomic.

Collectively, our study suggests a proposed role of CAV-1 KO in chemosensitivity of 4T1 cells to Dox treatment both in vitro and in vivo. Ongoing mRNA sequencing analyses aim to define the downstream pathways mediating these effects. Collectively, our data indicate that targeting CAV1 disrupts CSC-like phenotypes and reverses multidrug resistance, highlighting CAV1 as a therapeutic node to enhance chemotherapy efficacy in TNBC.

Funding: This work is funded by Cancer COBRE No. IP20 GM135000

Key Words: Triple Negative Breast Cancer, Caveolin-1, Cancer Stem Cells, Chemosensitivity

Cell and Molecular Biology



Shisheng Li, Ph.D.

Living organisms are constantly exposed to a wide array of DNA-damaging agents. To safeguard genomic integrity, they rely on robust DNA repair and damage-bypass mechanisms that either remove or tolerate these lesions, thereby ensuring survival. Defects in these processes can lead to genomic instability, which is associated with cancer, neurodegeneration, premature aging, and various other human diseases. Our research focuses on elucidating the molecular mechanisms of DNA damage, repair, and mutagenesis.



Alexander Murashov, MD, Ph.D.

Many noncommunicable diseases (NCDs) exhibit strong family clustering, including obesity, metabolic disorders, neurodegenerative diseases, heart disease, and cancer. Yet studying long-term generational effects under controlled conditions is nearly impossible in humans. *Drosophila* provides a powerful genetic model with conserved biological pathways, making it uniquely suited to dissect the basic mechanisms of transgenerational inheritance. What we eat, how we move, and even the air we breathe don't just shape our own metabolism and brain health—they can leave lasting marks on the next generation. My lab aims to uncover the role of microRNAs (miRNAs) in mediating familial susceptibility to obesity and related metabolic and neurodegenerative disorders. We will characterize offspring metabolic phenotypes and feeding behaviors following ancestral exposure to a Western diet and manipulate miRNA expression through knockdown or overexpression to define their contribution to inherited traits. The results are striking: choices made today can predispose offspring to neurological and metabolic disorders tomorrow, underscoring the powerful message that brain health is a legacy we pass on.

Oral Presentations

Roles of Spt6 in Nucleotide Excision Repair and DNA Damage Tolerance

Hannah Holmberg, Wenzhi Gong, and Shisheng Li

Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803.

Cellular DNA is under constant attack by various types of DNA damaging agents. Of these one of the most common is UV induced damage from Ultraviolet light. UV irradiation causes large helix distorting damage lesions of two main types: 6,4 Photoproducts and cyclobutane pyrimidine dimers (CPDs). Most of these lesions will be repaired by Nucleotide Excision Repair (NER). If the repair is slow or some sites are not repaired by the NER machinery the cellular stress response leads to DNA Damage Tolerance (DDT) pathways being activated for cell survival. Spt6 is a highly conserved histone chaperone that can be seen in organisms from simple Eukaryotes to complex Mammals. Spt6 plays roles in transcription, nucleosome remodeling and mRNA processing. Previous data has shown that mutations in Spt6 cause alterations in transcription and abnormal histone remodeling. This study aims to elucidate how the Spt6 mutant Spt6-1004 affects NER and DDT. CRISPR/Cas9 was used to delete AA931-994 of the Spt6 gene (Spt6-1004). Preliminary studies tested the UV sensitivity of the Spt6 mutants. These studies showed that Spt6-1004 mutation caused increased UV resistance in rad16 Δ rad26 Δ cells but showed increased UV sensitivity in otherwise wild type cells. Following these studies, the repair speed of cells in the Rpb2, Yef3 and Agp2 genes was tested using Laf-Seq. This showed that Spt6-1004 significantly restored TCR in the CD region of all three genes in the rad16 Δ rad26 Δ cells but had no effect in the rad16 Δ cells. Suggesting the Spt6-1004 region of Spt6 represses rad26 independent TCR. Further studies are being conducted to determine if this repression of rad26 independent TCR is seen across the whole genome. UV induced mutagenesis assays were used to test if Spt6-1004 mutation affected the DDT pathways. The results showed that this mutation significantly decreased UV induced mutagenesis.

Investigate the role of the Tfb1 Pleckstrin homology domain (Tfb1-PHD) and Spn1 in TCR and GGR

Cheng Lu, Shisheng Li

Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University

RNA polymerase II(RNAPII) plays an important role in the transcription-coupled nucleotide excision repair (TC-NER) with cooperation with the general transcription/repair factor TFIIH to do the TC-NER. Recent study shows that Rpb6 of RNAPII interacts with the PH domain (PH-D) of the p62 subunit of the general transcription/repair factor TFIIH and plays an important role in TC-NER in human cells. But their possible interactions and roles in the TC-NER in yeast cells are not determined. Our research tries to investigate whether the TFB1 PH-D and Rpb6 interact and cooperate to perform TC-NER. We do mutations with Rpb6 and TFB1 genes in different strains, test the sensitivity and repair ability of the mutants to see the interaction of TFB1 PH-D and Rpb6, and whether their interaction affects TC-NER. In this research, Rpb6 mutants will be subjected to sensitivity test, LAF-seq(Lesion-Adjoining Fragment Sequencing), and Bpa crosslinking test. Sensitivity test result shows truncation of rpb6 2-80aa Δ in rad16rad26(K Δ) strain generated strong UV sensitivity. LAF-seq results also suggest that the truncation of rpb6 2-80aa Δ in rad16rad26(K Δ) strain slows down the DNA repair after UV irradiation. These results suggest Rpb6 plays a role in TC-NER. We will test whether this effect is via the interaction with TFB1 PH-D through Bpa crosslinking test. Spn1 is a transcription elongation factor and histone chaperone that plays essential roles in RNA polymerase II-mediated transcription, chromatin remodeling, and mRNA processing. Spn1 may affect TC-NER as well. We truncated the C- and N-terminals of Spn1 and deleted Spn1 after the deletion of Pob3 in rad16(K Δ) and rad16rad26(K Δ) strains. Then sensitivity test shows that Spn1 Δ in rad16(K Δ) strain has increased sensitivity and C-terminal truncation in rad16rad26(K Δ) has decreased sensitivity. LAF-seq result shows Spn1 Δ in rad16(K Δ) strain has slower DNA repair, while C-terminal truncation in rad16rad26(K Δ) has faster repair.

Poster Presentations

Divergent functions of the RNA-binding protein, Mei-P26 in germline and somatic lineages of *Drosophila* testis

Shallinie Thangadurai and Alexander Murashov

Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA

The *Drosophila* TRIM-NHL family RNA binding protein, Mei-P26 has been linked to suppression of tumorigenesis in the female germline. Mei-P26 restricts growth and proliferation, but promotes differentiation in the ovarian stem cell lineage, yet its specific functions in different testis cell types during spermatogenesis remain unclear. Here we show that overexpression of mei-P26 in germline cells leads to truncated, agametic testes. These testes display germline cell cycle arrest and defective somatic cyst cell proliferation and differentiation, highlighting a failure in germline–soma coordination. By contrast, somatic overexpression of mei-P26 results in male-specific lethality. With the Gal80ts system to circumvent this lethality, induction of mei-P26 in adult somatic cells promoted proliferation and differentiation of both germline and somatic cells, accompanied by upregulation of phospho-S6K, indicating hyperactivation of the TOR signalling pathway. Our findings reveal that Mei-P26 exerts opposing, cell type–specific roles within the same tissue, restraining germline development while driving expansion of somatic cells. This study underscores the importance of cell-specific regulation by RNA-binding proteins and provides new insights into how misregulation of Mei-P26 may coordinate divergent cell fates in a shared tissue context.

Paternal Western Diet Induces Hyperphagia and Hyperactivity in Fathers and Offspring of *Drosophila melanogaster*, with Concomitant Neural Mitochondrial Remodeling.

Tolulope Olaolorun¹, Elena Pak¹, Analisa Taylor², Christopher Axelrod², Alexander Murashov¹

¹Comparative Biomedical Sciences, Louisiana State University, School of Veterinary Medicine

²Mitochondria Phenotyping Laboratory, Pennington Biomedical Research Center

Paternal nutrition is increasingly recognized as a determinant of offspring metabolic and neurological health, yet the underlying mitochondrial and epigenetic mechanisms remain poorly defined. To investigate these relationships, wild-type *Drosophila melanogaster* was used to examine how paternal exposure to a Western diet (WD) modulates neural and metabolic in both generations.

Adult males (F_0) were fed WD or control diet (CD) for five days and evaluated for behavioral and metabolic parameters. WD fathers exhibited hyperphagia and hyperactivity in FLIC and Con-Ex assays and showed elevated whole-fly respiration. To elucidate the metabolic basis of this phenotype, mitochondrial respiration was assessed using high-resolution respirometry (Oroboros O₂k, SUIT protocol). Across the major coupling states (LEAK, OXPHOS, and ETS) WD fathers displayed increased respiratory flux, consistent with enhanced mitochondrial capacity. This was accompanied by a ~30 % increase in triglyceride (TAG) content ($p < 0.0001$), elevated glucose levels ($p = 0.01$), and a three-fold increase in mitochondrial DNA copy number ($p < 0.05$), consistent with systemic metabolic remodeling. TUNEL staining revealed increased neuronal apoptosis, while dihydroethidium (DHE) fluorescence was stronger in the subesophageal zone (SEZ), indicating localized reactive oxygen species (ROS) accumulation. qPCR analysis in WD fathers confirmed significant upregulation of *marf* and downregulation of *mei-P26* ($p < 0.05$), together with altered expression of microRNAs (*miR-10*, *miR-1006*, *miR-277*) linked to developmental and metabolic regulation.

Given these pronounced paternal effects, we next examined the F_1 generation. Offspring of WD fathers exhibited complex-specific mitochondrial remodeling, with reduced respiration in complex-I and proline-linked states but increased glycerophosphate-linked respiration ($p < 0.05$). Behaviorally, both male and female offspring showed higher feeding and locomotor activity ($p < 0.001$), indicating inherited behavioral hyperactivity alongside altered mitochondrial regulation.

Collectively, these findings demonstrate that paternal WD induces hyperphagia and hyperactivity in fathers and offspring, with concomitant neural mitochondrial remodeling, suggesting potential epigenetic inheritance of metabolic and behavioral traits.

miR-10 Knockdown Reveals Cell-Type–Specific Regulation of Obesogenic Behavior

S. Bradley, S. Pandey, S. Thangadurai, E. Pak, and A. Murashov

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Previously, our laboratory showed that paternal exposure to a Western diet (WD), high in fats, salt, and sugars, changes protein and miRNA expression in the brains of *Drosophila melanogaster* offspring with concomitant increase in food consumption. (Murashov et al., 2023). Among these miR-10 was identified through miRNA-target enrichment analysis as a potential regulator of proteomic changes in offspring brains of WD-fed fathers.

Using the Gal4/UAS system, we used RNAi-mediated knockdown of miR-10 in dopaminergic and serotonergic neurons (Ddc-Gal4), as these neurons are involved in food and reward-seeking behavior. Using the Fly Liquid-food Interaction Counter (FLIC), we found that knockdown of miR-10 led to a nearly two-fold increase in licks. Additionally, qPCR showed a six-fold increase in miR-10 for WD-fed fathers and a two-fold increase in WD offspring. Based on these findings, we hypothesized that miR-10 may contribute to obesogenic behavioral traits, including hyperphagia, reduced sleep, and impaired learning and memory. Because miR-1006 shares an identical 3p seed sequence with miR-10-3p, we generated double knockdowns (miR-10 + miR-1006) to eliminate potential compensatory effects.

Behavioral analyses, including consumption/excretion, FLIC, locomotor, and passive avoidance, were conducted using neuronal (Elav-Gal4) and glial (Repo-Gal4) drivers with double knockdown UAS. Double knockdown in Ddc and pan-neuronal drivers resulted in two-fold increased locomotor activity and consumption, whereas glial knockdowns showed minimal feeding changes but impaired short-term memory in passive-avoidance assays. These phenotypes are consistent with WD offspring, suggesting miR-10 contributes to these behaviors through both neuronal and glial cells.

To probe potential downstream targets, we assessed Mei-P26 expression, a TRIM-NHL RNA-binding protein predicted to interact with miR-10-3p/miR-1006-3p (Agarwal et al., 2018). Overexpression of Mei-P26 in Ddc neurons phenocopied the double miRNA knockdown, suggesting miR-10/1006 regulate obesogenic behavior through Mei-P26-dependent pathways. These findings reveal a novel, cell-specific miRNA network linking Mei-P26 regulation to inherited metabolic and behavioral traits.

Lung and Cardiopulmonary Biology



Alexandra Noel, Ph.D.

Dr. Noël's research focuses on how prenatal exposure to vaping aerosols, engineered nanoparticles, and other airborne pollutants programs neonatal lungs for diseases such as allergic asthma. She pairs custom aerosol generation with inhalation toxicology, in-utero disease models, and epigenetic profiling to uncover pathways linking early exposure to lifelong respiratory risk.



Joseph Francis, DVM, Ph.D.

My research focuses on elucidating the role of cytokines and inflammatory pathways in the pathophysiology of chronic diseases, including heart failure, hypertension, metabolic syndrome, post-traumatic stress disorder (PTSD), and cancer. Over the past decade, I have concentrated on identifying the contribution of inflammatory molecules within the brain in PTSD and heart disease, exploring their impact on neuroimmune communication and stress-related pathology. More recently, my work has expanded to investigate plant-based therapeutic strategies, with a particular emphasis on cancer and PTSD, using both pharmacological and non-pharmacological interventions. This includes studying the bioactive potential of dietary agents, such as blueberries, in modulating inflammation and disease progression. In parallel, I am advancing research in multicellular organoid development to establish models for cancer precision therapy, enabling patient-tailored approaches and bridging experimental findings with translational applications.



Tammy Dugas, Ph.D.

Our toxicological research focuses on the role of the environment and environmental factors in the induction or exacerbation of vascular diseases including atherosclerosis and pulmonary hypertension. In particular, we are investigating the vascular toxicity of airborne particulate matter, of aromatic amines, compounds used industrially in the production of polyurethanes, and of HIV antiretroviral drug therapies. Mechanistic studies aimed at elucidating these toxicities explore drug/toxicant metabolism, endothelial cell injury (including mitochondrial oxidant stress), and stimulation of vascular smooth muscle cell proliferation. We furthermore explore whether females are more susceptible to these vascular toxicities. In other toxicological studies, we are examining the potential for combustion-generated ultrafine particulate matter (PM) to produce reactive oxygen species and induce oxidative stress in rodents exposed by inhalation. Finally, in our pharmacological studies, we are elucidating pathways by which red wine polyphenols might act on the estrogen receptor so as to promote vascular homeostasis and prevent the development of vascular diseases including atherosclerosis and restenosis.

Oral Presentations

Hydrogen Sulfide Donor Therapy Attenuates HFpEF by Regulating Calcium Signaling and Cardiac Hypertrophy

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Background: Reduced H₂S levels in HFpEF suggest that restoring its bioavailability could improve disease pathology. Previous work suggests that cardioprotective effects of H₂S are mediated through modulation of calcium ion channels and associated transporters. We hypothesized that H₂S donor therapy via oral polysulfide (SG1002) supplementation would improve HFpEF outcomes by modulating calcium signaling pathways.

Methods: 9-week-old male C57BL6/N mice were fed a combination of high fat diet and L-NAME (0.5 g/L) in drinking water to induce HFpEF. Five weeks after the onset of HFpEF (n=12 per group) were administered oral H₂S donor SG-1002 (95 mg/kg/day) for 5 weeks and compared to HFD + L-NAME alone mice (i.e., control). Exercise capacity was measured using forced treadmill running. Diastolic function was evaluated via transthoracic echocardiography and invasive hemodynamics. Bulk RNA Seq was performed on cardiac muscle to determine the transcriptional response to SG1002 therapy. Bioinformatic analysis was conducted using Ingenuity Pathway Analysis (IPA, Qiagen).

Results: SG1002 treatment significantly (153%) elevated plasma H₂S levels (p=0.0001), and enhanced exercise capacity (p=0.0360). SG1002 therapy reduced E/E' ratio (7.519±6.88, p=0.0016), and a markedly decreased in left ventricular (LV) end-diastolic pressure (12.31±5.14 p<0.0001) when compared to HFpEF control mice. Histological analysis demonstrated attenuated cardiac hypertrophy in SG1002-treated HFpEF mice, with reductions in cardiomyocyte size (10.65% reduction, p=0.0022) and increase in LV lumen area (68.59% increase, p=0.0006). RNA sequencing revealed significant downregulation of genes associated with dilated cardiomyopathy (Tnnt2 and Mhy6), and calcium signaling pathway (Pln and Atp2a2) in SG1002-treated HFpEF mice compared to HFpEF mice (p<0.001, z-score=-2.0).

Conclusion: The H₂S (SG1002) therapy improved diastolic function, and exercise capacity and reduced cardiac hypertrophy in HFpEF mice. The downregulation of calcium signaling and dilated cardiomyopathy gene expression pathway support therapeutic potential of H₂S therapy in HFpEF.

This work was supported by grants from the National Institutes of Health P20GM135002 and U54GM104940, P30AG050911 to T.D.A.

Poster Presentations

IL-10 Deficiency Improves Lung Function in 14-16-day-old Mouse Offspring Exposed in Utero to E-cigarette Aerosols

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Rationale: Electronic-cigarettes (e-cigs) refer to a variety of devices with the primary function of delivering nicotine via inhalation. These devices are commonly used by young adults in the U.S., including pregnant women, as recent studies report that 2.2–7.0% of individuals use e-cigs during pregnancy. Our previous studies showed in mice exposed in utero to e-cig aerosols, up-regulation of interleukin-10 (IL-10), a crucial immunoregulatory cytokine in lung homeostasis. This study aims to investigate both the impact of gestational e-cig exposure and the role of IL-10 on mouse offspring lung function.

Methods: C57BL/6 wild type (WT) and IL-10 knockout (KO) dams were exposed to either air or e-cig aerosol (free base nicotine: 24 mg/mL). Each group was exposed for 1.5-hour/day for 14 days prior to conception plus 20 days during gestation. Lung function in the offspring was evaluated using the flexiVent at 14-16 days of age.

Results: Compared to the air exposure, in utero e-cig exposure significantly reduced both the birth weight and length in WT pups, an effect driven by shorter female WT pups, while IL-10 deficiency improved these outcomes. In neonates, in utero e-cig exposure significantly affected the lung pressure-volume (PV) loops in WT offspring, reflecting a restrictive phenotype primarily driven by the WT females. The in utero e-cig exposure significantly increased the respiratory system elastance, lung tissue damping and tissue elastance compared to the air group in WT mice, whereas the removal of IL-10 in the e-cig group preserved these lung function parameters in the range of the IL-10 KO air exposed offspring. Also, sex-specific effects of IL-10 deficiency were identified.

Conclusion: In utero e-cig exposure has significant effects on birth outcomes and lung function in early-life in WT offspring, with the lungs exhibiting reduced ability to stretch and expand, whereas IL-10 deficiency protected from these impairments.

Funding source: Our Lady of the Lake Health and Louisiana State University Collaboration In Action Program 2024. AWD-006603.

Geek Bar E-cigarettes and Particulate Matter Containing Environmentally Persistent Free Radicals Impair Pulmonary Responses in Mice

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Electronic cigarettes (EC) deliver nicotine by heating liquids containing propylene glycol, vegetable glycerin, and various nicotine forms, including freebase, synthetic, and nicotine salts. The Geek Bar (GB), a newer EC device using synthetic nicotine salts, is rapidly gaining popularity. Particulate matter (PM) containing environmentally persistent free radicals (EPFRs), formed during the thermal treatment of hazardous materials found at Superfund sites, generate, among others, reactive oxygen species that drive oxidative stress and pulmonary dysfunction. Both Geek Bar aerosols and EPFR PM are inhalable pollutants. We hypothesize that the co-exposure of Geek Bar aerosol and EPFR PM would produce a synergistic effect on lung inflammation and oxidative stress.

Male C57BL/6J mice were exposed to either air, GB aerosol, EPFR PM aerosol, or in combination for a 7-week period. Lung function was assessed using whole-body plethysmography and the flexiVent system. Lung tissue was collected and analyzed via PCR for oxidative stress related gene expression, and bronchoalveolar lavage fluid (BALF) was evaluated for cytology analysis.

Exposure to EPFR PM maintained a consistent airborne concentration of ~317 µg/m³ across six days, while the 7-week average concentration for GB aerosol was ~0.32 mg/puff. Compared to air controls, GB exposure significantly impaired lung function at two weeks, evidenced by reduced minute volume and breathing frequency, with both parameters recovering the following weeks, suggesting exposure adaptation. Also GB alone caused a significant upward shift in the pressure-volume loop, consistent with an obstructive pulmonary phenotype. BALF from all groups were mainly composed of macrophages (> 99%), although not significant, the total cell counts in EPFR exposed mice were increased.

This study underscores the importance of evaluating co-exposure scenarios in inhalation toxicology, particularly as EC use increases and EPFR PM exposures become more prevalent, with ~53 million Americans living within 3 miles of a Superfund site.

Funding source: State of Louisiana - Governor Biotechnology Initiatives (GBI #013).

LS-200 maintains hemocompatibility and decreases thrombogenesis ex-vivo

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Peripheral artery disease (PAD) is an obstructive atherosclerotic and thromboembolic disease affecting the lower extremities. Obstructed arteries are treated using angioplasty and stenting. However, blockages can recur. One mechanism for re-obstruction is thrombus development following treatment. Polymeric nanoparticle (pNP) LS-200 developed in the Sabliov lab contains PLGA, a well-known medical polymer, grafted to a polyphenol that may have heparin-like activity. Indeed, LS-200 prolongs clotting time, which may reduce thrombotic risk. This finding led to the present study into LS-200 hemocompatibility and its anticoagulant effects. We hypothesize that 1) LS-200 possesses anticoagulant activity and 2) is hemocompatible.

Canine blood was obtained with an IACUC-approved protocol. Porcine blood was obtained from a commercial source. Coagulation was evaluated in LS-200 exposed canine blood using prothrombin time (PT), activated thromboplastin time (aPTT), and viscoelastic coagulation monitoring (VCM). Platelet activation was measured using thromboxane B2 and PDGF-BB ELISA. Hemolysis assays were performed using both canine and porcine blood. Endothelial viability and function were measured using ATP and endothelial NO assays following LS-200 exposure. A novel physiologic flow loop study measured dynamic thrombus development on thrombogenic and non-thrombogenic materials inserted into loops containing LS-200 exposed porcine blood.

LS-200 prolonged aPTT but shortened VCM clotting time ($p < 0.05$). LS-200 did not increase PDGF-BB or thromboxane B2. Mean hemolysis did not exceed 7%. Endothelial ATP production was unaffected below 0.36 mg/mL pNP at 72 h post-exposure ($p < 0.05$). Endothelial NO production did not significantly decrease 72 h post-exposure. Material thrombus coverage was significantly decreased in LS-200 exposed blood under flow ($p < 0.05$).

Overall, LS-200 reduced thrombus formation on otherwise thrombogenic materials and maintained hemocompatibility in measured endpoints. Investigations into immune cell activation, mutagenicity, and other key aspects of biocompatibility are required. Further studies will assess LS-200 as a hydrophilic coating for cardiovascular medical devices.

Neuroscience



Ahmed Abdelmoneim, DVM, Ph.D.

Our research program investigates how developmental exposure to environmental contaminants rewires the neuroendocrine stress axis and behavior, mapping mechanisms from receptor signaling to circuit function. Our goal is to determine how specific exposures cause stress-related disorders in animals and humans and to translate these mechanisms into strategies for diagnosis, prevention, and treatment. We use zebrafish as a scalable vertebrate model that adheres to the 3Rs (Replacement, Reduction, Refinement), supports high-throughput assays, and provides direct optical access to the developing brain.



Ethan Anderson, Ph.D.

Substance Use Disorders (SUDs) and Alcohol Use Disorders (AUDs) are major problems in our society today, and there is a great need for better therapies. Repeated substance and alcohol use causes neuroplastic adaptations in the brain through molecular mechanisms. These changes lead to cravings, increased motivation to take the drug, and can cause relapse during times of abstinence. I have studied these lasting effects of abused drugs on the brain since 2009. Almost all of my research experiences have focused on neuroplastic molecular mechanisms that underlie drug-induced behavioral changes in the nucleus accumbens (NAc), a brain area in the mesolimbic dopaminergic pathway vital for reward, stress, and anxiety. I employ cutting-edge viral and chemical tools to manipulate molecular pathways to reverse or mimic drug/alcohol-induced changes in NAc and study their behavioral relevance. The Anderson Lab aims to discover novel, translational treatments for SUDs and AUDs that are capable of reducing drug use by reversing neuroplastic adaptations that occur following chronic use.



Charles Lee, Ph.D.

My laboratory derives neural principles governing the sensory and cognitive functions of the brain using the central auditory system as a model. We interrogate neural circuits in the midbrain, thalamus, and cortex through physiological, anatomical and behavioral studies. We also address how alterations to these central neural circuits manifest in neurodevelopmental and neurodegenerative disorders, such as autism spectrum disorder, schizophrenia, and Alzheimer's disease.



Alexander Murashov, MD, Ph.D.

Many noncommunicable diseases (NCDs) exhibit strong family clustering, including obesity, metabolic disorders, neurodegenerative diseases, heart disease, and cancer. Yet studying long-term generational effects under controlled conditions is nearly impossible in humans. *Drosophila* provides a powerful genetic model with conserved biological pathways, making it uniquely suited to dissect the basic mechanisms of transgenerational inheritance. What we eat, how we move, and even the air we breathe don't just shape our own metabolism and brain health—they can leave lasting marks on the next generation. My lab aims to uncover the role of microRNAs (miRNAs) in mediating familial susceptibility to obesity and related metabolic and neurodegenerative disorders. We will characterize offspring metabolic phenotypes and feeding behaviors following ancestral exposure to a Western diet and manipulate miRNA expression through knockdown or overexpression to define their contribution to inherited traits. The results are striking: choices made today can predispose offspring to neurological and metabolic disorders tomorrow, underscoring the powerful message that brain health is a legacy we pass on.



Olalekan Ogundele, MS, Ph.D.

Dr. Ogundele's research focuses on synaptic and neural circuit mechanisms governing novelty learning and context discrimination. We are interested in midbrain excitatory projections that innervates forebrain centers, and the significance of the reciprocal circuitry between these regions. Our current research is focused on ventral tegmental area (VTA) glutamate projections that modulates hippocampal reward-sensing neurons during reward context learning or discrimination of reward value across spatial locations. We use in vivo neural recording with neuron-specific modulation in the VTA-hippocampus loop to decode single cell and multi-unit activity in freely behaving mice. The overarching goal of our research is to use neural ensemble coding outcomes, derived in behavioral tasks, to predict decision and better understand cognitive disorders.



Fabio A. Borges Vigil, Ph.D.

Dr. Vigil research focuses on traumatic brain injury, Alzheimer's Disease, and epilepsy, with a particular emphasis on the interaction between these three pathological conditions. Additionally, he is an expert in Kv7 K⁺ channels and investigates their relevance for the three pathological conditions mentioned above.

Oral Presentations

The Role of CeA-VTA Circuits in Decision-Making and Ranked Choice during Learning.

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The central amygdala (CeA) and ventral tegmental area (VTA) form a critical circuit regulating reinforcement learning, decision-making, and approach-avoidance behaviors. While VTA and amygdala (CeA) are known to influence learning, the extent to which CeA-VTA pathways shape the balance between reward and aversion learning remains unclear. In this study, we investigated how the inhibition of CeA GABAergic projections in the VTA, and VTA glutamate or dopamine releasing terminals in the CeA affects ranked choice decision-making in reinforcement paradigms. Neural projections were modulated using chemogenetic DREADDS, and its agonist Compound 21 (C21). In conditioned place preference and aversion tests, the target sides contained either 20% sucrose as the reward, and a bitter tasting mixture as an aversive stimulus. Double-floxed AAV5-DIO expressing hM4Di – an inhibitory GPCR – was injected into the CeA of VGAT-Cre mice while a cannula was positioned in the VTA. Similarly, in VGlut2Cre and THCre mice AAV5-DIO-hM4Di was injected into the VTA while a cannula was positioned in the CeA. For baseline preference (CPP) and aversion (CPA) tasks, animals were exposed to the reward and aversive stimulus without C21 modulation. After a wash period of 3 days, mice were tested in these tasks with chemogenetic modulation of CeA terminals in the VTA or VTA terminals in the CeA. Our results show that inhibiting CeA GABAergic terminals in the VTA enhanced reward learning but impaired aversion discrimination. Modulation of VTA terminals in the CeA had minimal impacts on reward or aversion learning. Notably selective modulation of VTA dopamine inputs caused modest changes in reward learning and mice maintained aversive responses. Furthermore, inhibiting VTA glutamate inputs to the CeA did not significantly change behavioral outcomes in both reward and aversion learning. Together, our results showed that CeA GABAergic modulation of the VTA plays a key role in balancing positive and negative valence in decision-making, shaping how organisms prioritize choices based on past reinforcement experiences.

Key words: CPP, CPA, Learning, CeA, VTA, Circuit, Reward, Avoidance

Acknowledgement: This work is funded by research support provided by NIMH and NSF.

Nicotine tunes dopaminergic and non-dopaminergic connectivity in ventral tegmental area local circuits

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The ventral tegmental area (VTA) is a heterogeneous midbrain nucleus and the main source of dopamine in the mesocorticolimbic pathways. These circuits are involved in reward prediction, aversion, learning, salience, novelty, and valence determination. In normal brain function, cholinergic excitation of VTA dopamine and non-dopamine neurons enhances presynaptic dopamine release in projection targets and modulates local inhibitory circuits within the VTA that, in turn, shape dopamine output. The current study elucidates a potential continuum of events driven by systemic nicotine in VTA local circuits toward attaining net dopamine (DA) excitation. After subcutaneous nicotine administration, we analyzed the response time and connectivity profiles of VTA DA and non-DA cells in anesthetized mice. Our results showed phasic co-activation patterns of both DA and non-DA putative units activated in two perievent firing rate peaks that span minutes. DA cells had a rapid onset, and longer peak periods compared with non-DA cells during the first peak response. The activation time was comparable for both DA and non-DA cells during the second perievent peak. Although more non-DA cells were responsive during the second response phase, the crosscorrelogram of putative pairs indicates a subtle decrease in inhibitory tone and increased excitatory tone for DA to non-DA connections. The results showed that the two mechanisms for nicotinic reinforcement of DA activity likely occur in a time-dependent sequence of events from co-activation of DA and non-DA units until attenuation of non-DA inhibitory tone. In addition, transsynaptic connectivity was enhanced among DA-DA pairs following nicotine administration.

Keywords: VTA, dopamine, GABA, firing rate, bursts, reward, reinforcement

Funding: This work was funded by National Science Foundation grant (IOS 2137023) and National Institute of Health grant (R01MH132018-01A1) awarded to OO. Also, IBRO-ARC and the Young IBRO Research Fellowship awarded to PAA.

Glutamatergic Hippocampal Circuits as Drivers of Aversion Based Learning

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The hippocampus (HPC) is central to spatial memory, consolidation, and value encoding, yet the relationship between stress and memory remains contradictory. While some rodent studies suggest stress enhances learning, others report impairments, highlighting a need to clarify the circuitry underlying this relation. In particular, the contribution of glutamatergic projections from the infralimbic cortex (IL) and paraventricular nucleus (PVN) to hippocampal function under stress with relation to value encoding and learning under stress conditions is poorly understood. This study examined how different stress modalities alter behavioral and molecular outcomes during a novel learning task. C57BL/6 mice were exposed to one of three stressors—(i) unconditioned non-adaptive, (ii) unconditioned adaptive, or (iii) conditioned stimulus stress—prior to performing a reward-driven alternation task in a T-maze. To assess molecular responses, c-FOS expression, a gene critical for stress adaptation, was measured via RT-PCR. To test the functional role of IL and PVN projections to the HPC, we employed chemogenetic modulation. Retrograde excitatory (HM3Dq) & inhibitory (HMD4i) AAVs were injected into the IL & PVN respectively, and a guide cannula was implanted in the CA1 of VGlut2-IRES^{Cre} male mice. Following recovery, the DREADD agonist compound 21 (0.5 µg/kg) was infused into the ventral CA1. Behavioral effects were assessed using an open field test to measure anxiogenic responses. Results indicated that stress disrupted learning and behavior in a modality-dependent manner, with distinct perturbations observed across conditions. Specifically, learning was strongly impaired in the conditioned aversion stimulus group, whereas the non-conditioned stimulus group showed no significant change. Chemogenetic modulation of the IL produced no notable behavioral effects, while modulation of projections from the PVN was associated with reduced anxiogenic responses.

Acknowledgement: This work was funded by the NIMH and NSF.

Ventral tegmental area (VTA) glutamate projections moderate CA1 encoding of space that is contextualized by reward placement

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Pyramidal neurons in the dorsal hippocampal CA1 (dCA1) are excitatory neurons modulated by environmental cues. VTA contains diverse neuron types which controls reward, aversion, etc., and VTA glutamate terminals in hippocampus are dominant type. While a population of dCA1 cells encode spatial location, a separate group is activated by reward probability and encounters. Since “rewards” are predicted at “locations”, we hypothesized spatiotemporal patterns in dCA1 ensembles resolve contextual preference relative to reward encounters. We examined these encoding patterns in biased place preference tasks for simple reward acquisition and complex discrimination of reward magnitudes. To test the hypothesis, we used behavioral tests (conditioned place preference test) for mice without neural implants, revealed a higher sensitivity to discriminating between two locations associated with reward magnitude (2% sucrose in preferred side /20% sucrose in non-preferred side), in comparison to detecting a reward (0% in preferred side /16% sucrose non-preferred side). The analysis of dCA1 single unit after neural implant, spatiotemporal activity during pre-conditioning, revealed that peak firing position of these cells is less preferred context. Therefore, when the contextual preference is biased with a reward (0%/16%) or a higher magnitude reward (2%/20%), a change in dCA1 firing rate around context entry events reflects updated spatial preference. Interestingly, the context of lower preference with no associated reward (0%) or a reward of lower value (2%) elicits a stronger firing response when compared with the alternative contexts with higher reward values (16% and 20% respectively). Together, we conclude that the spatiotemporal mapping of the dCA1 ensemble and the threshold of peak firing rate change encodes the initial contextual preference and is remapped by the biased presentation of a reward or a reward with a higher value. Now, we want to investigate VTA-glu innervation of dCA1 encoding spatial learning relative to reward paired with optogenetics.

Keywords: reward, absolute, magnitude, firing rate, and spatiotemporal

Funding sources: National Institutes of Health (NIH), National Science Foundation (NSF).

Contralateral Corticothalamic Projections from Cortical Layer 6b

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Higher forebrain operations are mediated through an extensive network of corticothalamic, thalamocortical and corticocortical connections. Within the corticothalamic network, two projection systems have been classically regarded to have complementary roles; the layer 5 corticothalamic pathways are considered 'drivers' of thalamic activity, while the layer 6a pathways are considered 'modulators' of thalamic activity. Recently through, a third corticothalamic pathway, originating from layer 6b, has received increased attention, in part due to its laminar role in mediating forebrain wake states. Moreover, we have shown that this sublayer may be a significant source of contralateral corticothalamic projections originating from limbic/prefrontal cortical areas. As such, layer 6b is uniquely poised to influence contralateral forebrain operations via overlooked ipsicortico-contrathalamocortical loops. Thus, to assess the organization of this circuit, we characterized the corticothalamic projections from cortical layer 6b of three cortical areas: primary auditory cortex (A1), insular cortex (IC), and prefrontal cortex (PFC). We employed the *Drd1a-Cre* mouse lines, which specifically expresses Cre-recombinase in layer 6b corticothalamic neurons. We stereotaxically injected floxed anterograde adeno-associated virus expressing various fluorophores, EYFP (green) and mCherry (red), and examined the terminations of ipsilateral and contralateral corticothalamic projections to specific thalamic nuclei. These projections differ according to the area injected, topographic segregation of terminal fields, characteristic morphological features, with no contralateral projections observed from A1. Our experiments addressed overlooked neuronal pathways involved in forebrain processing, which could offer insights into deficits in neurological disorders mediated by these pathways.

Keywords: Cortical layer 6b, Thalamus, Corticothalamic pathway, Neuroanatomical tracing, Sensory processing

Funding Sources: NIH, NSF

Combined behavioral effects of genes associated with autism spectrum disorder and Alzheimer's disease in mice.

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Autism Spectrum Disorder (ASD) and Alzheimer's Disease (AD) share unexpected yet profound symptoms. ASD is a neurodevelopmental disorder that arises in early childhood, primarily affecting social communication, cognition, and behavior. In contrast, AD is a progressive neurodegenerative disorder that impairs memory and executive function in older adults. Although these conditions manifest at opposite ends of the lifespan, they appear to share common mechanisms, including disrupted synaptic function, abnormal neuronal connectivity, and dysregulated neurotransmitter systems. Both disorders are also associated with overlapping genetic risk factors and pathological changes, such as abnormal processing of amyloid precursor protein (APP). Furthermore, patients with ASD have a higher risk of developing AD. This study investigated whether a SHANK3B mutation, frequently implicated in ASD, exacerbates neurodegenerative changes in an APP knock-in (APP-KI) mouse model of AD. Behavioral assessments were conducted at 6 and 12 months of age using a battery of tests: Y-maze, open field, light-dark box, novel object recognition, social interaction, and tail suspension tests. These assays were used to evaluate spatial working memory, anxiety-like behavior, social behavior, and depression-like behavior. Both APP-KI and SHANK3B/APP mice exhibited social impairments, reduced novelty preference, and increased depression-like behavior, with deficits becoming more severe with age, which varied among the different genotypes. These findings suggest that synaptic vulnerabilities linked to ASD may accelerate AD-related cognitive and behavioral decline, underscoring the importance of early intervention strategies that target shared molecular pathways between neurodevelopmental and neurodegenerative disorders.

CONVERGENCE OF BILATERAL AUDITORY TECTOTHALAMIC PATHWAYS

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The medial geniculate body (MGB) receives diverse and robust ascending and descending projections, making it a critical hub in the central auditory system. Ascending excitatory and inhibitory inputs to the MGB originate from the auditory midbrain (inferior colliculus: IC), which convey and regulate auditory signals. While the ipsilateral auditory tectothalamic pathways are well characterized, the contralateral tectothalamic pathways are largely unexplored. Therefore, to explore the cell-type specific organization of the contralateral pathways and to characterize their topographic and convergent organization across meso- and micro anatomical and physiological scales, we employed a Cre-lox mediated, dual anterograde viral tracing approach using C57BL/6J, VGlut2-Cre and VGat-Cre mice. Also, in vitro slice physiological approach will be used to assess the functional characterization of bilateral tectothalamic synaptic physiology using whole-cell patch clamp electrophysiology. Overall, our data highlights the overlooked roles of the contralateral tectothalamic projections in central auditory processing.

Keywords: Medial geniculate body (MGB), Inferior colliculus (IC), contralateral IC

Funding: NIH Grant No: RO1DCo19348, NSF Grant IOS 1652432

Poster Presentations

Novel understandings from studies on ion channel complexes in TBI and Alzheimer's disease

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Ion channel–scaffold assemblies in brain cells critically shape excitability and homeostasis. We sought to map specific Kv7 (KCNQ) channel interactions by Förster resonance energy transfer (FRET) binding microscopy assay in two disease models.

In Alzheimer's models, the enzyme beta secretase 1 (BACE1) has previously been shown to bind Kv7.2 channels. BACE1 cleaves the amyloid-beta precursor protein (APP), a necessary step for the production of beta-amyloid (A β) peptide in Alzheimer's disease. Our FRET experiments in HEK cells did not show binding between APP- and Kv7.2. Currently, we are testing if the binding of Kv7.2 to BACE1 is affected by the presence or absence of APP. A tripartite assembly would couple amyloidogenic APP processing to modulation of the M- current, linking A β production with neuronal firing. Supporting this, pharmacological Kv7 opening (retigabine) in APP/PS1 mice reduced A β plaques and tau phosphorylation and normalized neuronal excitability.

Our FRET experiments revealed for the first time that Kv7.4 binds to aquaporin-4 (AQP4) and the scaffold protein AKAP79. Such a complex could coordinate astrocytic K⁺ uptake and water permeability after a traumatic brain injury (TBI). We hypothesize that AQP4 and Kv7.4 are functionally coupled in astrocytes. Interestingly, we observed Kv7.4 knockout specifically in astrocytes to reduce the blood-brain barrier (BBB) breakdown after a TBI. These findings suggest a Kv7.4–AQP4–AKAP nexus that may limit edema and help preserve BBB integrity. Thus, Kv7–scaffold complexes represent promising neuroprotective/anti-edema targets across neurodegenerative diseases and traumatic brain injury.

Together, our data identify novel Kv7 channel complexes in AD and TBI. Targeting these assemblies (e.g., with Kv7 modulators or agents that perturb pathological binding) could yield dual benefits.

Mitochondrial function is altered during heroin seeking

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Introduction:

Chronic opioid use leads to long-lasting increases in drug-seeking behavior; however, the causal molecular and cellular mechanisms responsible are not fully understood. One mechanism involves signaling through phospholipase Cgamma1 (PLCg1) in the nucleus accumbens shell (NAcSh). We previously found that many mitochondrial proteins are altered by NAcSh PLCg1, by heroin, or by a combination of both, suggesting a novel interaction between PLCg1, heroin, and mitochondria. We hypothesized that heroin-induced NAcSh mitochondrial dysregulation occurs during heroin seeking, and that PLCg1 protects against heroin-induced mitochondrial dysregulation.

Experimental Approach:

We first infused a shRNA expression viral vector that reduces PLCg1 levels (AAV-shPLCg1) or a control virus bilaterally into the NAcSh of rats using stereotaxic surgery. Then we allowed rats to self-administer either heroin or saline for 12 days in a 2x2 design. Following seven days of forced abstinence, rats underwent a 30min heroin-seeking test and accumbens shell tissue was harvested. Next, the tissue was analyzed using high-resolution respirometry to measure rates of oxidative phosphorylation (OXPHOS) and mitochondrial efficiency.

Results:

Interestingly, we found that heroin and a PLCg1 knockdown together increased OXPHOS capacity, indicating that the mitochondria are generating a higher level of ATP in this context. Mitochondrial efficiency was also increased by both a PLCg1 knockdown and heroin.

Discussion:

These results show that mitochondrial activity is increased during heroin seeking, but that endogenous PLCg1 protects against these changes in OXPHOS and efficiency. These results suggest that these accumbal mitochondrial changes could be causal to the increased heroin seeking observed following a NAcSh PLCg1 knockdown. Future experiments will determine if these mitochondrial changes are causal to the behavior as these results could reveal new therapeutic targets for treating substance use disorders and lead to a better understanding of the role of mitochondria in addictive disorders.

Stress can both increase and decrease two-bottle choice ethanol consumption in female and male mice

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Background: Alcohol use disorder (AUD) is a chronic condition that is often comorbid with mental disorders like depression or post-traumatic stress disorder. Furthermore, prolonged stress and anxiety can increase the severity of AUD. However, the mechanisms controlling the effect of stress on AUD are still not fully understood. Thus, preclinical animal models of stress-induced changes in alcohol drinking are needed. Therefore, we aimed to determine how different stressors alter alcohol consumption in mice.

Methods: Male and female C57Bl/6J mice were allowed to self-administer ethanol in a two-bottle choice (2BC) protocol for at least five weeks (15% ethanol vs water, 2hr/day). Then, mice were split into a control (N=6-8M, 6-8F) or stress group (N=6-8M, 6-8F). Mice in the stress group were exposed to four different stressors: 1) injection of 5mg/kg i.p. U50,488, 2) repeated predator odor stress with 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) (1hr/day for 5 days), 3) 1-hour exposure to a live cat, and 4) injections of 1.25-5mg/kg i.p. of yohimbine. In a separate experiment, 3-week-old mice were separated into either group housing (N=2M, 6F) or single housing (N=2M, 5F) for 5 weeks to study the effect of isolation stress on 2BC as adults.

Results: After five weeks of 2BC, we exposed mice to U50,488 and saw significant increases in ethanol consumption in both sexes. After TMT exposure, female mice increased ethanol drinking, but males did not. In contrast, both yohimbine and a live cat predator exposure reduced ethanol consumption in both sexes. Finally, isolation stress did not produce any significant differences in alcohol drinking.

Conclusion: Our results show that different stressors can increase, decrease, or have no effect on alcohol consumption. Interestingly, female mice were more sensitive to the TMT stress, suggesting potential sex differences. Understanding the molecular events responsible for these different effects may determine mechanisms of how stress alters alcohol consumption.

Funding source: NIAAA R01 AA031007

Ahnak knockdown in the nucleus accumbens shell decreases heroin seeking in rats.

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Drugs of abuse such as heroin induce drug-seeking behavior, however, the underlying mechanisms remain unclear. Heroin use activates the nucleus accumbens shell (NAcSh), a key component of the brain's reward circuitry that plays a critical role in addiction, and likely cause heroin-induced synaptic plasticity. Previous results shows that reward signaling and synaptic plasticity in the NAcSh is mediated in part by phospholipase Cgamma1(PLCg1) an important protein in addiction. PLCg1 can be activated by an interaction with a protein called Ahnak. Ahnak, means 'giant' in Hebrew and is a very large 680 kDa protein. Due to Ahnak's interaction with PLCg1, we hypothesized that reducing NAcSh Ahnak would reduce drug seeking. To test this, we developed an adeno-associated virus (AAV) encoding a short hairpin RNAs (shRNA) to knockdown Ahnak (AAV-shAhnak). In this study, we first confirmed that AAV-shAhnak reduces Ahnak mRNA levels in vivo. Then, rats received a stereotaxic injection of AAV-shAhnak or a control AAV into the NAcSh. Two weeks later, a chronic, indwelling, jugular vein catheter surgery was performed, followed by a week of recovery. Rats were then trained to self-administer heroin for at least 12 days by lever pressing in operant conditioning chambers. Seven days after the last self-administration session, we then tested for heroin seeking by measuring lever pressing in the absence of heroin infusions. Our results show that knockdown of NAcSh Ahnak decreases the number of lever presses in the heroin-seeking phase. In conclusion, since Ahnak knockdown in the NAcSh decreases heroin-seeking behavior, this suggests that endogenous NAcSh Ahnak typically increases heroin-seeking, possibly through an interaction with PLCg1. These results deepen our understanding of addiction mechanisms and suggest Ahnak as a potential therapeutic target for reducing heroin-seeking and improving SUD outcomes.

Funding source: Anderson Start-up Funds

Role of Nucleus Accumbens Shell Ubiquitin Specific Protease 5 on Heroin Seeking

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The nucleus accumbens shell (NAcSh) is a part of the brain's limbic system that is implicated in addiction and reward. The addictive drug heroin can alter synaptic plasticity mechanisms in the NAcSh that may cause increased craving. Synaptic plasticity is dependent on changes in synaptic proteins, many of which play significant roles in addiction. Experimental alterations of NAcSh synaptic proteins can either enhance or reduce drug seeking, however, the mechanisms are not fully understood. Synaptically expressed proteins include ubiquitin, a protein that tags other proteins for degradation. Ubiquitin tags on proteins can be removed by ubiquitin specific proteases (USPs), thus USPs can prevent synaptic degradation. Previous studies have demonstrated that some USPs modulate many synaptic proteins, however, the role of USP5 has not been studied in relation to synaptic proteins and drug seeking. Since USP5 could regulate the strengthening and formation of opioid-induced synaptic plasticity, we hypothesized that knocking down NAcSh USP5 would increase heroin seeking. To test this hypothesis, we used RNA interference by cloning a novel short hairpin RNA (shRNA) plasmid, a type of RNA that silences genes by degrading their messenger RNA. This cloned shRNA targets USP5, and we next packaged it into an adeno-associated viral vector (AAV-shUSP5). A shRNA plasmid that targets the luciferase gene was used as a control vector (AAV-shLUC). Using stereotaxic surgery, these viruses were injected into the NAcSh of rats. An in vivo characterization of AAV-shUSP5 using qPCR resulted in a successful NAcSh USP5 knockdown. The rats were then allowed to self-administer heroin in operant chambers for at least 14 days and afterwards were tested for drug seeking. Our results show that NAcSh USP5 knockdown does not alter heroin-taking behavior, however, it does increase heroin seeking. Therefore, enhancing NAcSh USP5 levels could be therapeutic in the management of opioid use disorder.

Funding source: Anderson lab start up funds.

Gold Nanorod-Mediated Neuroanatomical Tracing and 3D Modeling

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Introduction: The uptake of metal nanomaterials in the brain is relevant for medical applications, and has potential in neuronal circuitry modeling, as well as neuromodulation. Gold nanorods are of particular interest as they have unique properties and a large surface area that can be utilized for conjugation. Gold nanorods can be conjugated to anterograde and retrograde tracers to alter their neuronal uptake. Additionally, their density and high X-ray attenuation make them an ideal contrast agent to be used with micro computed tomography (microCT), which is relevant in neuroscience applications as it has potential to be utilized for minimally invasive circuitry imaging.

Hypothesis and questions: Can gold nanorods provide sufficient contrast to be imaged via microCT, and can they be utilized for neuronal circuitry modeling?

Methods: Male and female mice underwent stereotaxic injection with gold nanorods in different regions of the brain including the medial prefrontal cortex, paraventricular nucleus, ventral tegmental area, and dorsal and ventral hippocampus. Gold nanorods conjugated to an anterograde neuronal tracer, PHA-L, and to a fluorophore, were used. Post-injection mice were perfused after 5, 6, or 7 days, and their brains were imaged with microCT. MicroCT data was reconstituted and imported into Avizo 3d, where segmentation and thresholding were utilized to precisely select for the gold nanorods. A 3D model was created to view uptake and diffusion of the gold nanorods post-injection.

Results: Gold nanorods diffused from their injection sites, and could be effectively separated from brain tissue based on micro CT voxel intensity in Avizo 3D. Through surface generation of labeled volumes in Avizo 3D, models were generated demonstrating gold nanorod localization.

Discussion: Gold nanorods were effectively visualized post-injection through microCT imaging and modeling, which has potential to enhance the understanding of neuronal connectivity and circuitry in the brain. Further experiments will explore the uptake and transport mechanisms of conjugated gold nanorods, and utilize alternative conjugations.

Funding: This work is funded through research support provided by the NIMH and NSF.

Downregulation of huntingtin in offspring brain is linked to paternal western diet (WD), behavioral changes, and alterations in miRNAs in *Drosophila*.

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a trinucleotide CAG repeat expansion in the huntingtin (HTT) gene, this mutation leads to atrophy of the basal ganglia. In HD, dysregulation of miRNA is associated with the disease's pathogenesis. Specifically, several transcriptional regulators, such as REST, TBP, CBP, RE1, and p53, are highlighted as biomarkers and therapeutic targets. We employed *Drosophila melanogaster* to study diet–miRNA–HTT interactions, as its HTT orthologs and neuronal pathways are conserved. In a previous proteomic study, we observed a downregulation of normal Htt levels in the brains of offspring following paternal exposure to a WD. This finding suggests that metabolic dysregulation in fathers—encompassing obesity and altered feeding behaviors—may heighten offspring susceptibility to neurological deficits. Given that mir-10 is predicted to target htt mRNA in this study employing *Drosophila* genetic tools, we explored whether altering this miRNA could replicate the HD phenotype. Our data indicate that miR-10 and miR-1006 appear to regulate metabolic and behavioral responses to WD. Their depletion led to increased food consumption, alterations in locomotor activity, and changes in sleep patterns. These miRNAs may contribute to the development of obesity by modulating appetite and energy expenditure, potentially influencing neural activity linked to feeding behavior. Their independent and possibly synergistic roles in managing diet-induced metabolic dysfunction echo HTT deficiency, suggesting they may play a part in HD-related pathways. We are investigating behavioral responses in symptomatic HD fruit flies to uncover the molecular mechanisms driving HD pathophysiology. Gaining a deeper understanding of this interaction could offer novel insights into miRNA-based therapeutic strategies for HD and other metabolic disorders.

Toxicology Specialties



Ahmed Abdelmoneim, DVM, Ph.D.

Our research program investigates how developmental exposure to environmental contaminants rewires the neuroendocrine stress axis and behavior, mapping mechanisms from receptor signaling to circuit function. Our goal is to determine how specific exposures cause stress-related disorders in animals and humans and to translate these mechanisms into strategies for diagnosis, prevention, and treatment. We use zebrafish as a scalable vertebrate model that adheres to the 3Rs (Replacement, Reduction, Refinement), supports high-throughput assays, and provides direct optical access to the developing brain.



Alexandra Noel, Ph.D.

Dr. Noël's research focuses on how prenatal exposure to vaping aerosols, engineered nanoparticles, and other airborne pollutants programs neonatal lungs for diseases such as allergic asthma. She pairs custom aerosol generation with inhalation toxicology, in-utero disease models, and epigenetic profiling to uncover pathways linking early exposure to lifelong respiratory risk.



Tammy Dugas, Ph.D.

Our toxicological research focuses on the role of the environment and environmental factors in the induction or exacerbation of vascular diseases including atherosclerosis and pulmonary hypertension. In particular, we are investigating the vascular toxicity of airborne particulate matter, of aromatic amines, compounds used industrially in the production of polyurethanes, and of HIV antiretroviral drug therapies. Mechanistic studies aimed at elucidating these toxicities explore drug/toxicant metabolism, endothelial cell injury (including mitochondrial oxidant stress), and stimulation of vascular smooth muscle cell proliferation. We furthermore explore whether females are more susceptible to these vascular toxicities. In other toxicological studies, we are examining the potential for combustion-generated ultrafine particulate matter (PM) to produce reactive oxygen species and induce oxidative stress in rodents exposed by inhalation. Finally, in our pharmacological studies, we are elucidating pathways by which red wine polyphenols might act on the estrogen receptor so as to promote vascular homeostasis and prevent the development of vascular diseases including atherosclerosis and restenosis.

Oral Presentations

Uncovering the Role of Glucocorticoid Signaling in Arsenic-mediated Stress Behavioral Alterations

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Arsenic (As) is a widespread environmental contaminant linked to numerous adverse health outcomes. Growing epidemiological and experimental evidence suggest its role as a risk factor for stress-related disorders such as anxiety and depression. However, the neuroendocrine mechanisms driving these stress behavioral alterations remain poorly understood. Our previous work demonstrated As-induced hyperactive stress behavioral phenotypes in larval zebrafish. Glucocorticoid (GC) signaling plays a key role in regulating behavioral stress responses and As has been shown to dysregulate it; therefore, in this study, we investigated the role of GC signaling in mediating physiological and As-induced stress behavioral phenotypes in larval zebrafish. To study this link, we used wild-type and transgenic zebrafish lines along with a combination of approaches, including pharmacologic GC modulations, co-exposures with As, high-throughput stress behavioral assessments, and in vivo imaging of corticotropin-releasing hormone (CRH) neuronal structure and activity—key regulator of GC signaling. Pharmacological modulation of GC signaling (dexamethasone, mifepristone, and metyrapone) produced behavioral phenotypes that mirrored As exposure; while co-exposure studies supported a mechanistic link. Structural imaging of CRH neurons revealed As-dependent changes in the neuronal count. In vivo calcium imaging of CRH neurons demonstrated changes in neuronal activity in response to GC pharmacological modulations and As-induced alterations that can be closely linked to previously observed stress behavioral phenotypes. Our findings provide deeper insights into the neuroendocrine mechanisms underlying As-mediated stress behavioral disorders and identify GC signaling as a possible driver of effects, highlighting GC-targeting strategies as potential avenues for mitigation and therapeutic interventions.

Keywords: Arsenic, glucocorticoid signaling, CRH neurons, developmental neurotoxicity, neurobehavior, stress-related disorders.

The role of noradrenergic (NA) regulation in lead (Pb)-mediated aberrant stress behavior in zebrafish (*Danio rerio*)

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Pb exposure during critical developmental periods disrupts neurophysiological systems governing stress and emotional regulation. Our previous work demonstrated that developmental Pb exposure impairs structural integrity of the NA innervation of the dorsal telencephalon, the amygdala homolog, causing dose-dependent reductions in volume and axonal complexity, and alters the locus coeruleus (LC) by increasing neuron number, disrupting neuronal organization, and suppressing spontaneous activity. However, the impact of Pb on adrenergic signaling, essential for stress-related behavioral responses, remains unclear.

We hypothesized that developmental Pb exposure disrupts LC NA neuron function, thereby altering signaling to α 1- and α 2-adrenergic receptors that regulate stress responses. By pharmacologically modulating these receptors, we aimed to test whether Pb-induced behavioral abnormalities could be rescued. To test this, zebrafish larvae were exposed to prazosin (α 1 antagonist) or yohimbine (α 2 antagonist) at 0.5, 2.3, 11.0, 56.5, and 280 μ M for 20 min or 12 – 16 hr. Stress responses were quantified using automated locomotor tracking during acoustic startle (100 Hz, 100 ms; AMR) and visual motor response (1200 lux, VMR) assays.

Acute yohimbine exposure produced significant hyperreactivity during AMR at 56.5 and 280 μ M and during VMR at 11.0 and 56.5 μ M. Prolonged yohimbine exposure (12 – 16 hr) caused hyporeactivity at 56.5 and 280 μ M in both assays. Prazosin exposure showed a biphasic dose-response, with moderate concentrations increasing locomotor activity and higher concentrations producing marked response suppression after both 20 min and 12 – 16 hr exposures.

These results demonstrate that α 1- and α 2-adrenergic receptor antagonists differentially modulate stress-response behaviors in zebrafish. Future work will test whether adrenergic receptor agonists and antagonists can normalize Pb-induced behavioral abnormalities. This approach will advance identification of receptor-specific mechanisms of action and potential therapeutic targets to mitigate the long-term neurotoxic effects of developmental Pb exposure.

Funding: NIEHS R21 ES037092

Larval Zebrafish Dark Avoidance: Linking Anxiety-Like Behaviors With Environmental Contaminant Exposure

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Epidemiological studies link developmental exposure to environmental contaminants with stress related disorders, yet the mechanisms underlying these behavioral maladaptations remain underexplored. Reflexive behaviors—such as response to sudden stimulus—are simple and therefore widely adopted in toxicological investigations. However, behaviors in relation to choice—such as searching for food or predator avoidance—offer richer insight, but are underutilized. This study aimed to optimize a larval zebrafish light/dark preference assay as part of a standard platform for detecting chemical-induced stress and anxiety-like behaviors. We investigated the earliest age appropriate (5-, 6-, or 7-days post-fertilization), the effects of rearing conditions adopted in toxicological investigations (dark raised vs circadian cycle), and the most appropriate holding volume (7, 5, or 4mL). The time larvae spent in light versus dark zones, while under unstressed or heat stressed conditions, was quantified as a choice index. Subsequently, we validated the optimized assay with anxiety modulators (caffeine and buspirone) and applied it following developmental exposures to bisphenol A and its alternatives; bisphenol F, bisphenol S, and bisphenol AF. Bisphenols have been detected among children and have been associated with anxiety-related behaviors. Our results supported testing 6-day old larvae in 7mL holding volume as the optimal configuration, and that rearing regimen did not significantly affect outcomes. Caffeine and buspirone produced the expected anxiogenic and anxiolytic profiles, and bisphenol exposures altered anxiety-like behavior. BPA exposure trended towards increasing dark avoidance without stress, but not under stressful conditions. Exposure to BPF and BPS induced increased dark avoidance at .04uM concentration but reduced this behavior at 1uM concentration when stressed. BPAF exposure induced a greater difference in choice under stressed and unstressed conditions, warranting further investigations. These findings highlight a robust scalable assay for assessing contaminant-induced changes in choice-related anxiety behaviors.

Poster Presentations

Title: Weathered micro/nanoplastics induce cytotoxicity and oxidative stress in human bronchial epithelial cells

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Rationale: Exposures to micro/nanoplastics (MNPs) are an emerging public health concern due to their omnipresence in our daily life and risk of absorption by ingestion or inhalation. MNPs found in the outdoor environment have the potential to be photochemically weathered and to generate free radicals, and the ability to adsorb co-contaminants, including heavy metals. We hypothesized that the toxicity induced by MNPs in lung cells is dependent on weathering processes, e.g., photochemical aging. Thus, we tested whether weathered MNPs are more toxic to lung cells than pristine MNPs.

Methods: Human broncho-epithelial cells (BEAS-2B) were grown at the air-liquid interface (ALI) and exposed to pristine (PNPs), weathered (WNPs), and lead-adsorbed weathered MNPs (WPbs) at environmentally relevant doses of 30-40 ng/cm². Following a 24-hour incubation, we assessed cytotoxicity, oxidative stress, and gene expression.

Results: While MNP exposure did not significantly decrease cell viability, results showed clear signs of cellular stress. Exposure to WPbs induced significant cytotoxicity, increasing extracellular lactate dehydrogenase (LDH) by 247%, which indicates cell membrane damage. Similarly, WPbs caused a 254% significant increase in reactive oxygen species (ROS) production, demonstrating a strong oxidative stress response. All MNP treatments showed an upward trend in LDH, ROS, and nitric oxide (NO) production, suggesting the activation of inflammatory pathways. Gene expression analysis showed that WNPs and WPbs significantly downregulated Hmox1 and Tnf- α , suggesting a compromised ability to protect against oxidative stress and regulate the post-injury, pro-inflammatory response.

Conclusion: Overall, the severity of cellular damage followed a clear trend of WPbs > WNPs > PNPs, suggesting that environmental weathering and co-contamination increase MNP-induced cellular stress in the lung epithelium. To our knowledge, this is the first study to investigate these MNP effects in a BEAS-2B ALI model at doses relevant to human inhalation exposures.

Funded by State of Louisiana - Governor Biotechnology Initiatives (GBI #013)