UNIVERSITY OF GEORGIA

Interdisciplinary Toxicology Program

Spring Workshop 2024

Program and Abstracts

April 10th, 2024

UNIVERSITY OF GEORGIA
Interdisciplinary Toxicology Program

Annual Spring Workshop

Paul D. Coverdell Center for Biomedical & Health Sciences
Rotunda and Rm. 175
Wednesday, April 10, 2024

8:00am	Breakfast
8:30am	Welcome and Director's Update: **Dr. Nick Filipov**
8:40am	Graduate Coordinator Update: **Dr. Tai Guo**
8:50am	UGATOX Update: **UGATOX President Seth Currie**
9:00am	Graduate School Welcome: **Dr. Anne Shaffer, Associate Dean of the Graduate School**
9:10am - 10:10am	Keynote Presentation: "Environmental Pollutants as Drivers in Autoimmune Disease." **Sarah Blossom, Ph.D.,** Professor and Director NM IMSPIRES Environmental Health Science Core Center, University of New Mexico, Albuquerque, NM
10:10am - 10:25am	*Break*
10:25am – 11:40am	ITP Student Platform Presentations:
10:25am - 10:40am	"Testing the potential of nonlethal epigenetic profiling to predict individual differences in tolerance to heavy metal exposure" **Ethan Shealy. Advisor: Benjamin Parrott**
10:40am - 10:55am	"Impact of Tobacco Smoke Extract on In Vitro Spermatogenesis" **Kylie Tager. Advisor: Charles Easley**
10:55am - 11:10am	"Source apportionment of $PM_{2.5}$ personal exposure samples from the Household Air Pollution Intervention Network (HAPIN) trial in Guatemala **Erick Mollinedo. Advisor: Luke Naeher**
11:10am - 11:25am	"Effect of Manganese Exposure via Drinking Water on Neurotransmitters in Adult Male and Female Mice" **Danielle Ludwig. Advisor: Nick Filipov**
11:25am - 11:40am	"Electrooxidation Degradation of Per- and Polyfluoroalkyl Substances (PFAS) by Modified Titanium Suboxide Anodes" **Yufei Sui. Advisor: Qingguo Huang**
11:45am	Annual Photograph of Interdisciplinary Toxicology Program
11:50am - 1:15pm	*Lunch*
1:15pm - 1:30pm	Students in Rm. 334 to vote on New UGATOX Board
1:30pm – 3:15pm	ITP Student Poster Viewing – Judging
3:30pm	New UGATOX Board announced. Post-Survey; Recognition of Poster and Platform Student Award Winners
4:00pm – 5:30pm	Students Ice Cream networking roundtable with Dr. Sarah Blossom College of Veterinary Medicine, Rm. 2007

LIST OF STUDENT PRESENTATIONS

PLATFORMS

Ethan Shealy "Testing the potential of nonlethal epigenetic Profiling to predict individual differences in tolerance to heavy metal exposure"
Advisor: Benjamin Parrott

Kylie Tager "Impact of Tobacco Smoke Extract on in vitro Spermatogenesis"
Advisor: Charles Easley

Erick Mollinedo "Source apportionment of $PM_{2.5}$ personal exposure samples from the Household Air Pollution Intervention Network (HAPIN) trial in Guatemala**"**
Advisor: Luke Naeher

Danielle Ludwig "Exosomal histone drives inflammasome activation and a-synuclein aggregation"
Advisor: Nick Filipov

Yufei Sui **"**Electrooxidation Degradation of Per- and Polyfluoroalkyl Substances (PFAS) by Modified Titanium Suboxide Anodes"
Advisor: Qingguo Huang

POSTERS

Ignacio Llada Exploring the metabolome and microbiome of steers grazing tall fescue: impact of low-level ergot alkaloids and endophyte presence in the grass
Advisor: Nikolay Filipov

Seth Currie Decoding the Effects of Per- and Polyfluoroalkyl Substances (PFAS) Mixtures on Neurodevelopment: Transcriptomic Insights from Caenorhabditis elegans
Advisor: Lili Tang

Blake Benson A High-Throughput, High-Content Analysis of Dopaminergic Neuron Degeneration in *Caenorhabditis elegans* Exposed to Per- and Polyfluoroalkyl Substances (PFAS)
Advisor: Lili Tang

Arina Chernikova	Impact of SARA-COV-2 infection on the Blood-Testis Barrier in Humans and Nonhuman Primates **Advisor: Charles Easley**
Alejandra Bargues-Carot	Histones in Extracellular Cesicles Augment Inflammasome Activation and a-Synuclein Aggregation **Advisor: Anumantha Kanthasamy**
Alyssa Otto	Evaluating Gut to Brain Pathology with an α-synuclein Engineered Microbe – A Novel Animal Model for Parkinson's Disease? **Advisor: Anumantha Kanthasamy**
Fatma Eldefrawy	Early Glycation Products Protect against Type 2 Diabetes and autistic Through Modulating Gut Microbiome and Associated Metabolic Pathways **Advisor: Tai Guo**
Jonathan Hancock	Mechanism of estrogenic endocrine disruptors in regulating uterine fluid movement **Advisor: Xiaoqin Ye**
Taylor Martin	Mechanisms of hormonal contraceptive Mifepristone (RU486) in regulating uterine fluid movement **Advisor: Xiaoqin Ye**
Jake Smith	Evaluating Microcystin in Water and Fish Tissue from Four Reservoirs in the Georgia Piedmont USA **Advisor: Peter Hazelton**
Fabian Tejedor-Rojas	Use of delayed treatment approach with the immunoprophylactic Lacto-N-Fucopentaose III (LNFOIII) and/or insulin –supplemented diet for improvement of hippocampal synaptic plasticity in Gulf-War Illness (GWI) model: preliminary findings **Advisor: John Wagner**
Jiaqi Chen	Use of delayed treatment approach with the immunoprophylactic Sulindac sulfide enhances the chemotherapy of breast cancer **Advisors: Wentao Li and Yaguang Xi**

Trevor Kalinowski	Neurochemical analysis of monoamine perturbations in a preclinical Gulf War Illness model: effects of sex **Advisor: Nick Filipov**
Yuqing Ji	Electrochemical degradation of per- and polyfluoroalkyl acids (PFAS) on titanium suboxide anodes **Advisor: Qingguo Huang**

Notes

Spring Workshop 2024
Platform Presentations
April 10, 2024

Testing the potential of nonlethal epigenetic profiling to predict individual differences in tolerance to heavy metal exposure

Authors: Ethan P. Shealy, Marilyn W. Mason, Benjamin B. Parrott

Recent advances have demonstrated that variation in DNA methylation profiles can be modeled to predict organismal traits such as chronological age, as well as more elusive phenotypes like age at maturity and all-cause mortality risk. Within toxicological frameworks, variation in individual responses to contaminant exposure are commonly observed; yet, the molecular and physiological traits that underlie variation at the individual level are not resolved. Here, we combine the genetically and experimentally tractable medaka fish model with a novel experimental design to test if standing variation observed across individual DNA methylomes predicts outcomes associated with exposure to a common environmental contaminant. Whole genome methylation sequencing was conducted on non-lethally acquired fin tissues in a cohort of Japanese medaka fish raised under common environmental conditions. Fish were then individually exposed to a lethal dose of copper sulfate and time-to-death was assessed. Traditional modeling as well as machine learning approaches were used to examine the relationships between DNA methylomes and individual responses. While we were able to identify a small number of genomic loci whose methylation status appeared to correlate with survival time, the ability to predict time-to-death from this information was insignificant. If informative methods are eventually developed, epigenetic surveying may prove to be promising in predicting risk of harm from contaminants to specific populations and even individuals within a population.

Impact of Tobacco Smoke Extract on *in vitro* Spermatogenesis

Kylie R. Tager[1,2], Ian D. Bachli[1,2], Elizabeth Waters[1,2,3], Krista M. Symosko[1,2], Katherine Watkins Greeson[1,2], R. Clayton Edenfield[1,2], In Ki Cho[1,2], Arina Chernikova[1,2], Edward D. Levin[4], Charles A. Easley[1,2]

[1] Department of Environmental Health Science, College of Public Health, College of Public Health, University of Georgia, Athens, GA 30602
[2] Regenerative Bioscience Center, University of Georgia, Athens, GA 30602
[3]Biomedical and Translational Sciences Institute, University of Georgia, Athens, GA, 30602
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Tobacco, the second most used psychoactive substance worldwide, is used by 24% of all adults, with an estimated 21% in 2025. The World Health Organization reports that 80% of tobacco users primarily smoke cigarettes, exposing themselves to more than 7,000 identified chemicals, including nicotine. Given the prevalence of male smokers, the addictive nature of nicotine, and the adverse effects of cigarettes, public health specialists are increasingly interested in how tobacco smoke affects male reproductive health and subsequent offspring health and development. Recent studies suggest cigarette smoke induces significant changes in semen parameters, such as significant reductions in sperm density, total sperm counts, and reduced sperm motility in addition to significant changes in axoneme structure. Smoking may also increase levels of seminal oxidative stress, and impact fertilization through various mechanisms including changes in acrosin activity, mitochondrial membrane potential, glutathione levels, apoptosis, post-translational modifications, and DNA fragmentation. To assess the effects of tobacco smoke on human spermatogenesis, we exposed differentiating spermatogenic-like cells to environmentally relevant levels of tobacco smoke extract using two *in vitro* spermatogenesis models. A series of assays revealed significant changes in cell death and cell cycle stage distribution when comparing the two differentiation protocols, including decreased cell counts at the G0/G1 phase, increased levels of late apoptosis, and increased haploid cell counts in the stepwise co-culture differentiation model. However, individual cell line data showed nonsignificant trends, suggesting minor direct effects of TSE on spermatogenesis. Future work will examine pathway-specific effects on spermatogenic and somatic support cells, including impacts on the blood-testis barrier, to fully elucidate how TSE negatively affects spermatogenesis.

Source apportionment of $PM_{2.5}$ personal exposure samples from the Household Air Pollution Intervention Network (HAPIN) trial in Guatemala

Authors: Erick E. Mollinedo[1], Katherine A. Kearns[2], Armistead G. Russell[3], Michael Johnson[2], Christian L'Orange[4], Ricardo Piedrahita[5], Ajay Pillarisetti[6], Jeremy Sarnat[7], John P. McCracken[1], Thomas F. Clasen[7], Jennifer Peel[8], William Checkley[9], Luke P. Naeher[1]

Author affiliations: [1]College of Public Health, University of Georgia, Athens, GA; [2]Berkeley Air Monitoring Group, Berkeley, CA; [3]School of Civil and Environmental Engineering, Georgia Tech, Atlanta, GA; [4]Department of Mechanical Engineering, Colorado State University, Fort Collins, CO; [5]University of Colorado, Boulder, CO; [6]School of Public Health, University of California, Berkeley, CA; [7]Rollins School of Public Health, Emory University, Atlanta, GA; [8]Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO; [9]Johns Hopkins University School of Medicine, Baltimore, MD.

Background: Fine particulate matter ($PM_{2.5}$) is a major air pollutant from household air pollution. Human toxicity to $PM_{2.5}$ depends on its chemical and physical composition which varies by the sources of emission. Source apportionment is a methodology that aims to identify sources of pollution to reconstruct their impacts, so strategies to improve air quality can be implemented. This study aimed to determine the potential sources contributing to $PM_{2.5}$ in rural Guatemala and determine how they are impacted by shifting from biomass cooking fuels to cleaner cooking options.

Methods: We selected 629 archived personal exposure filter samples from the Household Air Pollution Intervention Network (HAPIN) randomized-controlled trial Guatemala study site. $PM_{2.5}$ and black carbon (BC) concentrations were estimated by gravimetric and transmittance analyses, respectively. The concentrations of 24 inorganic chemical species were determined using x-ray fluorescence. Source apportionment was computed via positive matrix factorization (PMF) using the Environmental Protection Agency PMF 5.0 software. The PMF models were run for 3 to 6 factors, and judged based on known potential sources, the Q-function, and error estimates.

Results: Eleven chemical species, including BC, were detectable in the filter samples. A 5-factor model was chosen as the best fit and four potential sources were identified: Biomass burning, crustal, gasoline fuel, and agricultural residues. A fifth source was also observed but was not resolved due to its possible association with compounds not included in this analysis. BC, Ca, K, Mg, Mn S, Si and Ti had significantly lower concentrations ($p < 0.05$) among samples from households cooking with liquefied petroleum gas (LPG) compared to those cooking with biomass.

Conclusion: Five potential sources of air pollution were detected in $PM_{2.5}$ exposure samples from Guatemala. While cooking with LPG reduces overall exposures, other sources of exposure are important beyond the combustion of cooking fuels.

Effect of Manganese Exposure via Drinking Water on Neurotransmitters in Adult Male and Female Mice

Author: Danielle Ludwig

Author List: H. D. Ludwig[1,2], N. M. Filipov[1,2]

[1]Department of Physiology and Pharmacology, Athens GA, United States
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Manganese (Mn) is an essential metal and is important for several biological processes. However, Mn overexposures can cause adverse neurological effects at the structural, functional, and behavioral level. Additionally, Mn acts as an (neuro)inflammatory adjuvant and has been associated with basal ganglia dysfunction in cases of occupational as well as environmental overexposures. Consumption of Mn contaminated drinking water (DW) is the route of concern for non-occupational exposure. Most rodent studies addressing Mn neurotoxicity use primarily males and sex differences in terms of nervous and immune system responsiveness to excessive Mn in the DW are underexplored. Earlier, we investigated sex differences in neurobehavioral and neuroinflammatory alterations in response to subchronic Mn DW treatment. Adult male and female C57BL/6 mice, with GFP-tagged monocytes/microglia, were exposed to Mn via the DW (0.4g/L) for 8 weeks and behavioral assessment was conducted after 6 weeks of treatment. After 8 weeks of Mn treatment, a subset of the mice were given lipopolysaccharide (LPS), to characterize sex and Mn dependent inflammatory responses. Mn exposure resulted in gait deficits in both sexes and caused perturbations in multiple mood tests that persisted in the males after the LPS challenge. Inflammatory marker in the periphery of males exposed to Mn and challenged with LPS were potentiated, but the reverse was true in the female mice.

Using brains from the same study, the objectives of the current study were to determine the effects and sex specificity of Mn exposure on regional brain monoamine and amino acid neurotransmitters in the presence and absence of an inflammatory challenge in both males and females. Samples from the (i) striatum (STR), a brain region involved in motor control, (ii) prefrontal cortex (PFC), an executive function brain region involved in decision making, and (iii) the ventral hippocampus (vHIP), a region important for the regulation of stress responsivity and emotion in addition to learning and memory, were collected , processed, and analyzed by HPLC-ECD for all major monoamines and their metabolites and the amino acid neurotransmitters glutamate and GABA. In the STR, LPS challenged mice had increased levels of the dopamine (DA) metabolite HVA, as well as of serotonin (5-HT) and its metabolite 5-HIAA in both sexes. Mn did not affect striatal monoamines/metabolites in the absence of LPS challenge in either sex. However, STR DA levels were decreased in Mn-exposed, LPS-challenged mice overall, with

the effect being more pronounced in the females. Additionally, females had higher striatal DA and 5-HT than males. Within the vHIP, most monoamines were higher in the female mice. Only 5-HIAA levels were significantly increased after LPS challenge and the main effect was female-driven. Even though Mn DW exposure increased Mn levels in the brain of both sexes, the PFC was the region where Mn exposure caused most significant perturbations of monoamine homeostasis. In the PFC, Mn-exposed females, but not males, had increased levels of norepinephrine (NE); PFC 5-HT was decreased by Mn overall, but the decrease was still present after the LPS challenge only in the males. 5-HIAA and DOPAC were increased after the LPS challenge; this effect was female-driven and only in the females was potentiated by Mn, i.e., Mn-exposed, LPS-challenged females had increased levels of 5-HIAA and DOPAC. While males might be more susceptible to the effects of Mn on mood and locomotion without many perturbations in monoamine neurotransmitter levels, females may have alterations in mood that are more subtle than the effects on locomotion even with changes in monoamine levels. Both sexes had increased monoamine levels when exposed to LPS; however, the potentiated effect of Mn-exposure was only observed in the PFC of females. This further suggests that Mn DW exposure effects are sex biased. All monoamine analyses have been completed to date, and amino acid neurotransmitter analysis of the PFC, vHIP, and the STR are ongoing. This project is supported by grant number ES026383 (NIEHS).

Electrooxidation Degradation of Per- and Polyfluoroalkyl Substances (PFAS) by Modified Titanium Suboxide Anodes

Authors List: Yufei Sui, Xi Zhu, Yaye Wang, Gengyang Li, Lei Li, Shuping Dong, Yifei Wang, Hui Lin, Ke Li, Qingguo Huang*.

Per- and Polyfluoroalkyl Substances (PFAS), also known as "forever chemicals," are a large group of synthetic chemicals that have been widely used in industrial and consumer products. PFAS are persistent in the environment and do not break down easily. Perfluorooctanesulfonic acid (PFOS) is among the PFAS with frequent detections in the environment, biota, and human tissues. In this work, we studied the electrochemical oxidation (EO) of PFOS using modified titanium suboxide anodes. Magnéli phase titanium suboxides (TSO), such as Ti_4O_7, are favorable anode materials to degrade PFAS via EO. This work reveals that modified TSO anodes by incorporating Niobium (Nb) and Cerium (Ce) through sintering enhance PFAS degradation during EO. The results show that the Niobium-modified TSO anode (Nb-TSO) obtains higher PFOS degradation rates by EO compared with the pristine Ti_4O_7 or the Cerium-doped TSO anode (Ce-TSO). As a result, Nb-TSO displays the lowest energy consumption, which is reflected in its energy efficiency (EE/O). At 20 mA/cm^2, the EE/O of PFOS degradation by EO with Nb-TSO is approximately 1.8 times lower than that of the pristine Ti_4O_7 anode. The improved degradation efficiency of Nb-TSO anodes can be attributed to their increased specific surface area, while the elevated intrinsic reactivity of dopant Ce likely enhances the potential of Ce-TSO for PFAS degradation. This work provides insights into how TSO material structures may impact their electrocatalytic activities towards PFAS, and facile methods to prepare modified TSO anodes with high PFOS degradation efficiency.

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Poster Presentations

April 10, 2024

Exploring the metabolome and microbiome of steers grazing tall fescue: impact of low-level ergot alkaloids and endophyte presence in the grass

Author: Ignacio Mariano Llada

I.M. Llada[1], J.M. Lourenco[1], M.M. Dycus[1], G. Suen[2], D.P. Jones[3], Z.R. Jarrell[3], N.S. Hill[1], and N.M. Filipov[1].

[1]University of Georgia, Athens, GA; [2]University of Wisconsin, Madison, WI; [3]Emory University, Atlanta, GA.

Fescue toxicosis (FT) is a mycotoxin-related disease caused by ingestion of tall fescue infected with the ergot alkaloid (EA)-producing endophyte *Epichloë coenophiala*. Decreased weight gain is a hallmark of FT and a major concern for the beef industry. A non-toxic endophyte was introduced to fescue plants to mitigate the EA toxicity, but its impact on grazing animals is underexplored. This study aimed to investigate how the presence of toxic, low EA-producing, and non-toxic endophytes could influence the interaction between the animal metabolome and microbiome, and, ultimately, their performance. Eighteen steers were placed on nontoxic (NT), toxic (E+), and endophyte-free (E-) fescue pastures for 28 days. Fescue plants were collected for endophyte and total EA detection. Body weights were recorded pre, 14 and 28 days after pasture placement. Urine, rumen fluid (RF), solid (RS), and feces were collected at five-time points. An untargeted high-resolution metabolomics (HRM) approach was applied to urine and RF samples for metabolomic analysis, while the 16S rRNA gene was amplified (V3-V4 region) in RF, RS, and fecal samples for microbiome analysis. The microbiome and metabolome integration was evaluated with xMWAS. Weight gain decreased by 60% in steers grazing E+ compared to the other two groups. Endophyte presence in E+ was 78%, with an average total EA concentration of 12.6 ppb. There was a clear group separation of the urine and rumen metabolic features after sPLS-DA analysis obtained from the HILIC column, while C18 column metabolites between the E+ and NT groups overlapped. Features with discriminatory power due to E+ toxin(s) were associated with tyrosine-derived amino acids or lipid metabolism. Following pathway analysis, amino acid and carbohydrate metabolism-related pathways were affected by both endophytes present in the grazed fescue. However, lipid metabolism, phenylalanine, tyrosine, and tryptophan biosynthesis were impacted only in the E+ group. No differences were observed in alpha or beta diversity of the microbiota. Variations in bacterial abundance were only noted among the families *Prevotellaceae* (increased), *Rikenellaceae* (reduced), and *Clostridiaceae* (reduced) across the 3 matrices analyzed: RF, RS, and feces. Interactive analysis revealed 137 urine metabolites and 55 bacterial families unique in the E+ network. These metabolites were associated with several metabolic pathways, i.e., tryptophan and arachidonic acid. Overall, our study suggests that fall grazing of E+ fescue with low levels of EA affects the animal's metabolome more than it does the microbiome. *Epichloë* endophytes, whether novel or toxic, impact animal amino acid and carbohydrate metabolism. The E+ endophyte strain, potentially linked to EA production, affects lipid metabolism and this effect might be associated with the decreased weight gain in E+

steers. The E+ group exhibits a distinct metabolic-microbial network, connected to key pathways like tryptophan and arachidonic acid. Thus, even the ingestion of low levels of EA can adversely affect animal metabolism and impact weight gain. The presence of the novel endophyte, while not having an adverse impact on weight gain, influences the animal's metabolome. Supported by USDA (NIFA 67015-31301).

Decoding the Effects of Per- and Polyfluoroalkyl Substances (PFAS) Mixtures on Neurodevelopment: Transcriptomic Insights from *Caenorhabditis elegans*

Seth D Currie[1,2], Jia-Sheng Wang[1,2], Lili Tang[1,2]

Interdisciplinary Toxicology Program[1], Department of Environmental Health Science[2], University of Georgia, Athens, GA 30602

Per- and Polyfluoroalkyl Substances (PFAS) are a diverse class of industrial chemicals that have been used for decades in industrial and commercial applications. Due to widespread use and resultant environmental bioaccumulation, PFAS are consistently detectable in the bloodstream of humans. PFAS have been linked to several adverse health outcomes including hepatotoxicity, immunotoxicity, endocrine disruption, tumorigenicity, and neurotoxicity, *specifically for* developmental neurotoxicity (DNT). An increased prevalence of neurodevelopmental disorders in children has been observed and linked to pre- and postnatal exposure to PFAS; however, the mechanisms of adverse neurodevelopmental effects of PFAS are largely unknown. In order to determine PFAS mechanism of action, in-depth toxicological studies are required. The nematode *Caenorhabditis elegans* (*C. elegans*) serve as an ideal model organism for studying neurodevelopmental toxic effects due to the organism only having 302 neurons, a complete written diagram for its chemical and electrical connections available, and a short lifespan. In this study, behavioral and transcriptomic analyses were performed to assess the neurodevelopmental toxic effects of PFAS in developing C. elegans, as well as the potential toxicity mechanisms. The five PFAS compounds with a high occurrence frequency were selected to represent typical daily exposure in the United States, including perfluoroalkyl carboxylic acids (PFHxA, PFOA) and sulfonic acids (PFBS, PFHxS, PFOS). Wild-type worms at the first larval stage of development (L1) were exposed to either a single PFAS compound or a mixture of the five PFAS compounds at 0ppm, 5ppm, 10ppm for 48 hours. The neural behavior of the larvae was recorded and analyzed using a WormLab system (MBF Bioscience, Vermont, USA). The enrichment of functions and signaling pathways among differentially expressed genes (DEGs) was analyzed using the GO and DAVID databases. All PFAS compounds resulted in a significant reduction in locomotion ($p < 0.01$), and the PFAS mixtures exhibited an additive effect. Transcriptomic analysis revealed that more than 600 genes were differentially expressed (DEGs), and KEGG and GO enrichment pathway analyses indicated that the DEGs were involved in development, immunity, and enzyme activity. Our study provides novel evidence of the neurodevelopmental toxicity and the mechanisms of PFAS toxicity, which will be useful in assessing the adverse health effects of these emerging environmental pollutants.

A High-Throughput, High-Content Analysis of Dopaminergic Neuron Degeneration in *Caenorhabditis elegans* Exposed to Per- and Polyfluoroalkyl Substances (PFAS)

David Blake Benson[1,2], Seth D Currie[1,2], Lili Tang[1,2]
Interdisciplinary Toxicology Program[1], Department of Environmental Health Science[2], University of Georgia, Athens, GA 30602

Dopamine (DA) neuron degeneration plays a crucial role in Parkinson's Disease (PD) pathology, a common neurodegenerative disorder affecting over 10 million people worldwide. While the exact causes of PD remain unclear, the environmental pollutants are recognized as potential contributors. To develop effective prevention, management, and treatment approaches, a deep understanding of the morphological changes in dopaminergic neurons upon exposure is essential. This understanding is not only vital for PD research but also for studying other neurodegenerative disorders that are on the rise. However, the current low-throughput and labor-intensive methods have made it challenging in identifying DA neuron degeneration phenotypes. Therefore, the development of a high-throughput and high-content screening platform is urgent. The nematode *Caenorhabditis elegans* (*C. elegans*) is a promising alternative model to study neurotoxicity due to its simple nervous system of 302 neurons , and fully mapped chemical and electrical synapses. *C. elegans* assays are usually rapid, low cost, and amenable to high-throughput analysis. Moreover, the transparency of C. elegans allows for the visualization of dopamine neurons in vivo by using the green fluorescent protein (GFP) fused to the DAT gene promoter (Pdat-1::GFP), which enables the study of dopamine neuron degeneration directly within the organism. In this study, we developed a high-throughput, high-content analysis platform utilizing COPAS BIOSORT, vivoChips, and the transgenic C. elegans PD model (BZ555) to investigate the neurotoxic effects of per- and polyfluoroalkyl substances (PFAS) on DA neurons. The four PFAS, PFOS, PFOA, PFHxS and PFHxA, were selected based on their prevalence and concentrations in aqueous film-forming foam (AFFF)-impacted surface water. The L4 stage worms were exposed to six varying concentration of PFAS at 0, 100, 200, 300, 400, and 500μM, and the DA degeneration was quantified on Day 5, 7, and 10 following the exposure using our established scoring system. The scoring system incorporate 9 tiers, 0 being “completely healthy” and 8 being “dead”, with the tiers in the middle scoring the degeneration of dendrites, somas, or both. We found PFOS to have significant effects on neurodegeneration in *C. elegans* at almost every concentration. Our study not only elucidates the adverse effects of PFAS on dopaminergic neurons but also establishes a robust high-throughput high content neurotoxicity screening platform. The insights gained from this study contribute to the broader understanding of environmental pollutants - induced neurodegeneration, paving the way for future investigations into protective strategies and therapeutic interventions.

IMPACT OF SARS-COV-2 INFECTION ON THE BLOOD-TESTIS BARRIER IN HUMANS AND NONHUMAN PRIMATES

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SARS-CoV-2 has had an impact on over 320 million individuals. As strains like Omicron undergo changes to increase infectivity while sacrificing pathogenicity, the number of affected individuals is expected to rise significantly. Although the effects of SARS-CoV-2 on male fertility are not fully understood, clinical and experimental evidence suggests that the infection may have negative consequences for male reproductive health. There is also evidence indicating that the blood-testis barrier may be susceptible to infection. To explore this further, we established a blood-testis-like barrier using primary human and nonhuman cells and tested various SARS-CoV-2 variants, including WA1/2020, BA.1.1.529, BA.4.6, and XBB. We assessed barrier infection through transepithelial electrical resistance and Dye Flux assays. Regardless of the variant or species, SARS-CoV-2 infection led to disruption of the barrier. Interestingly, variants that were better adapted to human viral entry proteins caused more significant disruption in humans compared to nonhuman primates. Additionally, variants that were more adept at evading immune responses caused less disruption in both humans and nonhuman primates. These findings provide evidence that SARS-Cov-2 infection disrupts the blood-testis barrier, potentially impacting male fertility, with the extent of impact dependent on the adaptations exhibited by emerging variants.

Histones in Extracellular Vesicles Augment Inflammasome Activation and α-Synuclein Aggregation

Alejandra Bargues-Carot[1], Manikandan Samidurai[2], Adhithiya Charli[3], Chelva Janarthanam[1], Huajun Jin[1], Vellareddy Anantharam[1], Xuemei Huang[4], Arthi Kanthasamy[1], and Anumantha G. Kanthasamy[1]

[1]Department of Physiology and Pharmacology, Center for Neurological Disease Research, University of Georgia, Athens, GA; [2]SENS Research Foundation, Santa Clara, CA; [3]Biogen, Cambridge, MA; [4]Department of Neurology, Pennsylvania State University-Milton S. Hershey Medical Center, Hershey, PA.

Emerging evidence indicates that histones can be released into the extracellular space by damaged cells, and these circulating histones act as damage-associated molecular patterns (DAMPs) that augment inflammatory responses. The NLRP3 inflammasome, a multiprotein complex that activates caspase-1 and promotes maturation and secretion of the proinflammatory cytokines IL-1β and IL-18, has been implicated in the neuroinflammatory processes associated with environmentally linked chronic neurodegenerative diseases such as Parkinson's disease (PD). Nevertheless, the key signaling molecules and pathways involved in the circulating histone-mediated neuroinflammatory response to neurotoxicity are still largely unknown. In this study, we found that exposing dopaminergic neuronal cells to neurotoxicants increases the amount of histone 3 (H3) in released small extracellular vesicles. The extracellular vesicles isolated from the serum of PD patients, farmers exposed to pesticides, and a transgenic mouse model of PD also contain elevated H3 levels. Interestingly, the serum extracellular vesicles from PD patients demonstrate increased H3 modifications (H3K14ac, H3K18ac, and H3ser10P). We then investigated whether H3 plays a role in mediating neuroinflammation by activating the NLRP3 inflammasome via the Fyn kinase pathway. Exposing primary mouse microglia to recombinant (r)H3 promoted the generation of reactive oxygen and nitrogen species (ROS and RNS), the release of proinflammatory cytokines, and the activation of the NLRP3 inflammasome. Using the Fyn inhibitor saracatinib, or knocking down Fyn, dampened H3-mediated NLRP3 inflammasome activation, suggesting the involvement of Fyn kinase in this process. Interestingly, we found rH3 interacted with monomeric α-synuclein, augmenting its aggregation, thereby suggesting that H3 can serve as one of the co-factors regulating the pathogenic α-synuclein protein misfolding process. Also, rH3 treatment exacerbated α-synuclein-mediated NLRP3 activation, cytokine release, and RNS generation in mouse primary microglia compared to α-synuclein treatment alone. Collectively, these results demonstrate that H3 in extracellular vesicles may serve as a key pro-inflammatory amplifier of α-synuclein protein aggregation and neuroinflammation through the activation of NLRP3 inflammasome signaling via the Fyn kinase pathway. Funding support: NIH/NIEHS ES027245 and ES026892.

Evaluating Gut to Brain Pathology with an α-synuclein Engineered Microbe – A Novel Animal Model for Parkinson's Disease?

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Parkinson's Disease (PD) has been historically characterized by the loss of dopaminergic neuronal cells in the substantia nigra and the abnormal accumulation and aggregation of α-synuclein in the form of Lewy bodies and Lewy neurites. Current research endeavors to develop an enhanced animal model that more fully emulates disease pathology. Current hypotheses suggests that the onset of pathology begins either in the olfactory bulb/amygdala (brain centric) or in the enteric nervous system (body centric). These distinct subtypes are expected to be identifiable early in the progression of the disease through various imaging, clinical, and neuropathological indicators. While there are models that confirm gut-to-brain propagation of α-synuclein, to date there are no current models that fully link enteric nervous system disorders to Parkinson's disease. Hence, we aimed to investigate a model consistent with this recent evidence suggesting a bimodal relationship between the gut and the brain. To do this we utilized a bioengineered pathogenic α-synuclein producing *E.coli* strain in C57BL/6 mice and observed neurobehavioral and pathological changes. In our initial experiments we constructed various versions of human α-synuclein producing *E.coli* strains which were inducible with a simple sugar, rhamnose. The strains were then screened to test α-synuclein production. The confirmed the lead strain, EC^{rha} SYN 1.2 (SYN 1.2), produced α-synuclein by ELISA immunoassay. We next confirmed the aggregation kinetics via a RT-QUIC seeding assay. An increase in ThT fluorescence was observed within 3 h confirming α-synuclein aggregation. Subsequently, we began a pilot study where C57BL/6 mice were administered our SYN 1.2 ($1x10^9$ CFU, p.o, daily, n=6) or vehicle (n=4) for 35 days. Blood collections were taken every other week and behavior studies were performed from day 28-35. On day 35, animals were euthanized and processed for plasma ELISA assays, gut phosphorylated serine-129 α-synuclein (pS129 α-synuclein) immunohistochemistry (IHC) and mucin staining. Behavioral assessments included a pole test, rotarod, a tail suspension test, and a forced swim test. Preliminary results showed a trend indicating motor deficits after the SYN 1.2 agent when compared to vehicle. ELISA confirmed elevated levels of plasma α-synuclein, confirming microbial-mediated production and delivery, *in vivo*. Increased positive pS129 α-synuclein chromogenic immunostaining in colon tissue confirm pathology. These results align with reduced mucopolysaccharide staining suggesting a compromised intestinal mucosal barrier in SYN 1.2 mice. Overall, our results suggest that microbial agent SYN 1.2 can produce pathogenic α-synuclein, which triggers modest behavioral and gut pathology aberrations. Additional studies with larger cohorts are warranted to corroborate these findings.

Early Glycation Products Protect Against Type 2 Diabetes and autistic Through Modulating Gut Microbiome and Associated Metabolic Pathways

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The gut microbiota plays a pivotal role in human wellness, influencing various organ systems and contributing to the pathogenesis of conditions such as type 2 diabetes mellitus (T2DM) and autism spectrum disorder. Glycation products, commonly found in food, have received limited attention regarding their health effects. Early glycation products (EGPs) have been proposed to improve food protein properties, but their health implications remain largely unknown. Our previous studies on C57BL6 mice have shown that EGPs beneficially modulate T2DM and immune homeostasis by modulating cytokine secretion and gut microbiota. We hypothesized that EGPs could also modulate autism spectrum disorder through dysbiotic gut microbiota in the BTBR-mtB6 mouse model. Building upon this, this study aims to examine the effects of EGPs on the gut microbiota of male and female C57BL6 and BTBR-mtB6 mice. To achieve this, male and female mice are randomized into control, non-reactive (NR), and EGP treatment groups and administered EGPs derived from whey protein isolate-glucose system for six months. Fecal samples collected monthly for microbiota analysis, Sequencing analysis of 16S rRNA genes was conducted using the fecal samples from mice. Mice fed with a Western diet ad libitum and exposed to EGPs and non-reactive (NR) for 6 months at the physiologically relevant dose of 600 mg per kg body weight by gavage. The Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT) were conducted every month in addition to weekly glucose measurement. Flow cytometry was conducted using spleen cells to evaluate the effects of EGPs on the immune system. Our research strategy involves employing bioinformatics tools to explore the effects of EGPs on gut microbiota and associated metabolic pathways. We will also assess the impact of EGPs on immune system modulation and behavioral changes associated with depression, anxiety, and memory. Results indicated a significant alteration in gut microbiota composition, immune cell populations, and behavioral changes following EGPs consumption. This study aims to provide insights into the potential therapeutic role of EGPs in modulating T2DM and autism spectrum disorder, shedding light on the interplay between dietary factors, gut microbiota, and overall health. This study was supported by NIH R41DK121553, and partly supported by the Interdisciplinary Toxicology Program at UGA and the USDA National Institute of Food and Agriculture [grant no. 2016-67021-24994/project accession no. 1009090].

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Mechanism of estrogenic endocrine disruptors in regulating uterine fluid movement

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Estrogenic endocrine disrupting chemicals (**EDCs**) are EDCs that interfere with estrogen signaling. They are ubiquitous in the environment, such as mycoestrogens from fungi (e.g., zearalenone (ZEA)), phytoestrogens from plants (e.g., genistein), industrial phenolics (e.g., bisphenol A (BPA)), and organochlorine pesticides (e.g., DDT), etc. Estrogen is an ovarian hormone that is essential for uterine functions. We have demonstrated that high doses of ZEA and BPA cause uterine fluid accumulation and impaired early pregnancy in mice. Dynamic uterine fluid is critical for supporting early pregnancy events, for example, high uterine fluid volume assists sperm passage to reach oviduct/Fallopian tube for fertilization, while low uterine fluid volume facilitates embryos to attach to the uterine luminal epithelium (LE) for embryo implantation. In patients undergoing *in vitro* fertilization-embryo transfer (IVF-ET), excess uterine fluid at the time of embryo transfer leads to embryo implantation failure. *The mechanism of how estrogenic EDCs cause uterine fluid accumulation remains uninvestigated.* The uterine functions of estrogen are mainly mediated through estrogen receptor α (ERα / *Esr1*). Since estrogenic EDCs can bind to ERα and uterine fluid moves through the uterine epithelium, we hypothesize that estrogenic EDCs acts through uterine epithelial ERα to dysregulate uterine fluid movement. EEDC toxicity is believed to be ERα-mediated. *EpiERα*$^{-/-}$ (*Esr1*$^{fl/-}$*Wnt7a*$^{Cre/+}$) mice deficient of ERα in the uterine epithelium are infertile. Day 0.5 post-coitum (D0.5) *Esr1*$^{fl/-}$ control mice have distended uteri filled with easily drainable uterine fluid, while D0.5 *epiERα*$^{-/-}$ mice lack drainable uterine fluid. The D0.5 *epiERα*$^{-/-}$ uterine luminal epithelium (LE) layer is significantly shorter than the control. We recently developed a novel method using Alexa hydrazide (AH) to visualize uterine fluid absorption via bulk absorption (seen as smeared cellular staining). We also demonstrated LE as the main site for bulk uterine fluid absorption during early pregnancy. mRNA-seq of isolated D0.5 LE reveals upregulation of genes involving cellular sodium ion (Na^+) homeostasis, including three subunits (*Scnn1a*, *Scnn1b*, *Scnn1g,* 3-46 folds) for epithelial Na^+ channel (ENaC), in the D0.5 *epiERα*$^{-/-}$ LE. ENaC transports Na^+, the dominant electrolyte in the uterine fluid, to generate an osmotic gradient for bulk water absorption through the uterine epithelium. These preliminary data imply a novel role of E2 and uterine epithelial ERα in uterine fluid absorption. We hypothesize that E2-ERα signaling dynamically regulates uterine fluid absorption via genes involved in LE Na^+ homeostasis and endocytosis. This hypothesis will be first be tested by determining how uterine epithelial ERα-deficiency temporally alters uterine fluid absorption in preimplantation mice. Additionally, the molecular mechanisms of EEDC disruption of preimplantation uterine fluid movement will be investigated through *Esr1*$^{fl/-}$ and *epiERα*$^{-/-}$ mice. The completion of these studies will provide a novel function and related mechanisms of E2/EEDC-ERα signaling in regulating uterine fluid movement. It will also provide insights for future development of approaches to manage uterine fluid to facilitate successful early pregnancy.

Mechanisms of hormonal contraceptive Mifepristone (RU486) in regulating uterine fluid movement

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Uterine fluid absorption during early pregnancy facilitates uterine lumen closure, which enables intimate contact of the implanting embryo with the uterine luminal epithelium (LE) to initiate embryo implantation. Studies from ovariectomized rodent models reveal that ovarian hormones estrogen (E2) and progesterone (P4) induce uterine fluid secretion and absorption, respectively, primarily through the uterine epithelium. P4 is primarily synthesized from the developing corpus luteum during early pregnancy and its increasing levels inversely correlate with the reducing uterine fluid volume. Many women rely on the use of contraceptives to prevent pregnancy. Some hormonally based contraceptives such as Mifepristone (RU486) can disrupt uterine fluid movement as a potential side effect of use. Dysregulated uterine fluid volume has been associated with impaired early pregnancy both in animal models and women undergoing in vitro fertilization-embryo transfer. Despite the known function of P4 in uterine fluid absorption, the cellular and molecular mechanisms by which the use of contraceptives can disrupt P4 induced uterine fluid absorption are not known. Bulk uterine fluid absorption is expected to be mediated by a number of ion channels, such as epithelial Na^+ channel (ENaC), and aquaporins. In uterine fluid, the dominant electrolyte is Na^+. The transport of Na^+ by ENaC and potentially other sodium channels from the uterine fluid to the uterine epithelium generates an osmotic gradient, which facilitates bulk fluid absorption through the uterine epithelium. Na^+/K^+ ATPases then facilitate the movement of sodium ions across the basal membrane of LE cells into the underlying stromal layer. Our lab developed a novel method using Alexa Hydrazide (AH) to visualize uterine fluid absorption and demonstrated reduction of bulk uterine fluid absorption via LE from day post-coitum 0.5 (D0.5) @ 11 h to D3.5 @ 11 h. Our recent novel finding shows that bulk absorption on D3.5 increases from 11 h to 17 h. I have generated a mouse model with conditional deletion of PR in the uterine luminal epithelium (*epiPR*$^{-/-}$) with the genotype *Pgr*$^{f/-}$*Wnt7a*$^{Cre/+}$ to investigate how P4-PR signaling in the uterine epithelium mediates uterine fluid trafficking in early pregnancy and to explore potential methods by which contraceptives mediate fluid retention in the uterus. Preliminary data of *epiPR*$^{-/-}$ mice on D3.5 @ 17 h shows increased fluid retention, decreased bulk absorption, and altered expression of Na^+/K^+ ATPase subunit ATP1A1. Additionally, a single intrauterine injection of 10^{-4}M Terbutaline (TB), a potent agonist of ATP1A1, demonstrates induction of uterine fluid absorption on D3.5 @ 11h when bulk absorption is generally low, indicating a potential therapeutic method for alleviating uterine fluid retention. Together, these results provide novel insights into the intricate P4-PR signaling mechanisms in regulating uterine fluid trafficking during early pregnancy.

Evaluating Microcystin in Water and Fish Tissue from Four Reservoirs in the Georgia Piedmont USA

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Harmful algal blooms (HABs) are among the greatest inland water quality threats to aquatic wildlife, fish, and humans. Certain cyanobacteria in HABs produce cyanotoxins, such as microcystins, which are highly toxic to terrestrial and aquatic organisms. The primary routes of microcystin exposure are through dermal contact with or consumption of contaminated water, and consumption of accumulated toxins in aquatic animal tissue. We evaluated microcystin (MC) concentration in largemouth bass and water samples using an enzyme-linked immunosorbent assay (ELISA). Samples were collected across a six-month timespan from four reservoirs around Athens, GA (Bear Creek, Chapman, Herrick, Oglethorpe). MC concentrations in largemouth bass did not show a consistent trend across months when compared between lakes. The MC concentrations in the fillets ranged from to 0.0000106 to 0.206 µg/g whereas, the MC concentrations in the water ranged from 0.014 to 0.426 µg/L and generally increased throughout the growing season. Water and fillet concentrations were generally below Environmental Protection Agency (EPA) and the World Health Organization (WHO) benchmarks for recreation and fish consumption. However, the ELISA concentration values are preliminary as high-performance liquid chromatography-mass spectrometry (HPLC-MS) is still needed for verification.

Key Words: Harmful algal blooms; microcystin; freshwater fish; enzyme-linked immunosorbent assay

Use of delayed treatment approach with the immunoprophylactic Lacto-N-Fucopentaose III (LNFPIII) and/or insulin-supplemented diet for improvement of hippocampal synaptic plasticity in a Gulf-War Illness (GWI) model: preliminary findings

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Gulf War Illness (GWI) is a chronic multisymptomatic condition that affects approximately one-third of the 700,000 U.S. military personnel deployed in the 1990–1991 Gulf War (GW). Neurological, immunological, and gastrointestinal deficits feature in GWI prominently. We have shown that the immunomodulatory glycan Lacto-N-Fucopentaose III (LNFPIII) enhances gut health, improves neurogenesis, and has positive neurobehavioral and neurophysiological effects in preclinical GWI models. Recent studies have explored potential multi-modal GWI treatments where two or more potential therapeutics have been tested. Inulin, a safe soluble fiber supplement to the diet has been demonstrated to be beneficial on both gut and brain health in aged mice, as well as for human microbiota. Because inulin has not been tested in a GWI context and LNFPIII improved some, but not all, behavioral deficits caused by GWI-related exposures, herein, we examined whether the beneficial effects of LNFPIII on hippocampal synaptic plasticity, a neurophysiological correlate for learning and memory, are augmented by an adjunct dietary treatment with inulin and if inulin would be beneficial for synaptic plasticity on its own.

To emulate GWI, we used a well-established preclinical model. Male C57BL/6J mice were exposed for 14 days to pyridostigmine bromide (PB) and N,N-Diethyl-3-methylbenzamide (DEET) plus corticosterone via drinking water on days 8-14 and a single diisopropylfluorophosphate (DFP, a sarin surrogate) injection on day 15. Beginning at month 9, mice from the GWI or control groups were randomly assigned to receive LNFPIII treatment, inulin-supplemented diet or both (and their respective controls). At month 12, mice were sacrificed, and *ex-vivo* hippocampal slices electrophysiology was performed. Briefly, Schaffer collaterals were stimulated at the CA3 side of the CA1 region of hippocampal slices. Field excitatory postsynaptic potentials (fEPSPs) at the *stratum radiatum* were recorded to assess synaptic transmission and synaptic plasticity. Stable fEPSPs were recorded for at least 30 min at baseline before stimulating the slices with a high frequency tetanic stimulation (HFS) consisting of 3 trains of 100 pulses at 100Hz, with 20s intertrain intervals to induce long term potentiation (LTP). Data was digitized at 10kHz, low-pass filtered at 1kHz, and analyzed with pCLAMP software.

In addition to the decrement of hippocampal LTP caused by the GWI chemical exposure, preliminary results confirm that delayed LNFPIII treatment alone largely restores the LTP deficit 12 months after GWI chemical exposure and it might be beneficial to control animals as well. Within the GWI mice, treatment with the combination of LNFPIII and inulin diet further improved hippocampal LTP to a modest extent. Inulin diet, by itself, appears to have no effects on LTP magnitude. Upon completion of remaining slice recordings, the effects of the LNFPIII-inulin combination and inulin-supplemented diet by itself will be fully characterized in this GWI model. Our results confirm our prior findings that delayed LNFPIII treatment ameliorates GWI-related impairments in hippocampal synaptic plasticity in a preclinical GWI model. Notably, the inulin-supplemented diet might be a good adjunct therapeutic option to enhance the beneficial actions of LNFPIII.

This research was supported by a DoD grant to NMF *(W81XWH2110661)* and funding to FTR from the Interdisciplinary Toxicology Program (ITP) at the University of Georgia.

Sulindac sulfide enhances the chemotherapy of breast cancer

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Background

Sulindac is a prodrug of the nonsteroidal anti-inflammatory drug (NSAID) that affects prostaglandin production by inhibiting cyclooxygenases, commonly used for the treatment of inflammation, analgesia, and fever. Its active metabolite sulindac sulfide (SS) is responsible for its anti-inflammatory properties. Sulindac has been of interest for decades because of its attractive chemopreventive activity against cancer progression. However, the mechanism of the antitumor activity of sulindac has not been fully elucidated.

Principal Findings

Treatment of human BRCA1-WT breast cancer cell lines with SS, we find that SS significantly inhibits the capability of Nucleotide Excision Repair (NER) independent of p53. These results indicate the benefit of sulindac combination with DNA-damaging chemotherapies.

Neurochemical analysis of monoamine perturbations in a preclinical Gulf War Illness model: effects of sex

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Research Background and Purpose: The 1990-1991 Persian Gulf War was one of the first armed conflicts for the United States after the Cold War. During the war, soldiers were prescribed and took, in many cases excessively, the nerve agent prophylactic pyridostigmine bromide (PB), a short-acting reversible acetylcholinesterase inhibitor. Most were exposed to excessive levels of pesticides, such as permethrin (PM), while some soldiers were inadvertently exposed to the nerve agent's sarin and cyclosarin. Approximately one-third of the 700,000 returning soldiers, approximately 49,000 of them women, presented multi-system abnormalities, including chronic fatigue, gastrointestinal distress, musculoskeletal pain, headaches, mood disorders, and memory or concentration deficits. This culminated into a diagnosis of Gulf War Illness (GWI). These symptoms have been linked to GWI pathology and, potentially, are a result of neurochemical dysregulation. Research into this area of GWI is limited, mostly focused on short-term effects, and male subjects in models of the disease. Hence, to address these data gaps and account for female GWI veterans, we report on sex-specific neurochemical changes, months after exposure to GWI chemicals has ended using an established preclinical model of GWI.

Methods: To study GWI, male (n=19) and female (n=18) 8-week-old C57BL/6J mice received the PB/PM combination (0.7 and 200mg/kg, respectively), or DMSO vehicle-control for 10 days via intraperitoneal injections. Following GWI chemical treatment termination, all mice were maintained under normal housing conditions with food and water *ad libitum* for 9 months, with body weights checked bi-weekly. Nine months post GWI treatment termination, mice were sacrificed, brains were collected, and one-half rapidly frozen and kept at -80 °C until sectioned for neurochemical analysis via HPLC-ECD. Eight analytes, 3-methoxytyramine (3-MT), 3,4 dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA), dopamine (DA), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine (NE), and 3- methoxy-4-hydroxyphenyglycol (MHPG), were quantified across 6 targeted brain regions: prefrontal cortex (PFC), striatum (STR), dorsal hippocampus (DHIP), ventral hippocampus (VHIP), cerebellum (CERE), and brainstem (BS). Micro-punches (1.5-mm diameter) were

collected from 500-µm thick sections, placed in 100 µl of 0.2 N perchloric acid, sonicated, and then centrifuged (13,200 x g at 4 °C for 10 min). Monoamines and their metabolites were measured using HPLC with electrochemical detection, and protein normalization was performed according to previously described protocols. Neurochemical data were analyzed using SigmaPlot 12.5 and graphs were generated using GraphPad Prism 10.1. A two-way ANOVA was used to determine main effects and interactions followed by Student-Newman-Keuls post hoc comparisons to segregate treatment and sex differences. A P-value of < 0.05 was considered significant.

Results: In the STR, the PB/PM treatment was associated with a significant, sex-independent decrease of the DA metabolite 3-MT; the other two DA metabolites, HVA (significantly) and DOPAC (numerically), were decreased only in the PB/PM males. Same male-specific decrease was also observed in striatal serotonin (5-HT). Interestingly, striatal DA was higher and its metabolite DOPAC was lower in the females. In the PFC, female mice had significantly higher concentrations of both 5-HT and its metabolite 5-HIAA. This sex-difference in PFC 5-HIAA levels disappeared in the female PB/PM mice where 5-HIAA was significantly reduced to male levels. Similar to the PFC, female mice had higher concentrations of 5-HT in the BS, but PB/PM caused sex-independent 5-HT increase in this brain region in males. In the VHIP, there were trends for sex-independent increases of DA and its metabolite HVA in PB/PM-treated mice, 5-HIAA was lower only in the PB/PM treated males, while HVA and the NE metabolite MHPG were higher only in the VHIP of the female mice.

Conclusions: These findings show brain region specific neurotransmitter dysregulation many months after treatment with PB/PM has ended. Further, several important sex differences in neurotransmitters and/or their metabolites are apparent. Several of the observed PB/PM effects were either more prominent or specific to males, providing some justification for performing early investigative work with male rodents in this preclinical model of GWI. Nonetheless, it is important to study the effects in both sexes when evaluating potential therapeutic interventions for GWI.

Support: I would like to acknowledge and thank the Department of Defense, grant number W81XWH2110661, for their funding of this research and the assistance and support of the ITP.

Electrochemical degradation of per- and polyfluoroalkyl acids (PFAS) on titanium suboxide anodes

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Our studies indicate effective electrochemical degradation of per- and polyfluoroalkyl substances (PFAS) in aqueous solutions using novel Magnéli phase titanium suboxide (TSO) anodes, providing a potentially promising technology to treat PFAS in wastewater and contaminated groundwater. The results delineated the mechanisms of PFAS degradation on TSO anodes and identified important properties of the TSO materials governing PFAS degradation efficiency. PFAS degradation by TSO-based electrooxidation (EO) was evaluated using different reactor setups, including batch and reactive electrochemical membrane (REM), under different operation conditions, and their performance was compared. Tests are being conducted on PFAS-containing groundwater samples to evaluate the stability of long-term performance. Attempts are being made to further improve the reactivity of TSO anodes towards PFAS degradation by doping with selected elements, guided by density functional theory (DFT) computations. The overall results of our studies provide a basis for design and optimization of the TSO-based EO systems for applications to remediate PFAS contamination.

Notes: