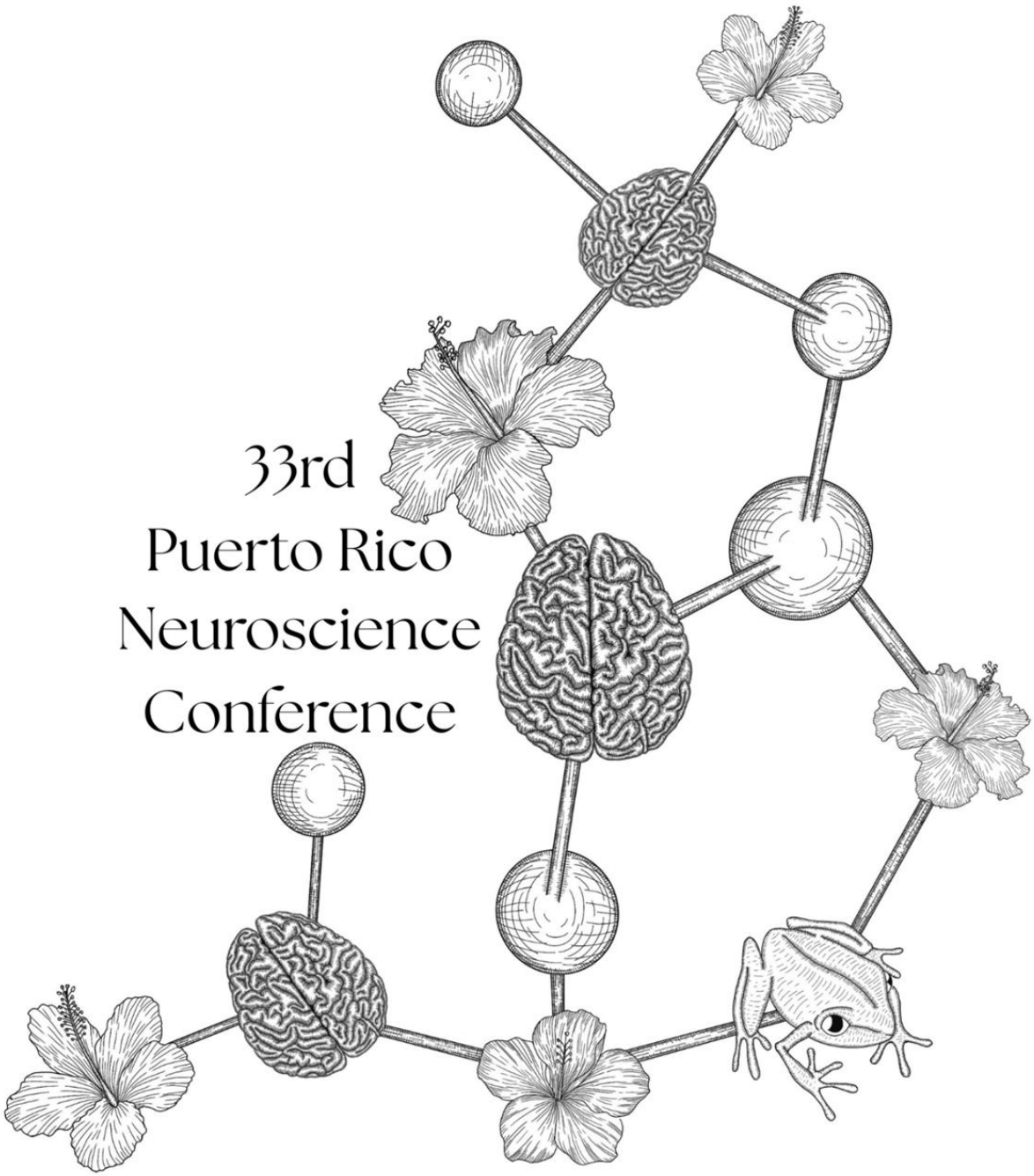

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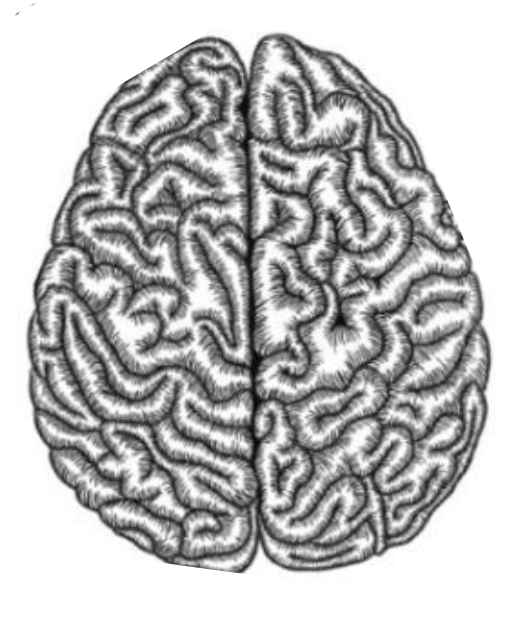

33nd Puerto Rico Neuroscience Conference

December 6, 2025

Jaime Benítez Rexach Amphitheater

San Juan, PR

2025 Host Institution

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Distinguished Speakers

Claudia Lugo-Candelas, PhD

Dra. Claudia Lugo-Candelas is a licensed clinical psychologist and Associate .Ph.D ,Claudia Lugo-Candelas .Dr where she holds both the Bender-Fishbein Scholar in ,Professor of Medical Psychology at Columbia University Child and Adolescent Psychiatry and the Florence Irving Associate Professor of Medical Psychology (in Her research centers on the perinatal programming of risk and resilience for psychiatric .Psychiatry) titles with a particular focus on the role of perinatal sleep in the development of inhibitory control difficulties ,disorders that elevate risk for childhood psychopathology

Dr. Lugo-Candelas is dedicated to advancing scientific understanding of the environmental exposures and lived experiences most relevant to minoritized, underserved, and underrepresented communities. She firmly upholds that diversity and equity are foundational to rigorous science and public health advancement. She earned her B.A. from the University of Puerto Rico, Río Piedras, and her Ph.D. in Clinical Psychology from the University of Massachusetts Amherst. She completed her postdoctoral fellowship in the Division of Child and Adolescent Psychiatry at Columbia University.

Distinguished Speakers

Robert Friedlander, MD, MA

Robert Friedlander, MD, MA, is the Walter E. Dandy Distinguished Professor and Chair of Neurological Surgery at the University of Pittsburgh and Co-Director of the UPMC Neurological Institute. A neurosurgeon and neuroscientist of international recognition, Dr. Friedlander was previously a faculty member at Harvard Medical School and Brigham and Women's Hospital.

He is a member of the National Academy of Medicine, the American Society for Clinical Investigation, and the Association of American Physicians. His research on apoptosis and neurodegeneration has been published in Nature, Science, Nature Medicine, PNAS, and other leading journals, and has received continuous NIH funding for over two decades.

Clinically, he specializes in complex cerebrovascular surgery, brain tumors, and Chiari malformations. He has held leadership roles across national neurosurgical organizations, including the Society of Neurological Surgeons and the Congress of Neurological Surgeons.

Born in Caracas, Venezuela, Dr. Friedlander completed his MD at Harvard Medical School and neurosurgical training at Massachusetts General Hospital.

For over two decades, he has had continuous NIH support as a principal investigator, as well numerous foundation awards, and his areas of interest include: Aneurysms, vascular malformations, brain tumors, carotid disease, cerebrovascular disease, Chiari malformation, spinal cord tumors. Research focuses on mechanisms of apoptosis, Huntington's disease, ALS, and stroke.

Distinguished Speakers

Victor Ambros, PhD

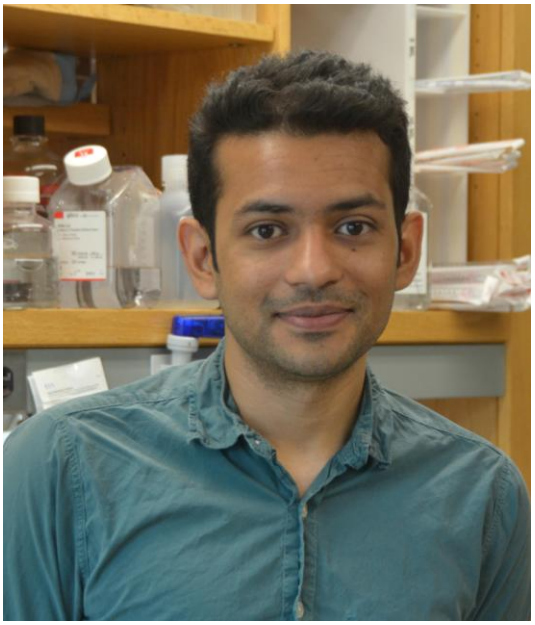
Dr.Victor R. Ambros, Ph.D. is a developmental biologist renowned for discovering the first microRNA (miRNA), a breakthrough that revolutionized understanding of gene regulation. Born in New Hampshire and raised in Vermont, he completed both his B.S. (1975) and Ph.D. (1979) in Biology at the Massachusetts Institute of Technology, training under Nobel Laureate David Baltimore. He later conducted postdoctoral research with H. Robert Horvitz, another future Nobel Laureate, at MIT.

Dr. Ambros joined the faculty at Harvard University in 1984, later moving to Dartmouth College in 1992. Since 2008, he has been a professor at the University of Massachusetts Chan Medical School, where he holds the Silverman Professorship of Natural Sciences, recognizing both his scientific excellence and mentorship. His landmark 1993 discovery of microRNA in *C. elegans*, in collaboration with Rosalind Lee and Rhonda Feinbaum, revealed a new class of small non-coding RNAs that regulate gene expression at the post-transcriptional level.

For his pioneering work, Dr. Ambros has been elected to the National Academy of Sciences (2007) and the American Academy of Arts & Sciences (2011). **In 2024, Dr. Ambros was awarded the Nobel Prize in Physiology or Medicine for the co-discovery with Gary Ruvkun of microRNA—small single-stranded RNA molecules, now recognized as key regulators of gene expression at the post-transcriptional level.**

Distinguished Speakers

Dhananjay Bambah-Mukku, PhD



Dr. Dhananjay “DJ” Bambah-Mukku is an Assistant Professor of Psychology at the University of California, San Diego. He earned his Ph.D. in Neuroscience at Mount Sinai/NYU in 2013 and completed his postdoctoral fellowship in Catherine Dulac’s laboratory at Harvard University in 2021.

His research investigates the neural and molecular mechanisms underlying social behaviors such as mating, parenting, and aggression, with a focus on how biological factors like sex, age, and physiological state shape behavior. His lab integrates single-cell and spatial transcriptomics with genetic, behavioral, and systems neuroscience approaches, and also uses comparative studies in mice and naked mole rats to explore evolutionary flexibility of neural circuits. Dr. Bambah-Mukku has published in leading journals including Science, Cell, and eLife, and his innovative work has been recognized with major awards, including the NIH Director’s New Innovator Award, an NIH K99/R00 Pathway to Independence Award, a Whitehall Foundation Fellowship, and a Kavli Institute for Brain and Mind Research Grant.

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Meeting Itinerary

TIME	ACTIVITY
8:00 AM	**Registration, Poster Set-up, and Breakfast**
8:30 AM	**Opening Remarks** President, Puerto Rico Neuroscience Conference Dr. Deborah Silva, UPR MSC Dean of Medicine Dr. Yasmin Pedrogo-UPR MSC -Chancellor Representative, Dean of Academic Affairs
9:00AM	**First Lecture: Host Dr. Caleb Feliciano** Speaker: Dr. Robert M. Friedlander MD, MA Walter E. Dandy Distinguished Professor, Chair of the University of Pittsburgh Department of Neurological Surgery ***"Synaptic mitochondrial dysfunction in Huntington's disease"***
10:00 AM	**Second Lecture: Host Dr. Carmen Maldonado** Speaker: Dr. Claudia Lugo Candelas, PhD, clinical psychologist and Florence Irving Associate Professor of Medical Psychology at Columbia University. ***"Perinatal sleep disruptions: intergenerational implications for neurodevelopment"***
11:00 AM	**First Poster Session and Coffee Break**
12:30 PM	**Lunch**
1:30 PM	**Second Poster Session and Coffee Break**
3:00 PM	**Third Lecture: Host: Dr. Critsina Velazquez** Speaker: Dr. Victor Ambros, Ph.D. Professor at UMASS Medical School. Internationally renowned geneticist and pioneer of microRNA, who shared the 2024 Nobel Prize in Physiology or Medicine. ***"MicroRNA regulation of gene expression in development and disease"***
4:00 PM	**Fourth Lecture:Host Dr. Emmanuel Cruz** Speaker: Dr. Dhananjay Bambah-Mukku, Ph.D.. D. Assistant Professor at the University of California San Diego. ***"Molecular and cellular dissection of social behavior circuits"***
5:00 PM	**Closing Remarks and Award Ceremony for Best Poster Presenters**

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Category: Behavioral Neuroscience

Optimizing Mifepristone (RU486) Administration to Investigate Alcohol-Induced Neuroadaptations in *Drosophila melanogaster*

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Introduction: Prolonged alcohol consumption induces progressive structural and functional changes in the brain, producing neuroadaptive responses that contribute to the transition from controlled use to excessive drinking. These adaptations underlie alcohol tolerance and physiological dependence, key features of Alcohol Use Disorder (AUD), yet the molecular mechanisms remain unclear. *Drosophila melanogaster* provides a valuable genetic model for studying these mechanisms, as it allows precise spatial and temporal control of genes related to alcohol tolerance and sensitivity. The UAS/GAL4 system enables spatial regulation, while the modified GeneSwitch system allows temporal control through administration of the hormone Mifepristone (RU486). Alcohol-induced neuroadaptations are influenced by epigenetic mechanisms such as histone acetylation, though their relationship has not been fully explored. **Methods:** In this study, we investigated how the duration of RU486 exposure affects alcohol tolerance by knocking down the histone acetyltransferase Tip60 in PDF-expressing ventrolateral neurons (LNv), where the neuropeptide PDF serves as a key signaling pathway. We hypothesized that varying RU486 exposure times would produce temporal differences in Tip60 knockdown, thereby altering alcohol-induced neuroadaptations. To test this, we examined two genotypes: PDF-GS-GAL4>UAS-Luc-RNAi (control) and PDF-GS-GAL4>UASTip60-RNAi (experimental). Age-matched adult female flies were administered RU486 for three intervals—three consecutive days, 48 hours before, or 24 hours prior alcohol exposure—after which they underwent the Alcohol Tolerance Assay. On day one, half of the flies were exposed to water vapor and half to 75% ethanol vapor; on day two, all flies were exposed to ethanol vapor, and behavioral responses and sedation rates were recorded. **Results:** Preliminary results show that longer RU486 exposure (three consecutive days) and earlier administration (48 hours before exposure) effectively induced Tip60 knockdown and prevented flies from developing alcohol tolerance, whereas 24 hours of treatment was insufficient. In contrast, flies that were not administered RU486 developed normal alcohol tolerance. **Conclusion:** These findings demonstrate that Tip60 activity in PDF neurons is critical for alcohol-

induced neuroadaptations and the timing of gene knockdown is essential for modulating alcohol tolerance. These findings lay the groundwork for further exploration of the epigenetic mechanisms underlying AUD.

Acknowledgements: This research was supported by the Louis Stokes Alliance for Minority Participation (PR-LSAMP) under grant number HRD-28186, the Research Initiative for Scientific Enhancement (RISE) at UPR-Rio Piedras under grant number 5R25GM061151-22, and the Institutional Development Award (IDeA) from the National Institute Of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Glyphosate Exposure Alters Neuronal Activity in the Prefrontal Cortex on Female Rats

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Introduction: Glyphosate is a widely used herbicide that has been associated with increased anxiety-like behaviors. Heightened anxiety may exacerbate defensive responses, such as avoidance. Avoidance is a defensive behavior in which an individual takes action to prevent potential harm. We hypothesized that glyphosate exposure enhances avoidance behavior and alters neuronal activity in brain regions implicated in avoidance. Avoidance can be modeled in rodents using platform-mediated avoidance. In this paradigm, rodents learn to avoid a foot shock by stepping onto a safe platform during presentation of a conditioned auditory stimulus (tone). Stepping on the platform protects the rodent from shock but does not eliminate the auditory stimulus. Importantly, when the animal steps onto the avoidance platform, it cannot access a sugar-pellet reward. Thus, platform-mediated avoidance creates a conflict in which rodents must choose between avoidance (no shock) and reward (receiving sugar pellets). The neurobiological basis of avoidance involves activity in cortical and subcortical regions, which can be studied using neuronal markers such as NeuN, a neuron-specific nuclear protein commonly used to identify and quantify neuronal populations. Method: To test our hypothesis, female rats were trained in platform-mediated avoidance. Subsequently, the rats were exposed to glyphosate (2.0 mg/kg/day, n=10); or filtered water (control group, n=10) for 12 weeks. Finally, the rats were euthanized, and their brains were extracted for histological analysis. Immunofluorescence using the NeuN antibody was performed to assess neuronal activity in the prefrontal cortex (PFC), including the prelimbic (PrL) and infralimbic (IL) subregions. Results: Glyphosate exposure significantly decreased avoidance expression compared to controls ($p = 0.0294$). On the other hand, Glyphosate altered neuronal count in the prefrontal cortex of female rats ($p= 0.0378$), but not in the subregions individually. Conclusion: These findings suggest that glyphosate may modulate avoidance behavior through alterations in neuronal count within the prefrontal cortex, particularly in regions implicated in decision-making and regulation of defensive responses.

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Intersection of neuroscience and law: speech decoding brain-computer interfaces and exploring the boundaries of the Fifth Amendment of the U.S. Constitution

María M. Cabrera-Torres, J.D., L.L.M.[1,2] and Gabriel Lázaro-Muñoz, M.B.E., J.D., PhD[1]

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Speech is the ability that humans developed to express thoughts and feelings by articulating sounds. Speech can be affected when people experience conditions like amyotrophic lateral sclerosis or spinal cord injuries that can impact a person's ability to make the movements necessary to produce speech. Different brain regions work together to handle the conversion of ideas into speech. Brain-Computer Interfaces (BCI) can record activity from parts of the brain involved in the movements that produce speech. A relevant example of BCIs is speech decoders, a type of neuroprosthesis, that can restore verbal communication for people who have lost the ability to speak. Speech decoders involve single-unit electrophysiology. Here, small wires are surgically implanted into speech areas of the brain and record electrical signals correspondnig to words that the person is intending to say. The small wires can be thought of as little microphones interviewing physiological activity from individual neurons at a timescale resolution of milliseconds. The electrical signals are then relayed to a computer device. Next, speech decoding devices can interpret the physiological activity from the brain and translate it into words almost instantaneously. For instance, the person intends to say "Hello", then the implanted device registers those brain signals and transfers them to a computer. Once in the computer, an artificial intelligence program decodes the signals and makes the word "Hello" audible in real time, and has even been performed using the patients' voice pre-injury or disease. This technology is promising in clinical settings where speech is impaired. The development of Brain-Computer Interfaces that can decode speech from brain activity raises legal and ethical questions regarding the nature of evidence in criminal law. As these technologies can potentially reveal a person's intended speech, courts must determine whether this information is classified as testimonial evidence, which is protected under the Fifth Amendment's right against self-incrimination. This is important since it is one's legal right to remain silent while being interrogated by law enforcement. Thus, with BCIs, physiological recordings of a person's inner speech could arguably be subpoenaed and used against the person. In sum, the intersection of neuroscience and neurotechnology with legal standards highlights the need for clear guidelines on how such data should be handled in a legal context.

Acknowledgments: Thanks to the generous support of the Neurotech Justice Accelerator at Mass General Brigham (NJAM) sponsored by the Dana Foundation.

Discovery of Anxiolytic Potential of *Stypopodium zonale* in a *Drosophila melanogaster* Model

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Introduction. Anxiety disorders affect nearly one-third of the population, representing the most common class of mental illnesses worldwide (World Health Organization, 2019). Current pharmacological treatments, such as benzodiazepines and antidepressants, are effective but limited by their potential to generate tolerance and dependence (Griffin et al., 2013), making the discovery of natural products with anxiolytic potential a priority. Tropical marine algae, particularly *Stypopodium zonale*, produce bioactive metabolites with neuroactive properties (Dorta et al., 2002). **Methodology.** This study aimed to evaluate the anxiolytic potential of crude *S. zonale* extracts using *Drosophila melanogaster* as a behavioral model, and to complement findings with proteomic profiling of control and experimental groups. Organic extracts from *S.zonale* were obtained using an ultrasonic bath and a mixture (2:1) of dichloromethane: methanol. *D. melanogaster* adults were exposed to crude *S. zonale* extracts under acute (2h) and chronic (oviposition to adulthood) conditions. Behavioral assays, including the Open Field Test (OFT), assessed anxiety-like responses by measuring locomotor activity and distance traveled from the arena center to the periphery. Comparative proteomic analysis of fly heads was conducted using LC-MS/MS to identify proteins differentially expressed in control versus experimental groups. **Results.** Both acute and chronic exposures to *S. zonale* produced significant anxiolytic-like behavioral effects, comparable to diazepam (positive control), with p-values <0.001. Proteomic profiling revealed 53 dysregulated proteins, predominantly downregulated odorant-binding and signaling-related proteins, suggesting a possible molecular basis for the observed behavioral changes. **Conclusion.** These findings demonstrate that *S. zonale* exhibits anxiolytic effects in a translational *Drosophila* model, linking behavior with proteomic mechanisms. This dual approach advances the understanding of marine natural products as potential sources for novel anxiolytic agents.

Acknowledgements: We thank the PR-INBRE program, UPR Cayey Biology Department, the Translational Proteomics Center, and the Puerto Rico Science, Technology, and Research Trust for supporting the project.

Rhythms of Aggression: Circadian Influence on Behavioral Ontogeny in Astyanax mexicanus

***Homar A. Cardona-Sierra** [1,2,3,] *Adrián J. Rivera-Rodríguez [1,2,3], Antonio J. Morales-Cintrón [1,2,] Dhalma I. Bayrón-Ho [2,3], Valeria De La Rosa-Reyes [1,2], Roberto Rodríguez-Morales [2,3].

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Background: External pressures, including temperature, light, and competition, play an essential role in the ontogeny of behaviors and are crucial for the survival, adaptation, neural development, and evolution of an animal. Specifically, our lab is interested in how access to light and darkness can influence the ontogeny of aggression in fish with diverse behavioral phenotypes, resulting from thousands of years of adaptation to cave habitats. **Methods:** To test this, we use the Mexican tetra, Astyanax mexicanus, a species of fish that provides a unique opportunity to study behavioral adaptation within a single species. This fish exists as two morphotypes: social, river-dwelling surface fish and blind, asocial, cave-dwelling cavefish. Our project explores whether exposure to circadian variable light cycles (14/10 light-dark cycles), or complete darkness modulates the ontogeny of aggression in A. mexicanus. Adapting previously published resident/intruder assays performed in adult Astyanax, we quantified and manually annotated emerging aggressive-like behaviors in surface fish and cavefish during juvenile development. **Results:** Our preliminary results, in fish raised in 14/10 light-dark cycles, indicate both morphotypes of A. mexicanus display primitive aggression through their juvenile stage, with strikes peaking at 50 days post fertilization (dpf). Interestingly, even though surface fish display double the number of strikes compared to the cavefish, cavefish' "aggressive state" lasts longer than that of surface fish during earlier development. However, our data also suggests that cavefish reduce aggression as they grow into adulthood, while surface fish exhibit a reduction in their aggression after 50 dpf, but

display aggression as they reach adulthood. **Conclusion:** These findings suggest that light exposure influences the developmental trajectory of aggression, offering insight into how sensory experience shapes neural and behavioral adaptation within a single species. This project paves the way for future behavioral studies involving rearing in total darkness, to determine the full impact of light on the adaptation of aggressive behavior in fish models.

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IACUC Protocols: A741123, A790124, 2024-0004.

The impact of lateral line ablation on sensory system adaptation and behavior in surface fish of the Mexican tetra

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Background: Sensory systems play an essential role in an animal's perception of their immediate environment, allowing them to find food, escape predators, choose and colonize habitats, and engage in social interactions. Previous studies have linked dysregulation in both sensory processing and social behaviors with neurodevelopmental disorders, including autism spectrum disorder (ASD). However, whether social behaviors are affected because of sensory system disturbances remains unanswered. We asked whether this was the case by using aggressive behavior as a proof of principle in the Mexican tetra, Astyanax mexicanus, a species of fish that exists as social populations with eyes (surface fish), and asocial, non-aggressive populations that are blind (cavefish), which compensated for vision loss with an expansion of their hair cell-containing mechanosensory system: the lateral line. **Methods:** To determine the role of lateral line expansion in reducing aggressive behavior in cavefish across evolution, we performed pharmacological ablation of the lateral line in surface fish under both light conditions and total darkness (mimicking the cave condition). After ablation, we transferred surface fish to resident/intruder arenas to quantify striking behavior (a metric of aggression) and the dynamics of time spent freezing during the assay. **Results:** Surface fish are repeatedly aggressive in light and dark conditions. However, we expect aggressive behavior to decrease in surface fish recorded in total darkness, suggesting that darkness is a primary driver of social behavior adaptation in this species. **Conclusion:** Our work will shed light on the contributions of environmental/external variables to the adaptation of sensory systems, which may lead to changes in complex behavior associated with neurodevelopmental disorders.

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The effects of early life stress in motivational conflict

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Introduction: Stress inducing experiences during early development can have lasting effects on subsequent emotional regulation, reward processing, and decision-making later in life. Despite extensive evidence linking early life adversity to hallmarks of neuropsychiatric, its mechanism remains elusive. To address this issue, we are probing how models of early life stress (prenatal and postnatal stress) shape decision-making under conflict, beginning with a model of prenatal stress (Pre-NS) during the Platform-Mediated Avoidance conflict task (PMA) in mice. **Methods:** In the Pre-NS paradigm, pregnant dams are subjected to restraint stress in a conical tube for a daily session of 4 hours three times a week for the last two weeks of pregnancy. In the postnatal stress paradigm (Post-NS), home disorders -cages are provided with only half of nesting and bedding material from post-natal day 2-10. Control groups did not undergo any stress procedures. We then waited until pups reached adulthood (2 months) before starting behavioral assessments. We then trained mice in the PMA task, where mice learn that a light cue signals reward (sucrose) availability, and that an auditory cue (20-sec) co-terminates with a 2-sec foot shock. A safety platform is located in the corner far from the reward port. Mice are trained first in a low conflict contingency, in which there is reward available during safe periods, and then in high conflict, in which there is only reward available during the warning tone. **Results:** We found no differences in approach or avoidance behaviors in the low or high conflict contingencies between groups. However, a variety of sex differences were observed in low- and high-conflict conditions. During low conflict and high conflict contingencies, Pre-NS males earned more rewards compared to male controls. Despite both sexes avoiding a similar number of shocks, Pre-NS males mounted the platform slightly later during the tone allowing them to obtain more rewards, while Pre-NS females showed increased avoidance. Following tone extinction, all groups reduced avoidance behavior comparably, suggesting a conserved extinction learning. Interestingly, after reinstatement (shock reminder), Pre-NS females exhibited dampened avoidance reinstatement. Altogether, these findings suggest that the effects of Pre-NS may be sex-specific and may impair flexibility in updating behavior based on changing threat-reward contingencies. **Conclusion:** Ongoing work in the lab

extends this framework to Post-NS models of limited bed nesting to determine whether the developmental timing of adversity differentially shapes conflict-driven decision-making and vulnerability to maladaptive behaviors.

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The impact of dietary composition on social behavior in *A. mexicanus*

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Introduction: Social dominance patterns are ubiquitous across the animal kingdom and are often dependent on resource acquisition and availability. We asked whether dietary variation could modulate aggression through neurochemical, metabolic, and hormonal mechanisms. Dietary variation can influence aggression and, consequently, survival in the animal kingdom. Method: To explore this, we used the Mexican tetra, *Astyanax mexicanus*, which exists as two morphotypes: river-dwelling, eyed surface fish that are highly aggressive and social, and blind cavefish, which are considered "asocial" and show little to no aggression. We conducted a dietary experiment in which we fed three diets—vegetarian, pellets, and frozen worms—to both surface fish and the Pachón cavefish for 4 weeks. To determine the effects of dietary variation on social behaviors, we conducted a resident/intruder assay in which we measured striking behavior, freezing, and zone-specific freezing in both the middle and corner areas of the tank. Results: Our preliminary data suggest that surface fish were consistently more aggressive than Pachón cavefish across all diets. Notably, the surface fish on the vegetable diet were more aggressive than those on the other diets, suggesting that diet composition may affect aggression. Cavefish were completely non-reactive during the assay and showed no hostility regardless of nutrition. Conclusion: Our preliminary data suggests that diet can influence how adaptation of social behavior, with aggression here used as a proof-of-principle.

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Preliminary findings on resting-state QEEG power profiles in Puerto Rican patients with Post-Acute Sequelae of SARS-CoV-2 (PASC)

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Introduction: Persistent symptoms following COVID-19 infection known as post-acute sequelae of COVID-19 (PASC), or long COVID. Epidemiological studies in the United States estimate that 10 to 35% of patients develop this syndrome after SARS-CoV-2 infection. Neuroimaging and clinical studies have identified disruptions in frontal brain networks. However, the specific electrophysiological signatures underlying these alterations remain unclear. **Methods**: This preliminary study employed quantitative electroencephalography (QEEG) resting-state to assess brain activity in individuals with PASC. The objective was to characterize neurofunctional dynamics by analysing topographic maps of absolute and relative power. Sixteen participants (12 women, 4 men; aged 31–50 years) with confirmed PASC and ongoing cognitive complaints were recruited from a COVID clinic. Participants underwent a 19-channel EEG recording (~10 minutes) while resting with eyes closed. Recordings were processed and z-score normalized using the Applied Neuroscience, Inc. (ANI) normative database. **Results**: Analysis of relative power and topographic mapping revealed a frequency shift toward slow-wave dominance, particularly in delta and theta bands, with alterations in the frontocentral regions. A reduction in posterior alpha activity was detected within occipito-parietal networks, which typically show high alpha power during relaxed wakefulness. Global alpha power was reduced, while the increased frontal alpha may reflect atypical or compensatory executive control processes. These electrophysiological patterns are consistent with reduced cortical arousal, attentional instability, and cognitive fatigue, symptoms commonly reported in PASC. Although beta-band activity was elevated in frontal regions, its inconsistent spatial distribution indicates variable engagement of cognitive mechanisms. **Conclusion**: These findings suggest widespread alterations in brain activity that may be associated with disrupted connectivity between critical brain regions. Despite the limited sample size, these results highlight the utility of QEEG as a non-invasive tool for detecting neurophysiological changes in individuals with PASC. Future studies should include larger cohorts, integrate cognitive

assessments, and identify region-specific biomarkers that link EEG alterations to cognitive symptoms. This approach will advance the neurocognitive characterization of PASC and guide the development of targeted neurorehabilitation strategies.

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Drosophila *melanogaster* as a Research Model to Study the Impact of PFAS on Neuroplasticity of Complex Motor Behaviors

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Introduction Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants known for their stability and bio accumulative properties, raising concerns about their potential to disrupt biological systems. **Methods** This study used *Drosophila melanogaster* to evaluate the effects of a long chain PFAS, Perfluorooctanoic acid (PFOA) exposure on survival, reproduction, locomotor behavior, and ethanol tolerance—traits associated with neuroplasticity. For the survival analysis, male and female flies were exposed to PFOA concentrations ranging from 1 µM to 1 mM for 28 days. Reproductive effects were assessed by allowing 10 mating pairs to reproduce for 3 days under 100 µM, 300 µM, 700 µM, and 1 mM PFOA, and offspring were counted over 7 days. Locomotor performance was evaluated using the Rapid Iterative Negative Geotaxis (RING) assay after 14 days of exposure to 1□M to 1mM concentration range. To assess alcohol tolerance, flies were exposed to 300 µM, 700 µM, and 1 mM PFAS for 14 days, followed by an acute ethanol tolerance assay. **Results** Our findings indicate that PFAS impacts survival in a concentration- and sex-dependent manner. Reproductive output decreased significantly with increasing concentrations. Locomotor ability was affected in a dose-dependent manner, with 300 µM producing the most significant reduction, though no sex differences were observed. Ethanol tolerance development shows a significant difference in time to reach 50% sedation time by PFOA exposure. **Conclusion** These results suggest that PFOA may impair survival, reproduction, and complex motor behaviors in *Drosophila* in a concentration-dependent fashion. Further research is needed to confirm these preliminary findings and to understand the mechanisms involved in PFOA-induced neurotoxicity.

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Exposure to the herbicide glyphosate disrupts activity in the hippocampus, but not medial prefrontal cortex, in male rats

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INTRODUCTION: Glyphosate, one of the most widely used herbicides, has raised concerns regarding its impact on mental health. Recent work from our lab indicates that glyphosate, even at levels considered safe by the Environmental Protection Agency (2.0 mg/kg/day), increases anxiety-like behaviors. Unfortunately, increases in anxiety may exacerbate other behaviors, such as avoidance. As part of a larger behavioral project, we are exploring how glyphosate influences the neurobiology of brain regions implicated in avoidance. Specifically, the prelimbic (PL) subregion of the medial prefrontal cortex is crucial for expression of avoidance, whereas the infralimbic (IL) subregion is thought to inhibit avoidance. Furthermore, the hippocampus is implicated in the contextual modulation of fear-related behaviors. For these reasons, we hypothesized that glyphosate exposure would disrupt activity in these brain regions. **METHODS:** To test this idea, adult male rats (3 months of age upon commencement of experiments; Glyph: n=16; Ctrl: n=16) were exposed to glyphosate (2.0 mg/kg/day) or filtered water for controls for 12 weeks. Next, rats were sacrificed for histological analysis using c-Fos labeling. Experimenters performing data analyses were blinded to the condition of the treatment to minimize bias. **RESULTS:** We did not observe any changes in either PL (Glyph: 280 cells/cm^2; Ctrl: 220 cells/cm^22; p = 0.3970, T-test) or IL (Glyph: 200 cells/cm^2; Ctrl: 200 cells/cm^2; p = 0.69, T-test). Next, we assessed four subregions of the hippocampus. Here, we observed that glyphosate increased cellular activity in the CA1 (Glyph: 75 cells/cm^2; Ctrl: 12 cells/cm^2, p = 0.0001, T-test), CA3 (Glyph: 50 cells/cm^2; Ctrl: 12 cells/cm^2, p = 0.0031, T-test) and dentate gyrus (Glyph: 65 cells/cm^2; Ctrl: 11 cells/cm^2, p = 0.0001, T-test). Contrary to this, there was no difference in activity in CA2 (Glyph: 30

cells/cm^2; Ctrl: 20 cells/cm^2, p > 0.08). **CONCLUSIONS:** Collectively, this exploratory work suggests that glyphosate disrupts contextual modulation of fear-related behaviors thus impairing avoidance.

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Ethanol Preference in Puertorrican Honeybees

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Introduction: Studies have shown that honeybees (Apis mellifera) undergo a physiological transition from nursing to foraging in approximately 21 days. Yet, sometimes this developmental step is delayed by a primer pheromone spread by older foragers bees. The major component in this primer pheromone is ethyl oleate (EO) which is produced by the older forager from exposure to fermented nectar. However, studies have failed to determine whether the honeybees collect the ethanol purposefully or accidentally. Gaining insight on their preference is the first step in rectifying this. **Methods:** In this study, we investigated the ethanol preference in a representative sample of gentle Africanized bees of 3-4 weeks of age since they are active foragers known to be capable of learning and memorizing specific locations. These bees were sourced from colonies maintained at the Estación Experimental Agrícola (EEA) at the University of Puerto Rico in Gurabo. To assess preference, we used an artificial flower patch consisting of 36 uniformly blue flowers. Eighteen flowers contained a 1 M sucrose solution, while the remaining eighteen contained a 1 M ethanol solution at one of three concentrations: 5%, 7%, or 10%. The experiment is conducted in three phases with recorded foraging visits (35) across each: a control phase, an acquisition phase, and an inverted flower patch phase. In the control phase, all flowers contained sucrose, allowing us to observe baseline foraging patterns. In the acquisition phase, ethanol solutions were introduced to half of the flowers, revealing initial preferences or aversions. During the inverted patch phase, the positions of ethanol and sucrose solutions were systematically switched to account for positional biases, ensuring foraging behavior was driven by the solution rather than flower location. **Results:** Results suggest varied responses across the ethanol concentrations. At 5% ethanol, approximately 59% bees showed indifference, 33% avoided the solution, and 1% exhibited a preference. For the 7% concentration, approximately 100% bees were indifferent. At 10% ethanol, 67% bees remained indifferent while 33% actively avoided the solution. **Conclusion:** We discussed these results regarding the timeline of ethanol exposure of foragers and concluded that ethanol acts as a gustatory

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Coconut Oil Enhances Sensory Perception in a *Caenorhabditis elegans* Model of Alzheimer's Disease

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INTRODUCTION: Millions of people worldwide suffer from Alzheimer's disease (AD), a progressive neurodegenerative disorder. A central biomarker of AD pathology is beta-amyloid (Aβ) plaques in the brain, which alter neuronal connectivity, promote neuroinflammation and lead to neuronal death. Beyond cognitive impairments, early stages of AD may also involve sensory deficits and impaired glucose metabolism as Aβ deposits alter sensory-related neuronal circuits and biochemical functions. Coconut oil (CO), rich in medium-chain triglycerides, may increase the availability of ketone bodies, providing an alternative energy source to neurons with impaired glucose metabolism and offering potential neuroprotective benefits. **METHODS:** To test whether CO mitigates Aβ-induced sensory dysfunction, we used a *Caenorhabditis elegans* transgenic model of AD. Synchronized eggs of strain CL2355, which pan-neuronally expresses the human Aβ gene, and control strain CL2122 were treated before Aβ induction, from hatching to the L4 larval stage, or after Aβ induction, from the L4 stage onward, with 1.0% CO or 0.5% dimethyl sulfoxide as vehicle, and compared with their respective untreated controls. Chemotaxis assays were performed at the L4 developmental stage to evaluate the ability of animals to detect 0.5% diacetyl as a chemoattractant. Our **results** showed that CL2355 animals exhibited deficits in their chemotactic response; however, this response was significantly improved with CO treatment under both pre- and post-induction conditions. **CONCLUSIONS:** This suggest that CO may partially counteract the Aβ-induced disruption of sensory circuits in *C. elegans*. Future studies will examine other AD-associated processes to determine whether the effects of CO extend to other relevant phenotypes. These findings attempt to promote metabolic strategies that contribute to preserving neuronal function during Aβ neurotoxicity in AD.

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Effects of anthropogenic noise exposure on exploratory performance and cognitive behavior in the Caribbean hermit crab (*Coenobita clypeatus*)

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Introduction: Recent studies have shown that anthropogenic noise pollution in coastlines has a negative impact on animals inhabiting these areas, potentially affecting their intraspecific communication, essential survival skills, physiology, and reproduction. This study investigates the effects of noise that endangers the biodiversity within the littoral ecosystem, using the Caribbean hermit crab (*Coenobita clypeatus*) as the experimental model. To assess the effects of noise exposure on short-term memory and thigmotaxis (centerphobia), we used a behavioral test battery including the Novel Object Recognition (NOR) and Open Field Arena (OFA) tests. **Methods:** Laboratory-habituated animals were divided into three groups: a control group (no exposure to anthropogenic noise), an acute group (exposed to noise during the test), and a chronic group (exposed to noise for 14 hours prior to the test). Animal behaviors were analyzed using the EthoVision XT software program. Within the NOR test, two analytical indices were incorporated to quantify memory performance and exploration: the Discrimination Index (DI = (T_novel – T_familiar)/(T_novel + T_familiar) and the Recognition Index (RI = T_novel / (T_novel + T_familiar), along with the total exploration time (TE). These variables allowed a refined interpretation of whether the observed behavioral changes were due to memory impairment or alterations in motivation and exploration. **Results:** Results showed that chronic noise exposure significantly reduced total exploration time (TE) compared to both control and acute groups ($p < 0.05$), while DI and RI values remained positive and comparable across groups, indicating that the animals retained the ability to recognize the novel object. This suggests that noise did not impair short-term recognition memory but instead reduced exploratory motivation in the chronically exposed crabs. Furthermore, in the OFA test, both acute and chronic groups exhibited increased thigmotaxis compared to control animals ($p < 0.05$), suggesting a change in their normal exploration patterns and general interaction with the environment. **Conclusion:** Together, these findings indicate that while memory

functions remain preserved under noise exposure, chronic exposure alters exploration, possibly reflecting reduced motivation. These results highlight the significant behavioral effects of sustained anthropogenic noise on *C. clypeatus* and emphasize the importance of regulating coastal noise pollution to protect species' natural behavioral patterns and cognitive integrity.

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Characterizing development of mechanosensory organs in the lateral line of the larval river-dwelling and cave-adapted Mexican tetra

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Introduction: Over 1.5 billion people live with hair cell damage due to natural aging, noise pollution, or underlying genetic conditions across the globe. A central question in neuroscience is whether natural variation affects hair cell formation to counteract environmental disadvantages and influence auditory and mechanosensory functions. Non-mammalian species, like amphibians and fish, provide an opportunity to address this because they contain a superficial structure called the lateral line that contains hair cells homologous to ours. While significant strides have been made towards understanding the genetic basis underlying hair cell development in the lateral line of zebrafish, less is known about natural variation in hair cells across closely related species. **Methods:** To ask this broader question, we looked into the Mexican tetra, *Astyanax mexicanus*, a fish species that exists as two morphotypes: (1) a river-dwelling surface fish and (2) a blind, cave-adapted fish that has an expanded lateral line. By using this model system, we asked if the expansion of the lateral line occurs in early development stages in the blind cavefish and if these supernumerary or “extra” neuromasts have more hair cells compared to their ancestral surface fish counterpart. We performed immunohistochemistry, FM1-43 and DAPI to label and quantify hair cells and neuromasts in both surface fish and cavefish across different early larval development stages. **Results:** Our data suggests differences in the lateral line across early development. This could mean that in one morphotype (cavefish) the lateral line is developing slower and eventually catches up and expands compared to surface fish. Distribution of hair cells across body regions indicates variation within the same morphotype. **Conclusion:** This means that morphological expansion begins early during larval development. By understanding how these developmental expansions occur, new mechanisms can be uncovered to become another avenue to counteract hearing and balance disorders, or to eventually interrogate hair cell regeneration mechanisms in a model with natural variation of the hair cell sensory epithelia.

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Discovery and Development of Anxiolytic Agents from Tropical Marine Algae using *Drosophila melanogaster* as a Behavioral Model

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Introduction. Based on reports from the World Health Organization and the National Institute of Mental Health (NIMH), one third of the population is affected by anxiety disorders. These are often treated with benzodiazepines and antidepressives, which can lead to tolerance and dependence. This highlights the need for safer and more effective anxiolytic therapies. Marine natural products, particularly from tropical brown algae, offer promising alternatives due to their bioactive metabolites with neuroprotective properties. *Stypopodium zonale* has emerged as a potential source of such compounds. Our research focuses on studying the anxiolytic effects of natural products derived from tropical marine algae, including *S. zonale*, found in the coastal waters of Puerto Rico, using the fruit fly, *Drosophila melanogaster* as a model organism. We aim to chemically characterize algae extracts, conduct anxiety-related behavioral tests in *D. melanogaste*r, and propose a mechanism of action underlying the observed anxiolytic effects. The **methodology** involves exposing fruit flies to algae extracts through both chronic (from oviposition to adulthood) and acute (6 hours) treatments. Following exposure, anxiety-like behaviour is assessed using Open-Field (OFT) and Dark-Light Box (DLB) tests, where wall-following behaviour — *Drosophila's* natural tendency to stay close to the arena's edges— is quantified. A Wall-Following Decrease Index (WFDI) is calculated to measure reductions in wall-following behavior, based on the distance the flies travel away from the walls toward the center of the arena. RING assays were performed as a counter-essay to confirm that the algae extracts do not affect locomotor activity. In the DLB test the latency time measures how long it takes for *Drosophila melanogaster* to enter the dark compartment for the first time, where a shorter latency indicates lower anxiety. The time spent in each area reflects anxiety levels: spending more time in the illuminated zone suggests less anxiety, while less time indicates greater anxiety. **Results.** Recent data has proven that the brown algae, *Stypopodium zonale,* has anxiolytic effects, similarly to the effect of Diazepam, a benzodiazepine used as our positive control. Statistically significant anxiolytic effects were also obtained for *Dictyota cervicornis* and *Padina boergesenii*, although no anxiolytic effects were observed

for *Lobophora variegatta*, *Ulva, Symploca*, and *Sargassum sp.* species. **Conclusion.** Our research represents an unprecedented approach to anxiolytic drug discovery as it improves our understanding of Puerto Rico's marine algae and their natural product's chemo-diversity.

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Reanalysis of Personality Outcomes in "Neural and Subjective Effects of Inhaled N,N-Dimethyltryptamine in Natural Settings": A Bayesian Model Approach

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Introduction: The study by Pallavicini et al. (2021) investigated the psychological effects of inhaled N, N-dimethyltryptamine (DMT) in natural settings, reporting increases in agreeableness and absorption and decreases in anxiety after DMT use. However, these findings were based on an independent t-test that did not account for individual or measurement-level variability. Thus, the present study aimed to conduct a more robust reanalysis using Bayesian models that account for uncertainty and inter-individual differences in subjective psychometric outcomes. **Methods:** This study reanalyzed publicly available data from Pallavicini et al. (2021), which included 35 healthy, experienced psychedelic users from Buenos Aires, Argentina, recruited between May and December 2019. Eligible participants inhaled freebase DMT in their preferred environments. Statistical reanalysis included pre-to-post differences in psychometric measures such as the Big Five Inventory (BFI), State-Trait Anxiety Inventory (STAI-State), and Tellegen Absorption Scale (TAS), and were evaluated using a hierarchical Bayesian model estimated via Hamiltonian Monte Carlo in the *brms* RStudio package. Models accounted for within-subject change and cross-measure dependencies using weakly informative priors, with posterior means and 95% credible intervals (CrI) back-transformed to the original scales. **Results:** Compared with the original paired *t*-tests, the Bayesian model yielded smaller and more uncertain estimates of change. Posterior means indicated slight increases in BFI Agreeableness (0.18 [– 0.76, 1.10]), Conscientiousness (0.25 [–1.05, 1.54]), Extraversion (0.17 [–0.74, 1.09]), Neuroticism (0.20 [–0.87, 1.27]), Openness (0.15 [–0.64, 0.93]), and TAS Absorption (0.30 [– 1.26, 1.84]), with a slight decrease in STAI-State Anxiety (0.29 [–1.25, 1.82]). None of the 95% CrIs excluded zero, and posterior probabilities of improvement (~0.65) indicated modest but uncertain evidence for change. **Conclusions:** Our Bayesian reanalysis suggests that DMT's psychological effects in natural settings are modest and highly variable across individuals. While trends toward increased agreeableness and absorption and decreased anxiety were observed, the results highlight uncertainty and the importance of individual and contextual factors. Further studies should be conducted to confirm these findings.

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Strategies of incorporating deep learning to classify the phases of the estrous cycle in the rat

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The estrous cycle plays a critical role in modulating neural processes in female rodents, influencing a range of body functions and behaviors (Alvord and Pendergast, 2024; Markus, 2013). Accurate identification of estrous stages is therefore fundamental for studies examining sex differences and changes of neural activity in the brain. Indeed, there are some potential limitations while analyzing the estrous cycle in rodents. First, manual classification of vaginal cytology remains subjective and variable, leading to inconsistencies that compromise reproducibility across laboratories. Second, considering the number of animals used in experiments and number of days of assessment of the estrous stages, hundreds or thousands of images requiring analysis may be generated leading to additional difficulties. To address these issues, we identified the open-access software *EstrousNet*, which is a recently developed deep learning–based platform designed to automate estrous cycle classification. Interestingly, *EstrousNet* reportedly achieves approximately 90% accuracy in predicting stages from cytology images (Wolcott, 2022). As we began to incorporate the technology, we came across a set of obstacles which we had to overcome to use *EstrousNet* in our laboratory. In this project, we sought to validate *EstrousNet*'s reported performance by comparing its automated classifications against our manual analyses. Using a dataset of vaginal cytology images collected from adult female rats, we evaluated *EstrousNet*'s predictions across multiple cycles, assessing accuracy, consistency, and time efficiency relative to manual classification. Preliminary results indicate that *EstrousNet* performs comparably to human observers while substantially reducing analysis time (Wolcott, 2022). Minor discrepancies between automated and manual classifications highlight potential areas for refinement in both algorithmic training and standardization of cytological labeling criteria. Overall, these findings support *EstrousNet* as a reliable and efficient tool for estrous stage identification and advances reproducibility in sexbased neuroscience research.

Extinction learning of morphine conditioned place preference according to the estrous cycle

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Opiate addiction continues to be a public health crisis in the United States, responsible for approximately 75,000–100,000 yearly overdose-related deaths. While men have a higher prevalence of opiate use disorders (OUD), some studies have suggested that women may be more susceptible to developing OUD. As women have been underrepresented in clinical studies, there is a gap in understanding how the hormonal fluctuations of the estrous cycle impact susceptibility to addiction. We have previously used a conditioned place preference model (CPP) to study the effects of extinction training on extinction of morphine-conditioned place preference in male rats. In males, we previously found that extinction-trained animals could be classified into two groups: those who extinguished their conditioned behavior, and those who failed to do so. We also found an increase in brain derived neurotrophic factor (BDNF) in the hippocampus (HPC)and amygdala of animals who successfully extinguished their preference. Here, we replicate this model in female rats. Additionally, we monitored estrous cycle stage using vaginal lavages to study the impact of rat hormonal stage on extinction. We also measured the levels of BDNF expression as well as expression of downstream effectors such as, p-TrkB and p-Erk using Western blots. Similar to males, we found two extinction phenotypes, however, female rats that were in the estrous stage during the extinction test were more likely to extinguish their drug conditioning than their counterparts in the diestrus stage. Preliminary data also suggest a trend toward increased p-Erk levels in the HPC of animals in the extinction group which coincides with our previous observations in females, of increased BDNF in the HPC and nucleus acumbens, and p-TrkB. Together, these findings suggest the potential involvement of hormonal states in the extinction learning process and may indicate a potential link between extinction learning and BDNF signaling.

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Behavioral Strategies and Sex Differences During Motivational Conflict in Platform Mediated Avoidance

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Introduction: Although reward-approach and threat-avoidance are often studied in isolation, their interplay is central to many psychiatric disorders, for example, depression involves diminished reward approach and heightened avoidance, while addiction reflects compulsive reward pursuit despite threat. Addressing this requires a rodent model of active avoidance with adjustable conflict levels. We expanded the platform-mediated avoidance (PMA) task to support both low-conflict (LC) and high-conflict (HC) contingencies in mice. **Methods**: In this task, a light cue in the nose-poke port signals reward availability (20% sucrose solution, 6μL), while an auditory warning cue (8kHz tone, 75dB, 20s) co-terminates with a mild footshock (0.2mA, 2s). Mice were first trained in LC PMA for 10 days, during which reward was available throughout the session, including inter-trial intervals, and delivered on a variable interval 30-sec schedule. Each daily session included 20 warning tones spaced ~2 minutes apart. This was followed by 10 days of HC PMA training, in which reward (1:1 fixed ratio) was available only during the warning tone. In LC, animals acquired avoidance over time, reducing shocks received from 64% on day 1 to 35% by the end of training ($p = 0.0021$). **Results**: Under HC, animals increased reward intake (104 to 176 rewards, $p = 0.0081$) while reducing shocks received from 63% to 39% ($p = 0.0430$). The contingencies elicited distinct strategies: in LC, animals avoided cues rapidly (mean latency to avoid = 6.95 s), whereas in HC, they increased latency to avoid (12.6 s) to obtain rewards ($p = 0.0004$). During extinction, avoidance declined from 59% to 4% by day 3 ($p < 0.0001$), while cue-driven reward approach increased from 25% to 67% nose-poking time ($p = 0.0022$). Re-exposure to a single shock reinstated avoidance, with platform time returning to 62% ($p = 0.0066$). Sex differences were evident across learning, conflict, and reinstatement. In LC, males showed more reward approach during intertrial intervals ($p = 0.0007$) and earned more rewards ($p = 0.0235$), while early tone-platform time in females predicted later avoidance ($p = 0.0101$). In HC, females initially spent more time on the platform during the tone but converged with males by mid-training ($p = 0.0008$), while males gradually increased avoidance ($p = 0.0009$). During reinstatement, females showed significantly higher

avoidance ($p = 0.0469$). **Conclusion**: These findings reveal distinct behavioral strategies across motivational contexts and highlight sex-specific differences in how animals integrate threat and reward during learning, conflict resolution, and extinction.

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The impact of dietary composition on stress behavior in *Astyanax mexicanus*

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Introduction: Environmental pressures may underlie adaptation of stress-related behaviors. *Astyanax mexicanus*, a teleost fish, is an ideal model for investigating the evolution of stress and behavioral adaptation in response to environmental variability due to its two distinct morphotypes that evolved in different habitats. These are composed of surface fish, which inhabit illuminated, predator-rich rivers, and Pachón cavefish, which have adapted to isolated subterranean environments with constant darkness and lack of food resources. Most cavefish have lost stress and anxiety -like behaviors compared to their surface fish ancestors. We aimed at uncovering whether diet variation, one of the many environmental pressures that cavefish were subjected to, between the morphotypes was associated to adaptation of stress behaviors. **Methods:** To answer this, we used a novel tank test, which has been previously validated as a measurement of stress levels in fish models. We determined the following experimental groups based on diet acquisition for one month: (1) vegetable-based, (2) commercial pellets, or (3) frozen worms. Each morphotype was evaluated independently to control for potential confounding effects of interpopulation interactions on behavioral outcomes. Behavioral endpoints included the duration spent in the upper and lower halves of the tank, which served as indicators of exploratory activity (top-dwelling) and anxiety-like behavior (bottom-dwelling). **Results:** Surface fish displayed a significant increase in time-spent bottom-dwelling across all diet treatments compared to Pachón cavefish, suggesting a generalized heightened anxiety-like response that is independent of diet. Pachón cavefish displayed generally low anxiety levels; however, individuals on pellets and bloodworm diets exhibited anxiety levels similar to those of surface fish, suggesting that certain nutritional conditions may partially restore anxiety-like responses in this otherwise low-reactivity, cave adapted morph. **Conclusions:** Overall, these results suggest that morphotype is the primary determinant of behavioral stress response in *Astyanax mexicanus*, while dietary composition has a minimal or dispensable effect on stress adaptation. Other environmental conditions, including darkness,

water temperature or water chemistry may be associated to adaptation to stress, and will be evaluated in future work.

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Role of antibiotics on avoidance and anxiety-related behaviors in female vs male rats

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Introduction: Antibiotics are essential for treating infectious diseases but can disrupt the gut microbiota, leading to behavioral and neurological alterations. According to the 2020 National Survey on Drug Use and Health (NSDUH), nearly eight million (18.4%) Hispanic/Latino adults report having a mental illness, underscoring the need to address biological and environmental factors that may contribute to mental health disparities in this population. This study aims to evaluate the behavioral and neurological effects of oral broad-spectrum antibiotics to better understand their implications for minority and underserved groups. We hypothesize that daily oral antibiotic exposure (1 mg/kg per day) will increase avoidance behaviors, alter brain activity, and disrupt gut microbiota composition. **Methods:** Rodents received either an antibiotic cocktail (ampicillin, streptomycin, and clindamycin; 1 mg/kg; n = 16) or filtered water (n = 16) per sex and were assessed for avoidance and anxiety-like behaviors, as well as neuronal activation in brain regions implicated in avoidance. **Results:** Female animals exposed to antibiotics spent significantly more time on the platform during the tone (p = 0.0402), less time in the center of the open field (p = 0.0190) and exhibited a higher anxiety index in the elevated plus maze (p = 0.0266). In contrast, male animals exposed to antibiotics showed no significant differences in avoidance or anxiety-like behaviors compared to controls (p > 0.8). Preliminary c-Fos immunolabeling revealed increased neuronal activation in the nucleus accumbens (NaC) and basolateral amygdala (BLA) of female animals, whereas male animals showed no detectable changes. Conclusion: These findings suggest that antibiotic exposure increases avoidance and anxiety-like behaviors in females but not in males, corresponding with enhanced neuronal activation in NaC and BLA regions. This sex-dependent vulnerability highlights the importance of considering biological sex when evaluating the neurobehavioral effects of microbiota disruption and its potential contribution to anxiety and fear-related disorders.

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Neuroproteomic Effects and Behavioral Characterization of Anxiolytic Natural Products from Tropical Marine Algae

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Introduction: Anxiety disorders are the most prevalent psychiatric conditions worldwide, and the limitations of current treatments, particularly dependence and tolerance associated with benzodiazepines, highlight the urgent need for safer anxiolytic alternatives. Marine natural products offer a unique opportunity for drug discovery due to their chemical diversity and neuroactive potential. **Methods:** This interdisciplinary study integrates natural product chemistry, behavioral neuroscience, and proteomics to evaluate the anxiolytic potential of tropical marine brown macroalgae from Puerto Rico, using *Drosophila melanogaster* as a translational model. Flies were exposed to crude algae extracts under acute and chronic treatment paradigms. Anxiety-like behavior was quantified through open-field testing, and locomotion controls were performed through ring assays. Proteomic profiling of dissected fly heads was conducted using LC-MS/MS to identify differentially expressed proteins associated with anxiolytic effects. **Results:** Extracts from *Stypopodium zonale*produced robust anxiolytic-like effects without impairing locomotor activity, while *Dictyota cervicornis* and *Padina boergesenii* exhibited moderate effects under chronic exposure. No anxiolytic activity was observed for *Lobophora variegata*, *Symploca*, *Ulva*, or *Sargassum* species. Proteomic analysis revealed dysregulation of more than 50 proteins, including odorant-binding, synaptic, and signaling-related proteins linked to threat detection and stress pathways in the fly's brain. **Conclusion:** These findings demonstrate that tropical marine algae, particularly *S. zonale*, represent promising sources of neuroactive compounds with anxiolytic potential. This work presents the first neuroproteomic screening of Puerto Rican marine algae for anxiolytic applications and underscores the value of integrating behavior, neurobiology, and chemical ecology to advance the discovery of next-generation anxiolytic agents.

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Ventral Pallidum Astrocytes Regulate Approach–Avoidance Conflict Behavior

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Introduction: Balancing appetitive and aversive drives is essential for adaptive decision making, yet the cellular and circuit mechanisms that support this process remain poorly understood. The ventral pallidum (VP) is a major limbic output structure implicated in motivation and reward, where GABAergic neurons and astrocytes modulate synaptic transmission and behavioral vigor. While neuronal contributions to motivation have been extensively characterized, the role of astrocytes in integrating competing drives during conflict remains largely unexplored. Methods: Here, we examined VP astrocyte activity in mice performing the platform-mediated avoidance (PMA) task, a model of approach–avoidance conflict in which animals must choose between approaching a light-signaled sucrose reward and avoiding a tone-signaled foot-shock by stepping onto a safety platform. To probe flexibility across motivational contexts, we tested mice in low conflict (LC) and high conflict (HC) contingencies across 10-day training blocks, as well as in a mixed-cue (MC) paradigm that dynamically alternates between safety, threat, and conflict trials within a single session. Results: Fiber photometry recordings revealed distinct patterns of VP astrocyte calcium activity across these conditions. During LC sessions, astrocytes showed sustained activation to threat-predictive cues, whereas in HC they exhibited inhibition during the cue presentation and elevated activity linked to avoidance responses. Optogenetic activation of VP astrocytes accelerated avoidance behavior ($p = 0.0054$) and increased time on the platform across LC and HC conditions. In HC, this effect was accompanied by reduced reward-seeking during tone presentations, suggesting that astrocyte activation biases behavior toward threat avoidance under conflict. Importantly, stimulation during reward-only sessions, where there is no threat of foot-shocks, failed to alter behavior, indicating that astrocyte-driven modulation is contingent on motivational conflict rather than cue exposure alone. In the MC paradigm, astrocyte activation further increased avoidance and reduced seeking during dynamically interleaved conflict trials, consistent with a contex tdependent regulation of motivational balance. Conclusion: Together, these findings identify VP astrocytes as active contributors to conflict resolution, dynamically modulating behavioral output

according to motivational context. This glial mechanism highlights a previously underappreciated component of VP circuitry that may be critical for adaptive choice behavior and whose dysregulation could contribute to maladaptive decision-making in disorders such as addiction, anxiety, and depression. **IACUC Approval number:** 700222 **Acknowledgements:** This work was supported by NIH grants K00MH136687-03 (C.M.R.) and R21MH137593 (C.B.R.). We thank Pamela Pérez-Vázquez, Vanelisse Rivera-Marzán, Camila Regalado-Franco, Verónica Rodríguez-González, Alanis Rivera-Santos, José González-Báez, Carlos Rodriguez and Alejandro Santos-Bermúdez for their assistance with behavioral testing and animal care.

Caffeine-Mediated Effects on Neuromuscular Activity in Biceps' Myofibers

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Introduction: Caffeine, the most widely consumed psychoactive compound worldwide, acts as a central nervous system stimulant known to enhance neurotransmitter activity, promoting alertness and improved concentration. On average, Americans consume approximately three cups of coffee per day, underscoring the pervasive presence of this stimulant in daily life. Caffeine exerts its effects on neuromuscular performance primarily through three mechanisms: increasing calcium ion release from the sarcoplasmic reticulum, preserving muscle glycogen via phosphodiesterase inhibition, and blocking adenosine receptors in the brain. These mechanisms collectively enhance muscular contraction strength, reduce fatigue perception, and elevate energy levels. **Methods:** This study aimed to determine whether caffeine intake produces measurable benefits in muscle resistance during a 90-degree isometric biceps curl hold, and whether habitual physical activity influences this response. Participants ingested 5 ounces of caffeine prior to testing. Neuromuscular activity was recorded using the Backyard Brains Muscle Spiker Box system for subsequent analysis and data interpretation. **Results:** The average participant age was 19 ± 0.32 years (mean±St.err). Approximately 21% of subjects reported never consuming coffee, while regular consumers reported an average intake of 1.2 ± 0.1 cups per day(mean±St.err). Phenotypic data suggest that caffeine increased the amplitude of muscle action potentials, indicating enhanced excitability and contractile efficiency in biceps myofibers. **Conclusion:** Our findings suggest that caffeine consumption may enhance neuromuscular performance by increasing action potential amplitude and improving contractile response in biceps' myofibers. This effect appears beneficial in both physically active and inactive individuals, indicating that caffeine may enhance muscular efficiency independent of baseline activity level.

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Prenatal Stress Induces Sex-Dependent Behavioral and Immune Alterations in Mice

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Introduction: Perinatal stress is associated with long-lasting alterations in neural systems that regulate threat, reward, and social behaviors. Method: To model prenatal stress (PNS) in mice, we employed a chronic restraint stress paradigm during gestation (4 hrs./day, 3× per week for 2 weeks). Adult male and female offspring were then tested in a battery of behavioral tests when they reached adulthood: we used the open field test (OFT)/elevated plus maze (EPM), social interaction (SI), and sucrose preference tests to assess anxiety-like behavior, sociability, and anhedonia, respectively. Urine and blood samples from dams and adult offspring were analyzed for interleukins and related inflammatory markers. Results: PNS dams did not differ from controls in pup retrieval, sucrose preference, or SI tests, but displayed increased distance traveled in the OFT and EPM, suggesting heightened locomotor activity. Among offspring, PNS males exhibited reduced social interaction compared to controls. Interestingly, in PNS offspring, center time in the OFT increased with the number of cage mates in both sexes. PNS females, however, showed reduced distance traveled in the OFT, indicating potential alterations in exploratory behavior. PNS offspring exhibited elevated IL-17a and IL-6 levels alongside decreased circulating TNFα. PNS females displayed higher IL-1a concentrations compared to controls. Conclusion: Collectively, these findings indicate that prenatal stress induces sex-dependent behavioral and immune pro-inflammatory alterations in mice, potentially reflecting mechanisms underlying differential vulnerability to prenatal stress.

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Effects of Auditory and Visual Stimuli on Autonomic Nervous System Arousal

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Introduction: The average American adult accumulates a significant amount of screentime each day. Excessive use of the internet can result in problematic internet use, a use of the internet that may cause interference and difficulties in their social, work, or school life. Problematic internet use has been related to reduced autonomic flexibility and autonomic unbalance. Studies have shown that reduced autonomic flexibility, specifically increased activation of the sympathetic nervous system and suppressed activity of the parasympathetic nervous system can have an impact on inflammatory response and is a risk factor for the onset of a series of age related metabolic, cardiovascular, and neurodegenerative conditions. The purpose of this research is to analyze how different types of auditory and visual sensory stimuli are presented in digital media, how they influence the activation of the autonomic nervous system, and their potential effects on our social and physical lives. This study aims to determine whether one of these types of stimuli generates a greater response of the autonomic nervous system and how this may relate to the effects of excessive internet use on users' health. It is hypothesized that visual and auditory stimuli will have an equal effect on autonomic nervous system arousal. Methods: We will utilize the Backyard Brains SpikerBox to record electrocardiogram signals from participants, using heart rate and heart rate variability as measures of activation of the autonomic nervous system. Participants will be exposed to four, one-minute-long bimodal sets of stimuli: calming imagery with calming audio, calming imagery with arousing audio, arousing imagery with arousing audio, and arousing imagery with calming audio. Conclusion: The results of this study can be used to determine which form of stimuli is more likely to result in a higher risk of autonomic unbalance and reduced autonomic flexibility.

Low-Frequency DBS of the NAc Modulates Extinction, Spontaneous Recovery, and Relapse of Morphine CPP: Evidence from BDNF–TrkB Pharmacology, and Chemogenetics

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Opioid addiction caused approximately 727,000 overdose deaths from 1999 to 2022 (CDC, 2024), and relapse remains the greatest barrier to treatment, with up to 91% of individuals relapsing within one year of abstinence. Although current pharmacological and psychosocial interventions alleviate withdrawal and reduce relapse, treatment resistance in some patients highlights the need for novel approaches. Deep brain stimulation (DBS), FDA-approved for Parkinson's disease since 1997, has emerged as a promising intervention for addiction by targeting reward-related circuits. In preclinical studies, low-frequency DBS (LF-DBS, 20 Hz) of the ventral striatum/nucleus accumbens (VS/NAc) during extinction training enhances extinction of morphine-conditioned place preference (CPP) and elevates brain-derived neurotrophic factor (BDNF) in the dorsal hippocampus (dHPC), although relapse persists when stimulation is applied only during extinction. The goal of this study is to dissect the molecular and circuit mechanisms by which LF- DBS promotes extinction and alters reinstatement vulnerability. Specifically, Aim 1 will evaluate whether LF-DBS applied during extinction and reinstatement can prevent relapse of morphine CPP, while also assessing spontaneous recovery after a drug- and DBS-free period, and whether LF-DBS alone is sufficient to drive BDNF expression in reward-related regions. Aim 2 will test whether BDNF–TrkB signaling in the NAc is required for extinction by pharmacologically blocking TrkB with ANA-12 or activating it with the agonist 7,8-dihydroxyflavone (7,8-DHF), with the prediction that antagonism will impair extinction while agonism will facilitate it. Aim 3 will determine whether dHPC→NAc glutamatergic projections are necessary for extinction using chemogenetic inhibition, with the hypothesis that silencing this pathway will disrupt extinction and promote relapse, thereby identifying hippocampal input as a critical substrate for DBS-induced plasticity. Preliminary behavioral data from Cohort 1a demonstrate that LF-DBS delivered during extinction training confers long-term protection against relapse-like behavior, as animals that received LF- DBS showed reduced preference for the drug-paired side during the spontaneous

recovery test compared to Sham-DBS. These findings suggest that DBS not only accelerates extinction but also provides enduring protection after a drug- and stimulation-free interval, supporting its potential as a relapse-prevention strategy. Together, these studies will establish whether LF-DBS protects against reinstatement by engaging BDNF–TrkB signaling and hippocampal–accumbal circuitry, providing mechanistic insight into neuromodulatory strategies for opioid addiction.

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Age-Dependent Effects of Cholinergic Circuit Manipulation on Memory and Plasticity

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Introduction: Memory is essential for an organism's survival but is vulnerable to disruption during aging. Agingrelated memory decline is hypothesized to be driven in part by changes in the brain's cholinergic system. As part of this system, the medial septum sends cholinergic projections to the hippocampus, which is essential for episodic and spatial memory processing. However, the impact of aging on this circuit, and how it relates to memory impairments associated with aging, remain unclear. Methods: Here, we used chemogenetics to manipulate the activity of medial septum cholinergic neurons during consolidation of object-location memory (OLM) and contextual fear memory (CFM) tasks, in male and female mice of varying ages. Following CFM recall, mice were sacrificed to characterize hippocampal network activity patterns associated with memory recall. Results: We found that chemogenetic activation in young mice resulted in a greater OLM performance, while chemogenetic inhibition had no effect on OLM consolidation. In contrast, we found sexspecific effects in the OLM task in older mice. OLM consolidation in younger females, but not males, was improved with chemogenetic activation of medial septum cholinergic neurons. Chemogenetic manipulation did not affect CFM, in either sex or age-group. However, successful CFM recall in older mice was associated with increased activity in some hippocampal subregions, compared with young mice. Conclusion: These data suggest that the regulation of memory consolidation by cholinergic signaling might differ between sexes, and as a function of aging. Understanding these mechanisms may lead to a better understanding of aging-related memory loss in humans.

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The impact of cocaine on the estrous cycle in female rats during conditioned place preference

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Introduction: Research has shown that women may experience a more rapid escalation in substance use, heightened cravings, and increased difficulty in cessation compared to men, potentially influenced by estradiol, a key female hormone. A clinical study indicated that cocaine-dependent women exhibited lower salivary estradiol levels throughout their menstrual cycles relative to controls, although the underlying relationship and its implications for addiction remain largely unexplored. This project investigates the effects of cocaine on estradiol levels and associated cocaine-seeking behavior in female rats. We hypothesized that exposure to cocaine will lead to alterations in estrous cycles and estradiol levels, thereby impacting cocaine-seeking behavior. **Methods:** To test this hypothesis, we employed a cocaine-conditioned place preference (CPP) paradigm utilizing female Long Evans rats, which received alternating injections of 10mg cocaine and saline over twelve days. Following a fifteen-day extinction period, two reinstatement sessions were conducted with 5 mg and 10 mg doses of cocaine. Throughout the experiment, vaginal smears were conducted to assess the estrous cycle of the animals. Blood and brain samples were collected to measure estradiol levels using an enzyme-linked immunosorbent assay (ELISA). All procedures were approved by Ponce Health Sciences University IACUC Protocol #2103000487A002. **Results:** Our findings revealed significant differences in the number of changes in estrous stages within the cocaine CPP group, while no such differences were observed in the saline group across the entire cycle. Additionally, distinct transitions between estrous stages were noted among the cocaine group within the same day compared to controls. Moreover, rats in the estrus and metestrus stages that received a 10mg dose of cocaine exhibited a significantly greater preference in the CPP test compared to those in proestrus. Notably, cocaine-exposed rats exhibited lower brain estradiol levels, while serum estradiol levels remained unchanged. A positive correlation was found between the second reinstatement and serum estradiol levels, suggesting that higher concentrations of estradiol are associated with a greater preference for the cocaine-associated chamber. **Conclusion:** Our results highlight the complex and differential effects of cocaine on estradiol concentrations in the brain versus serum. Elevated serum estradiol levels may enhance the motivational aspects of cocaine use. Understanding the hormonal states that affects cocaine's effects could inform hormone replacement therapy and cycle-aware drug rehabilitation, supporting the development of sex- and hormone-specific treatments for substance use disorders.

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Chronic Pain Following Mild Concussive-like Injury: Development of a Rat Model

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Introduction: Chronic pain is a common outcome of mild traumatic brain injury (mTBI), affecting over 60% of patients, yet its underlying mechanisms remain poorly understood due to the lack of a reliable animal model. This study developed a rat model of mTBI-induced chronic pain. **Methods:** Our model focused on a repetitive (3X, 48 hours apart) closed head injury (rCHI) weight-drop approach. Injury severity was evaluated through standard behavioral measures— time to wake, right, and ambulate—confirming mild injury without loss of consciousness. Painrelated behaviors were assessed via cephalic and extra-cephalic mechanical sensitivity using von Frey filaments. **Results:** Behavioral results showed no significant differences between rCHI and Sham groups in injury severity measures, supporting the mild injury criteria. However, rCHI rats displayed prolonged cephalic hypersensitivity ($p=0.002$) and hindpaw hypersensitivity ($p=0.0380$) at all time points compared to Sham rats. Ongoing experiments are validating injury level through immunohistochemistry for microglial (Iba-1), astrocytic (GFAP), neuronal density (Neu-N), and neurofilament (Nf-1) markers in the perilesional cortex. **Conclusion:** Closed head injury induces prolonged tactile cephalic and extracephalic mechanical hypersensitivities in this model. Loss of consciousness or gross motor loss was not evoked. This model emulates the mTBI-induced pathological pain phenotypes in humans; thus, it is a valuable tool for identifying the underlying mechanisms and contributing to the development of targeted therapies. Future work will focus on optimizing other pain-like behaviors and explore neural plasticity changes using markers like ERK and cFos in pain-processing brain regions. These findings set the basis to elucidate the mechanisms of pathological pain following mTBI. **IACUC:** A12021 (Medical Sciences Campus to DS-M) **Acknowledgements:** NIEHS R21ES034191 to DS-M and FG-V; NINDS R21NS119991, Brain & Behavior Research Foundation Young Investigator grant, PRCTRC Pilot, NIGMS COBRE II, RCMI8G12MD00760, Hispanics in Research Capability (HiREC), and Title V Pilot Project (PiP) to DS-M and LV-R; Yarimar Carrasquillo, PhD and Michael Whalen, MD for intellectual contributions to this project.

Ensembles in the ventral hippocampus exert long-term control over habit-based behavior

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Introduction: Habits are routinized behavioral patterns that can be elicited by environmental cues and occur almost automatically. The ventral hippocampus (vHC) is involved in the formation and execution of habits, and it can form stable ensembles controlling behavior. **Methods:** Using young mice, which are innately prone to display habit-like behavior, we tagged neurons that were active in the vHC during the execution of habit-like behavior, and later performed chemogenetic manipulation of these tagged ensembles. **Results:** Chemogenetic stimulation of these neuronal ensembles induced habit-like behavior under conditions in which mice typically display more goal-directed, flexible actions; however, chemogenetic inhibition of these ensembles did not block later habit-like behavior. Additionally, quantification of overlap between vHC neurons active during habit formation vs execution revealed minimal overlap, indicating that ensembles recruited by the vHC for habitual actions may drift over time. **Conclusion:** These findings demonstrate that vHC ensembles remain functional long term and are sufficient, though not necessary, to drive habitual behavior, suggesting dynamic recruitment of neuronal populations during different stages of habit formation and expression and motivating future work to determine the specific information encoded by these ensembles.

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Identifying Microbiome Bacterial Diversity in *Drosophila melanogaster* Gut During Acute Alcohol Exposure

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Introduction: Alcohol Use Disorder (AUD) is characterized by compulsive alcohol intake despite serious social, physical, and financial consequences. Each year, more than 7% of individuals aged 12 and older require treatment for AUD. Research indicates that alcohol consumption disrupts the gut microbiota, leading to an imbalance in its composition and abundance. This microbial community which includes bacteria, viruses, and fungi is essential to the gut-microbiota-brain axis, a system of two-way communication between the gut and brain. Our lab is investigating how alcohol-related changes in the microbiome contribute to the development of alcohol tolerance. We use the fruit fly *Drosophila melanogaster* as a model organism because of its short lifespan and the similarities its gut microbiome and alcohol response share with those of humans. We hypothesize that prior exposure of flies to antibiotics, by reducing or eliminating their intestinal microbiota, will cause a decrease in their tolerance to alcohol. Method: To test this, flies were exposed to 50% alcohol while their diets (high fat, high protein and normal diet) were altered, and they were treated with a cocktail of antibiotics. The flies were then dissected to extract their intestines, which were subsequently cultured on different agar plates to quantify the colony forming units (CFUs). Results: The results have shown that disrupting the microbiome with antibiotics is effective and supports the idea that bacteria influence ethanol tolerance. Conclusion: Next steps will be using techniques such as DNA sequencing or biochemical assays to identify microbiomes organism that are resistant to alcohol exposure.

Velocity-Driven Paradigm to Investigate Effort-Based Decision-Making in Rats

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Introduction: Flexible regulation of effortful actions is essential for adaptive behavior, yet the neural mechanisms that support value-based decisions under changing cost conditions remain unclear. We introduce a novel behavioral paradigm in that rats traverse a linear corridor to obtain rewards scaled to their crossing velocity. By aligning task demands with species-typical locomotor behavior, this paradigm offers a powerful framework for studying motivation and decision-making. Methods: Physical effort is modulated via incline changes (flat vs incline), and trial structure allows animals to skip trials without penalty, enabling spontaneous regulation of effort allocation. Results: Our data show that most rats (n=3) learn to initiate trials quickly (>5 seg) and adjust behavior according to task demands. Velocity-scaled rewards drive increased movement vigor, while trial omissions reflect sensitivity to effort cost (Flat: 12% vs incline: 20%; p=0.0033). Across sessions, rats displayed stable velocity profiles (1-5 seg latencies; 22-99 m/seg) and trial-by-trial adjustments in response to changing effort demands, demonstrating flexible cost-benefit integration. Conclusion: These behaviors emerged without extensive operant conditioning, highlighting the naturalistic structure of the task. Its ethological design and scalable effort-reward contingencies make it ideal for probing the neural circuits that regulate cost-sensitive, goal-directed action across physiological and pathological states.

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The impact of chronic stress prior to cocaine exposure on the motivation to seek cocaine

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Introduction: Stress has been identified as a significant factor in the onset of substance use disorders and plays a crucial role in relapse among individuals with drug addiction. Research highlights sex-based differences in stress responses and the incidence of addiction, often attributed to biological distinctions. These differences underscore the importance of investigating how stress affects brain function differently in males and females. This study aims to examine whether sex differences influence sensitivity to the rewarding properties of cocaine. We hypothesized that stress exposure in both sexes would lead to increased cocaine intake and a heightened drive to obtain the drug in females compared to their male counterparts. **Methods:** To test this hypothesis, we subjected rats to inescapable footshock as a form of chronic stress over 5 days. Following this stress exposure, the rats underwent extended-access cocaine self-administration for 10 days, with each session lasting 6 hours. Afterward, we implemented a 2-hour progressive ratio or dose-response protocol in both male and female rats to assess their motivation and sensitivity to obtaining cocaine. **Results:** The results indicated that chronic stress prior to extended access to cocaine self-administration did not increase motivation for cocaine seeking in either the male or female treatment groups, leading us to reject our initial hypothesis. Interestingly, preliminary data from the dose-response protocol showed that stressed males consumed less cocaine and demonstrated lower motivation to seek it compared to non-stressed males across all doses. **Conclusion:** This finding suggests that stressed males may be more sensitive to the reinforcing effects of cocaine. We will conduct a study with female rats to assess their behavioral responses and explore potential sex-based differences.

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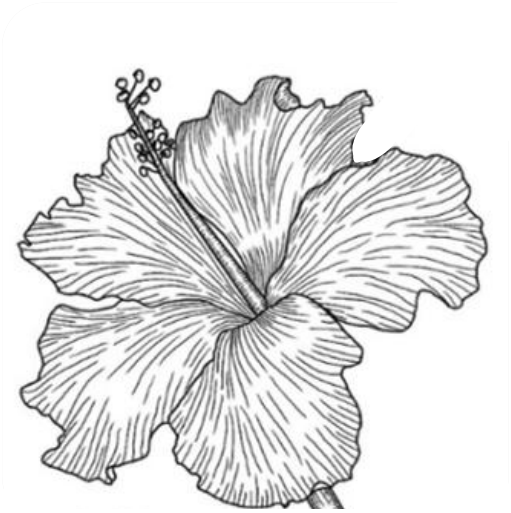

Category: Clinical Neuroscience

Temporal Trends in Thrombectomy Rates in Puerto Rico: A December Holiday Spike

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Introduction: Stroke risk has been demonstrated to fluctuate with seasonal and socio-behavioral factors. Seasonal changes in diet may impact the incidence of cerebrovascular events. Puerto Rico provides a unique setting to study this effect, as cultural practices lead to increased consumption of alcohol, sodium, fried foods, sugar, and carbohydrate-rich foods during the holiday season, particularly in December. The absence of major seasonal shifts in weather allows for the isolation of behavioral factors. Additionally, there is only one thrombectomycapable center on the island, allowing for analysis of the entire population through a single thrombectomy database. **Methods:** A retrospective analysis of thrombectomy data at Mennonite Hospital in Caguas, Puerto Rico was performed to determine the number of procedures performed during holiday and non-holiday months. Patient information, including age, sex, and comorbidities, was also collected. **Results:** There was a relative increase in thrombectomy cases in December compared with the average monthly rate for the rest of the year. In 2023, the average number of thrombectomies performed during non-holiday months was 9.3, compared with 19 in December, representing a 105% increase. In 2024, the non-holiday average was 9.2, and 12 thrombectomies were performed during December, representing a 30.7% increase. A combined T-test of December and non-holiday months for 2023-2024 showed no statistically significant difference ($p=0.321$), likely due to the limited sample size. **Conclusion:** A general trend of increased thrombectomies was observed in December 2023 and 2024 compared to non-holiday months. Given the absence of significant seasonal changes on the island, the observed trend may be due to behavioral factors, primarily cultural dietary patterns during December, which may increase the risk of cerebrovascular events. Further analysis of comorbid conditions, amongst other demographic characteristics, is needed to better elucidate the definitive cause of the observed trends. However, these findings underscore the potential impact of short-term behavioral patterns in stroke risk and may help better inform prevention strategies and public health interventions, particularly for patients with pre-existing risk factors such as hypertension, dyslipidemias, diabetes, and obesity.

DICER-1 Primary Intracranial Sarcoma in the 3rd Decade of Life

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We report a rare adult case of primary intracranial sarcoma, DICER1-mutant (PIS-DICER1) arising in the right temporal lobe of a 32-year-old man with a history of pediatric Hodgkin lymphoma without prior cranial radiation or temozolomide exposure. He presented with a 6.3-cm complex solid–cystic, contrast-enhancing temporal mass. Resection was followed by early recurrence requiring re-resection via right craniotomy. Histology showed a highly cellular, infiltrating pleomorphic spindle-cell neoplasm arranged in haphazard fascicles with multinucleated giant cells, brisk mitoses, microvascular proliferation, and necrosis. Immunohistochemistry (IHC) demonstrated diffuse vimentin and CD56, patchy p53, SALL4, and desmin, focal CAM5.2, and loss of ATRX nuclear staining; INI1 and BRG1 were retained. A broad IHC panel was negative (GFAP, OLIG2, SOX10, S100, EMA, synaptophysin, chromogranin, neurofilament, cytokeratin, progesterone receptor, INSM1, IDH1 R132H), helping exclude glial, neuronal, melanocytic, and meningeal differentials. Targeted molecular testing identified DICER1 RNase IIIb hotspot mutation (E1813D) with co-mutations in ATRX (R666*) and NF1 (Y489C), supporting the diagnosis of PIS-DICER1; DNA methylation profiling is recommended for orthogonal confirmation when available. Given the tumor's rarity and aggressive features, management centered on maximal safe resection with multidisciplinary deliberation regarding adjuvant therapy, balancing recurrence risk against the patient's prior malignancy history. Genetic counseling and germline testing were advised to evaluate for DICER1 syndrome and to guide family surveillance; systemic imaging was recommended to exclude additional primary lesions or metastasis. This case expands the clinicopathologic spectrum of PIS-DICER1 into adult hemispheric disease with ATRX loss and NF1 co-mutation, underscores the diagnostic value of integrated histology–IHC–genomics, and highlights practical considerations for postoperative surveillance, genetics-informed counseling, and individualized adjuvant planning.

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South Florida Morsani College of Medicine, for their expert guidance and mentorship throughout this project. Their insight into neuropathology and sarcoma diagnostics was invaluable in shaping the direction of this case study. We also thank the University of South Florida Morsani College of Medicine and Ruffalo Hooper & Associates for their institutional support, which was critical to the completion of this work. This research was supported by HCA Healthcare and/or an HCA Healthcare affiliated entity. The views expressed in this presentation represent those of the author and do not necessarily represent the official views of HCA Healthcare or any of its affiliated entities.

Assessing Fetal-Type Circle of Willis in Puerto Rican Cadavers

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Introduction: The Circle of Willis, a network of arteries at the brain's base, plays a crucial role in collateral circulation to the brain, preventing ischemia, transient ischemic attacks, or strokes. Karatas et al. (2015) highlights its importance and the significant anatomical variations it presents. One common variant is the Fetal-Type Posterior Circle of Willis (FTPCW), which is characterized by persistence of the embryonic caudal segment of the internal carotid artery (ICA). This results in carotid-dominant perfusion of the posterior cerebral artery (PCA), even after maturation of the vertebrobasilar circulation of the brain. This study documents the incidence of FTPCW and its subvariants in dissected brains. Methods: Conducted at the San Juan Bautista School of Medicine's Anatomy Lab using Puerto Rican cadavers, the study involved dissecting the posterior circulation to expose the Circle of Willis and identify the PCA. The variations of PCA were noted, following A. Fleur van Raamt's classification. Results: Of the forty-one brains examined, one was excluded due to significant damage. The study found sixteen Fetal-type PCAs, with thirteen being partial subtypes and only one full subtype. Two Fetal-type PCA subtypes were indeterminate. Notably, two brains exhibited bilateral partial Fetal-type PCAs. Conclusion: The study's clinical relevance lies in understanding the role of collateral circulation in ischemic stroke. Previous studies, including Fleur van Raamt et al. (2006), have linked FTPCW to an increased risk of intracranial aneurysms and reduced collateral circulation. This study found a high prevalence of the fetal configuration in the posterior Circle of Willis among the 41 brain specimens, suggesting a potential increase in cerebrovascular accidents in the Puerto Rican population. These findings underscore the need for further investigation into these variations for a broader understanding and increased statistical certainty.

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Advancing Parkinson's Disease Treatment through Noninvasive Precision Therapy: A Review of MRgFUS Subthalamotomy

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Introduction: Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor symptoms such as bradykinesia, rigidity, postural instability and tremor, for which there is no known cure. Despite the symptomatic relief provided by pharmacological therapies, surgical approaches are indicated in cases where medication efficacy diminishes over time. The loss of nigrostriatal dopaminergic neurons produces relative hyperactivity of the subthalamic nucleus (STN), making it a possible target for neurosurgical treatment. Magnetic resonance-guided focused ultrasound (MRgFUS) has emerged as a noninvasive alternative that allows for the ablation of deep-brain structures, including the subthalamic nucleus, without craniotomy and electrode penetration, thereby diminishing incision and hardware-related morbidities commonly associated with open stereotactic procedures. **Methods:** A literary review was conducted to identify current clinical trials, neuroimaging findings, and physiological studies related to MRgFUS in Parkinson's disease, with emphasis on its effects on brain metabolism and structure. The search was performed across PubMed, consisting of three clinical trials published in the *New England Journal of Medicine (NEJM), Npj Parkinson's Disease,* and *Movement Disorders*. Key words utilized in the review included "MRgFUS", "Parkinson's disease", "Subthalamotomy" and "STN". **Results:** Various studies have shown that beyond its ablative role, MRgFUS has also demonstrated the ability to transiently open the blood–brain barrier, allowing the targeted delivery of drugs. Neuroimaging has revealed that it modulates the brain's metabolism, producing local metabolic suppression in the subthalamic nucleus and coordinated changes across motor circuits. Moreover, it induces gray matter volume alterations associated with tremor improvement, suggesting neuroplastic reorganization. Clinical trials have demonstrated significant improvements in motor function following MRgFUS, as evidenced by reductions in MDS-UPDRS scores. In a 2020 randomized controlled trial, the mean MDS-UPDRS III score of the active-treatment group showed a mean improvement of 9.8 points, while the control group showed only a minimal reduction, from 18.7 to 17.1. The group difference of 8.1 points ($p<0.001$) demonstrates that patients who underwent treatment experienced greater motor improvement. **Conclusion:** Nevertheless, current research remains limited by small sample sizes and short follow-up durations. The long-term safety

and potential cognitive effects of MRgFUS have not yet been fully characterized, and most studies have focused primarily on tremor-dominant Parkinson's disease, leaving its applicability to other clinical phenotypes uncertain. Future studies should prioritize large-scale, longitudinal randomized controlled trials that assess outcomes across multiple lesion targets and include diverse patient populations to better establish the efficacy, durability, and broader therapeutic potential of MRgFUS in Parkinson's disease.

Atypical Rolandic Epilepsy with Neurocognitive Regression: A Longitudinal Pediatric Case Report

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Introduction: Atypical Rolandic epilepsy (ARE), also known as epilepsy with continuous spikes and waves during slow sleep (CSWS), represents the severe end of the benign Rolandic epilepsy spectrum. It is characterized by focal motor seizures, sleep-potentiated centrotemporal discharges, and progressive cognitive and behavioral decline. Emerging evidence implicates variants in genes such as CNTNAP2 in modifying disease phenotype and contributing to associated neurodevelopmental disorders. Methods: A retrospective review of clinical records, electroencephalography (EEG), neuroimaging, neuropsychological assessments, genetic studies, and treatment outcomes from 2012–2023 was conducted to characterize seizure evolution, therapeutic response, and long-term neurocognitive outcomes. Results: A female patient presented at age 2 with focal clonic seizures affecting the right face and arm, without loss of awareness. Serial EEGs revealed frequent left centrotemporal spikes evolving to near-continuous discharges during sleep, consistent with ARE. MRI, PET, and SPECT studies were unremarkable. Genetic testing identified a heterozygous CNTNAP2 K1127E variant of uncertain significance. Despite trials of levetiracetam, valproate, oxcarbazepine, topiramate, lamotrigine, clobazam, and corticosteroids, seizures persisted daily, with partial steroid responsiveness. The patient subsequently developed expressive language delay, attention deficits, anxiety, and mild psychomotor impairment. Long-term follow-up demonstrated stable seizure frequency but enduring cognitive and behavioral sequelae requiring educational accommodations, speech and occupational therapy, and psychological support. Conclusions: This 11-year longitudinal case underscores the chronic, multifaceted nature of atypical Rolandic epilepsy and highlights the possible modifying role of CNTNAP2 variants in epileptic and neurodevelopmental outcomes. Early recognition, serial EEG monitoring, and integration of multidisciplinary neuropsychological and behavioral interventions are crucial for optimizing long-term prognosis in children with ARE.

IRB Approval: EMSJBIRB- 14-2025

Recovery-Focused Care for First Episode Psychosis: The PorTi Integrated Treatment Model of the UPR

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Introduction: Early intervention after a first episode of psychosis (FEP) is vital, as timely treatment improves symptoms, functioning, and long-term prognosis. Evidence shows that combining pharmacological and psychosocial strategies such as cognitive-behavioral therapy, family psychoeducation, and vocational support achieves the best outcomes. The Coordinated Specialty Care (CSC) model provides a multidisciplinary, recovery-oriented framework that emphasizes rapid access, small caseloads, shared decision-making, medication management, social skills training, and integration of educational and employment services. This approach prioritizes quality of life, self-efficacy, and social integration beyond symptom remission. **Methods:** Participants are individuals aged 16-35 years old with FEP. Socio-demographic and clinical outcome data was collected from semi-structured interviews and from the medical records. Interventions include pharmacotherapy, psychotherapy, family support, and educational/vocational services. Standardized clinical and functional scales (SCID, BDI, BAI, CRDPSS, C-SSRS, Lehman QOL, MIREC-GAF) are administered alongside metabolic monitoring (BMI, blood pressure, glucose, lipids). Evaluations occur at intake and scheduled follow-ups.Results: Between 2015 and 2025, 104 individuals enrolled. A graduation rate of 65% was recorded; within the non-graduates, 83% were administrative discharge and 17% transferred. Participants were primarily male (64%), unmarried (93%), with a mean age of 23 years. Schizophrenia was the most common diagnosis (38%), followed by schizoaffective disorder, bipolar type (23%), and depressive type (21%). A 2025 follow-up (n=28), 71% were engaged in work, study, or volunteer activities. **Discussion:** Notable achievements include partnerships with health schools, a 24/7 crisis line reducing hospitalizations, and strong family involvement through therapy and advocacy.**Conclusion:** PorTi demonstrates that coordinated specialty care can promote recovery, functional engagement, and quality of life in first-episode psychosis by integrating medical and psychosocial approaches. Early intervention in first-episode psychosis is critical. Vocational and educational outcomes highlight the model's effectiveness.

Psychiatric Symptoms Among Hispanic Adults from Puerto Rico Living With Asthma During the COVID-19 Pandemic

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HSDG= Suman Subject Data Gatherer

Introduction/Objective: The lifetime prevalence of asthma in Puerto Rico (PR) is higher than in any other state or territory of the United States. Adults with asthma (AWA) were at high risk of poor health outcomes during the COVID-19 pandemic, including the emergence or worsening of psychiatric symptoms (PS), compared to adults without chronic diseases (AWOCD). However, few studies have documented the extent of differences in PS among these groups during this public health emergency among Hispanics. We examined the severity and odds for clinical levels of PS in AWA (G1=236) vs. AWOCD (G2=671) who completed an online survey during the outbreak (IRB-approved #1920-194). **Method:** Participants aged 21–78 years (*M*=42.84; 81.26% women) had to be PR residents for ≥3 months before enrollment, have internet access, and understand Spanish. Adults completed a Health/Treatment History Module and questionnaires assessing symptoms of depression, anxiety, posttraumatic stress disorder (PTSD), feelings of loneliness (FOL), fear of COVID-19 infection (FOCI), and death/self-harm thoughts (DSHT). Using ANCOVAs (adjusting for sex, age, and household size), we compared the mean severity of PS between groups. With logistic regression, we examined the crude and adjusted odds ratios of presenting clinical levels of PS when having asthma. Clinical levels of PS were identified using cut-off points that distinguished cases with at least moderate-level symptoms from those with mild or absent symptoms. **Results:** AWA had significantly ($p \leq .001$) higher continuous scores in symptoms of anxiety [$F(1, 900) = 43.49$], depression [$F(1, 900) = 49.04$], PTSD [$F(1, 900) = 58.88$], total FOCI [$F(1, 901) = 20.18$], and FOL [$F(1, 900) = 40.04$] than AWOCD. G1's adjusted odds of presenting clinical levels of PS were 1.53 (Obsessive/Agoraphobic FOCI; $p \leq .01$) to

2.85 (depressive symptoms; $p \leq .001$) times higher than in G2. AWA were 3.13 times as likely as their peers to present DSHT anytime during the pandemic ($p \leq .001$) and 2.86 times as likely to present DHST within the past two weeks ($p \leq .001$). **Conclusion:** As expected, AWA showed a higher severity and increased odds for clinical levels of PS than AWOCD. Public policies must emphasize strategies to address the mental health needs of AWA during health emergencies.

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Clinical Outcomes and Safety Profile of AAV2-Mediated *DDC* Gene Therapy for Pediatric AADC Deficiency

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Introduction: Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare autosomal-recessive neurometabolic disorder caused by biallelic *DDC* mutations, leading to impaired synthesis of dopamine and serotonin. Clinically, it presents with hypotonia, developmental delay, oculogyric crises, and autonomic dysfunction, resulting in severe motor disability and early mortality. Patients with severe presentations of this disorder have a reported mean life expectancy range of 4.6-8 years. Conventional therapies such as dopamine agonists, monoamine oxidase inhibitors, and pyridoxine provide only partial symptomatic relief. Eladocagene exuparvovec (Upstaza®/Kebilidi™) is the first FDA-approved gene therapy targeting this neurotransmitter-synthesis defect and the first AAV-mediated gene therapy used to treat a neurological deficiency. Using an adeno-associated virus serotype 2 (AAV2) vector, it delivers functional DDC cDNA directly to the putamen, restoring AADC activity and enabling endogenous dopamine production. **Objective:** To evaluate the clinical outcomes and safety profile of eladocagene exuparvovec in pediatric AADC deficiency through a comprehensive literature review (2010 - 2024). **Methods:** This was performed across PubMed and ClinicalTrials.gov, consisting of three intraputaminal open-label trials (AADC-1601, AADC-010, AADC-011; n=26) and one midbrain trial (NCT02852213; n = 7). Keywords utilized in the review included “AAV2” “gene therapy”, and “AADC deficiency”. **Results:** Across the intraputaminal program, 26 pediatric patients with severe AADC deficiency received bilateral AAV2-hAADC infusions of ~1.8×10^{11} viral genomes (vg) per putamen. Within 12 months, over 67 % achieved ≥ 1 new motor milestones in AADC-1601; 17% (2/12) walked backwards independently, 25% (3/12) gained head control, and 25% (3/12) learned to sit independently. PDMS-2 scores increased significantly (median +62; $p = 0.005$). Similar results were observed in the midbrain trial, although limited by a smaller cohort (n=7). PET imaging confirmed >50 % increases in putaminal [^{18}F]-DOPA tracer uptake and elevated dopamine metabolites. Oculogyric crises and autonomic instability markedly decreased, l significant quality-of-life gains ($p < 0.001$). No vector-related toxicities occurred; transient dyskinesia (77%) resolved within weeks to months. **Conclusions:** Long-term follow-up (5–10 years) showed sustained motor and biochemical improvements. Collectively, AAV2-mediated DDC gene therapy offers a novel, effective treatment for

AADC deficiency with favorable pediatric safety profiles. Future directions should focus on vector delivery optimization, assessing long-term efficacy and safety profiles, and expanding to related monoamine-based disorders.

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Phenotypic variability of X-linked adrenoleukodystrophy in siblings

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Background*:* Adrenoleukodystrophy (ALD) is a rare X-linked peroxisomal disorder due to a ABCD1 gene mutation resulting in the defective metabolism of very long chain fatty acids (VLCFA), leading to their accumulation in organs like the brain, spinal cord, and adrenal glands. ALD phenotypes include childhood cerebral ALD, associated with rapid demyelination and neurological decline, adrenomyeloneuropathy (AMN), characterized by progressive lower extremity weakness and adrenal insufficiency, and Addison's disease, which causes adrenal dysfunction typically without neurological symptoms. There is no known correlation between genotype and phenotype. ***Case Summary****:* We present two male siblings with hemizygosity for ABCD1 mutation and elevated VLCFA levels who exhibit a significant difference in clinical presentation and progression of the disease. Patient A presented at age 8 with symptoms of encephalopathy, initially misdiagnosed as encephalitis. Brain MRI showed bilateral symmetric white matter hyperintensities and biochemical testing revealed high levels of VLCFAs, confirming cerebral ALD. He was later diagnosed with adrenal insufficiency as well. Despite bone marrow transplant, the patient developed progressive neurodegeneration, spastic quadriparesis, intractable epilepsy, adrenal insufficiency, hearing loss and vision loss. He is currently bedridden with a tracheostomy and gastrostomy. In contrast, Patient B was diagnosed by screening at age 6. Multiple brain MRIs done over the years have shown mild white matter hyperintensities without progression. He did develop adrenal insufficiency during childhood which was treated and then resolved. At 17 years of age, he started to present symptoms of mild leg stiffness, hyperreflexia and difficulty ambulating. Whole spine MRIs and follow up Brain MRI were normal but electrodiagnostic studies confirmed the diagnosis of adrenomyeloneuropathy. Patient is currently only on baclofen to treat spasticity and has remained relatively stable for now. ***Conclusion***: This case report highlights the wide range of phenotypic variability of ALD, even among siblings with identical mutations. While one sibling experienced rapid decline despite treatment, the other has remained stable. These findings emphasize the importance of

early identification and regular monitoring in at-risk individuals. Further research is needed to better study these differences and improve care for patients with ALD.

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Adults With or Without Hypertension Living in Puerto Rico: Psychiatric Symptoms During the COVID-19 Pandemic

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/Objective: Adults with hypertension (AWH) are more likely to have psychiatric symptoms (PS) than adults without chronic diseases (AWOCD). Although the COVID-19 pandemic represented a severely stressful event to the general adult population in Puerto Rico (PR), few studies have documented the extent of differences in PS among these groups during this public health emergency. We examined the rates and severity of PS among AWH (G1=425) vs. AWOCD (G2=671) who completed an online survey during the outbreak (IRB-approved #1920-194). **Method:** Participants aged 21–79 years (*M*=46.06; 77.46% women) had to be PR residents for ≥3 months before enrollment, have internet access, and understand Spanish. Adults completed a Health/Treatment History Module and questionnaires assessing symptoms of depression, anxiety, posttraumatic stress disorder (PTSD), feelings of loneliness (FOL), fear of COVID-19 infection (FOCI), and death/self-harm thoughts (DSHT). Using ANCOVAs (adjusting for age and household size), we compared the mean severity of PS between groups. With logistic regression, we examined the crude and adjusted odds ratios of presenting clinical levels of PS when having hypertension. **Results:** AWH had significantly ($p \leq .001$) higher scores in symptoms of anxiety [$F(1, 1090) = 32.13$] depression [$F(1, 1090) = 53.36$], PTSD [$F(1, 1089) = 52.30$], total FOCI [$F(1, 1088) = 21.96$], and FOL [$F(1, 1089) = 28.77$] than AWOCD. G1's adjusted odds of presenting clinical levels of PS were from 1.53 (Obsessive/Agoraphobic FOCI; $p \leq .01$) to 2.64 (PTSD symptoms; $p \leq .001$) times higher than for G2. AWH were 2.42 times as likely as their peers to present DSHT anytime during the pandemic ($p \leq .001$) and 2.59 times as likely to present DHST within the past two weeks ($p \leq .001$). **Conclusion:** As expected, AWH showed a higher severity and odds for clinical levels of PS than AWOCD. Public policies must emphasize strategies to address the mental health needs of this population during health emergencies.

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Radiologically Isolated Syndrome in a Puerto Rican Female

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INTRODUCTION: Multiple sclerosis (MS) often begins with an asymptomatic phase where lesions are evident on magnetic resonance imaging (MRI) before the appearance of clinical symptoms, a condition known as radiologically isolated syndrome (RIS). This stage offers a window for early intervention; however, the benefits of initiating therapy prior to symptoms onset remain uncertain. **METHODS:** This case study aimed to characterize the early clinical course of a young patient with RIS and evaluated the impact of early immunomodulatory therapy on disease progression. A 24-year-old female with incidentally discovered RIS underwent proactive treatment with fingolimod, a sphingosine-1-phosphate (S1P) receptor modulator. She was monitored over two years with regular neurological examinations, patient-reported symptom tracking, and serial MRI and laboratory assessments to track disease activity and treatment effects. **RESULTS:** Over 24 months of follow-up, the patient remained free of acute MS relapses and showed no new MRI lesions. Neurological disability remained minimal, with only a slight increase over time, despite chronic fatigue, mild cognitive slowing, and intermittent sensory symptoms. **CONCLUSIONS:** Preliminary findings suggest that initiating immunomodulatory therapy at the RIS stage is feasible and may stabilize radiologic and clinical markers of MS and avoid progression or appearance of symptoms, highlighting the need for controlled studies to confirm the long-term benefits of early intervention.

Anatomical Study of the Vertebral Artery Transposition and its Clinical Relevance

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Aim: This study aimed to precisely measure the average length of V segments of the vertebral artery, with a focus on facilitating meticulous preoperative planning for lesions along the foramen magnum anterolateral to the brainstem and the odontoid process of C2. **Methods:** Twelve formalin-fixed specimens injected using the six-vessel technique were immersed in 70% ethanol for optimal tissue fixation. After a 48-hour waiting period for latex setting, specimens were dissected under microscopic magnification, exposing the vertebral artery. Measurements were taken after opening the foramen of C1 and C2. CT images were analyzed using the EPIC hyperspace production software. A detailed illustration of the measurement process and step-by-step dissection and craniotomy procedures were provided. **Results:** The mean percentage of vertebral artery mobilization after opening C1 and C2 foramen was 24.8% and 32.3%, respectively. CT scans from 200 patients provided mean lengths for vertebral artery segments. **Conclusion:** Understanding the patient's unique vertebral artery configuration in comparison to average measurements enhances surgical planning for far lateral approaches. This individualized strategy, mobilizing the vertebral artery instead of navigating around it, improves safety margins and patient outcomes in skull base pathologies. These findings offer valuable insights for optimizing alterations to the far lateral approach, particularly in challenging cases requiring complete exposure.

Pure Intracranial Schwannoma of Cranial Nerve X: Case Presentation and Literature Review

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Introduction: Schwannomas involving the intracranial segment of cranial nerve X (vagus nerve) are exceedingly rare and often present with nonspecific symptoms, making diagnosis and management particularly challenging. These benign tumors may mimic more common cerebellopontine angle masses and require careful radiologic and histologic evaluation. **Methods:** We report a case of a 43-year-old female who presented with two months of worsening left-sided headaches and associated nausea. Imaging with brain MRI revealed a 1.9 × 1.7 × 1.7 cm extra-axial mass in the left lateral cerebellomedullary cistern with mild mass effect on the medulla. **Results:** The patient underwent a left retrosigmoid craniotomy for gross total resection. Histopathologic examination confirmed a WHO Grade I schwannoma with Antoni A and B areas and strong S100 positivity, consistent with a vagus nerve sheath tumor. Preoperative workup also included evaluation for left upper quadrant abdominal pain, revealing a non-obstructing renal stone, but no definitive gastrointestinal pathology. Postoperatively, the patient had no neurologic deficits on exam, though she experienced mild oropharyngeal dysfunction requiring nighttime PEG tube support, which was subsequently removed. **Conclusion:** This case highlights a rare presentation of a primary intracranial vagus nerve schwannoma with isolated headache and vague abdominal symptoms, without lower cranial nerve palsy on exam. The case adds to the limited literature on Type A vagal schwannomas and emphasizes the importance of considering lower cranial nerve schwannomas in the differential diagnosis of cerebellomedullary cistern masses.

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Clinical profile and management of patients who underwent spinal fusion surgery admitted to a Pediatric Intensive Care Unit

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Introduction: Adolescent idiopathic scoliosis (AIS) is a multifactorial disorder involving not only spinal deformity growing evidence but may also suggest significant neurodevelopmental and neurophysiological components. Disrupted communication between the spinal cord, cerebellum, and sensorimotor cortex may contribute to the progression of the spinal curvature. Mechanical deformation of the spine can cause disfunction of spinal cord and cerebrospinal fluid dynamics, leading to asymmetric neural signaling, proprioceptive deficits, and impaired postural control. Surgical management through posterior spinal fusion (PSF) aims to restore spinal alignment but introduces risks of altered neural signaling, respiratory impairment, significant blood loss and central sensitization due to perioperative stress and opioid exposure. Although current evidence highlights certain perioperative factors as potential contributors to postoperative complications, their direct link to extended Pediatric Intensive Care Unit (PICU) stays has yet to be clearly demonstrated. **Methods:** Therefore, this study aimed to determine respiratory complications in pediatric patients with short versus prolonged length of stay (SLOS and LLOS respectively) in PICU monitoring following PSF surgery. Furthermore, it is essential to examine how these complications may be influenced by perioperative factors, particularly the total opioid dosage used for pain control and the extent of intraoperative blood loss. A retrospective study assessed the intraoperative blood loss and of opioids for pain management in PICU after PSF surgery in a tertiary teaching hospital (UPR-RCM IRB approved number: 2407252385). This study included 40 patients aged 10–21 years admitted between November 2018 and December 2023 (mean age 13.4 ± 2.7 years; 30 females, 10 males) with a mean length of stay of 3 ± 1.4 days. Mean hemoglobin was 11.8 ± 0.99 (SLOS 10.09, LLOS 9.86). **Results:** Cumulative opioid doses were higher in LLOS (17.9) versus SLOS (13.2). Hemoglobin showed lower variability (variance = 2.33, SD = 1.53)

compared to opioid cumulative dose (variance = 14.4, SD = 218.7), and correlations with LOS were weak and non-significant (Hb ρ = -0.09, p = 0.79; opioid ρ = -0.14, p = 0.69). Ongoing data collection aims to expand the sample and enable more conclusive analysis. **Conclusion:** This study provides insight into perioperative factors that may increase the risk of prolonged ICU stays in AIS patients, with potential implications for optimizing pain management and improving patient outcomes.

Characterization of Sex Differences in Epilepsy: A Retrospective Study in a Multiethnic Population in Hawaii

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Introduction: Research increasingly recognizes sex-based biological and clinical differences in people with epilepsy (PWE), influencing etiology, comorbidity profiles, and treatment outcomes. However, limited data exist on how these differences manifest within ethnically diverse island populations such as Hawai'i, where genetic, environmental, and social factors may interact uniquely. This study aimed to characterize sex-related differences in seizure etiologies and comorbidities among PWE receiving care at Hawaii Pacific Neuroscience (HPN). **Methods**: A retrospective chart review was conducted on 500 patients diagnosed with epilepsy who were seen at HPN between January 2019 and July 2020. Extracted data included age, sex, seizure type, epilepsy etiology, and comorbidities. Statistical analyses were performed using chi-square tests and one-way ANOVA to identify significant sex-based differences. **Results**: No significant sex differences were observed in age distribution or seizure types. However, notable distinctions emerged in both etiologies and comorbidity patterns. Women exhibited a higher aggregate burden of psychiatric comorbidities ($p < 0.001$), including anxiety ($p = 0.006$), depression ($p = 0.002$), and post-traumatic stress disorder ($p = 0.047$). The total burden of neurological comorbidities was also higher among women ($p = 0.031$), with particularly increased rates of migraines ($p < 0.001$) and sleep disorders ($p = 0.043$). In contrast, structural etiologies attributable to traumatic brain injury (TBI) were more common in men ($p = 0.006$), while malformations of cortical development (MCD) were more prevalent among women ($p = 0.031$). **Conclusion**: Findings indicate distinct sex based patterns in epilepsy etiology and comorbidity profiles within Hawai'i's multiethnic population. The higher prevalence of TBI-related epilepsy in men and the elevated psychiatric and neurological comorbidity burden among women suggest potential differences in disease mechanisms and lived experiences. These results highlight the importance of incorporating sex informed approaches in both clinical care and research to enhance diagnostic precision, treatment personalization, and quality of life for individuals with epilepsy. Future studies integrating genetic, hormonal, and sociodemographic factors are warranted to elucidate the underlying mechanisms of these observed disparities.

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Post Viral Respiratory Infections Syncope: An Uncommon Presentation of Chiari I Malformation and Atlanto-Occipital Assimilation

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Introduction: Cough syncope as a single symptom on a patient is an important indicator for a physician to study the craniocervical junction due to the probability of Chiari 1 Malformation as the cause and the progressive nature of this condition. Our case is added to the literature as the 3rd example of such a situation. If left undiagnosed and untreated, patients can develop severe quadriparesis impacting their quality of life greatly. Symptoms and progression can be corrected with prompt decompressive surgery. **Method**: We report a 37 y/o male patient who developed multiple cough-associated syncope after Covid-19 infection. The patient suffered an acute stroke like episode for which he sought medical attention in the emergency department. Workup at the time showed no ischemia, active bleeding or identifiable cause. This patient had further acute progressive neurological symptoms including dizziness, blurred vision, somnolence, face weakness, dysarthria, dysphagia, broad based gait, generalized tiredness and left hemiparesis with worsening to quadriparesis (weakness and hyperreflexia of extremities). MRI of the craniocervical junction was obtained and the diagnosis of a Chiari 1 malformation with displacement of tonsils 7.5 mm below the foramen magnum was made, patient was promptly scheduled and underwent decompressive surgery. **Results**: Follow-up 6 months later showed a marked increase in strength in all extremities and improved mobility as well as quality of life. The presence of cough syncope in patients should prompt physicians to add Chiari 1 malformation high on the list of their differential diagnosis and strive to obtain image the craniocervical junction and cervical spine for its diagnosis. **Conclusion**: Early recognition and expedited treatment prevent progression of neurological symptoms that severely impact a patient's quality of life and if severe enough may put their life at risk.

Establishing a Novel Standardized Approach for Lumbar Puncture in Cynomolgus Macaques: Interdisciplinary Collaboration and Outcomes

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Introduction: Cerebrospinal fluid (CSF) collection is a critical procedure for translational research. Previously, no standardized protocol existed for lumbar punctures (LP) in nonhuman primates (NHP). Failures in this procedure may lead to permanent disabling neurological aftereffects and further complications, including death. These often occur due to poor patient/subject positioning and inaccurate anatomical landmarks. This study presents a model for LP procedures in NHPs for potential use in intracranial pressure (ICP) measurement and modulation, leveraging an interdisciplinary approach to optimize fluoroscopic LP success. **Methods:** Forty-seven cynomolgus macaques from the Caribbean Primate Research Center underwent LP using two positioning strategies, fetal and neutral, guided by iliac crest landmarks under fluoroscopy, as approved by an IRB. Procedural success, number of attempts, opening pressure, sex, age, and corral of origin were recorded and statistically analyzed using chi-square and t-tests. **Results:** Overall LP success was 97.8% (46/47), with first-attempt success in 81% (38/47) of cases. The mean opening pressure was 6.36 ± 2.07 cm H_2O (n = 46). No statistically significant differences were observed in ICP or procedural success across sex (p = 0.67), age (p = 0.42), or corral origin (p = 0.59). Neutral positioning improved vertebral targeting and procedural ease. The single LP failure occurred due to an intraoperative death. **Conclusions:** Compared with prior studies reporting low LP success rates in NHPs, our approach yielded high LP success rates, particularly when fluoroscopy was used as an adjunct to anatomical landmarks. These results enable future ICP modulation studies utilizing NHP models. This study is limited by its relatively small sample size (n = 47) and the use of a single NHP species, cynomolgus macaques. The positioning technique was not randomized, and LP success may be biased by fluoroscopic availability and operator experience. Future directions include: implantation of LP shunt systems to chronically lower ICP in NHPs, multidisciplinary collaborations with ophthalmology to assess optic nerve disc morphology and visual outcomes, and expansion to other NHP species to increase translational relevance.

Influence of Alcohol and Western Diet on Cerebral Perfusion and Gut Microbiome Diversity in Alzheimer's Disease Risk

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Introduction: Alzheimer's disease (AD), a type of dementia, is characterized by amyloid-beta (Aβ) accumulation, tau pathology, progressive cognitive decline and often comorbid cerebrovascular disease. Modifiable lifestyle factors, particularly diet and alcohol consumption, have been implicated in dementia risk through their effects on cerebrovascular integrity and cerebral perfusion. Gut microbiome diversity influences brain health and may affect cerebral blood flow (CBF) through gut-brain axis pathways. Multi-post labeling delay arterial spin labeling (MD ASL) MRI provides a noninvasive measure of cerebral blood flow (CBF) corrected for arterial arrival time, enabling early detection of neurovascular dysfunction. This study examined associations between modifiable lifestyle factors including western dietary patterns and alcohol intake in cortical CBF and gut microbiome diversity from MD ASL in cohorts at risk for dementia due to AD. **Methods:** Data were collected from middle-aged to older adults at increased risk for AD (full cohort: n=122, mean age = 68 years), of whom 68% were female. One-third (34%) were *APOE* ε4 carriers, a known genetic risk factor for Alzheimer's disease. Participants were drawn from the Wisconsin Registry for Alzheimer's Prevention (WRAP) and the Wisconsin Alzheimer's Disease Research Center (ADRC). Western diet scores were derived via principal component analysis of food frequency questionnaires (FFQ); alcohol intake was quantified as average daily units; cortical CBF was derived from MD ASL MRI scans. **Results:** Adjusted linear regression models revealed a positive association between alcohol intake and gray matter CBF (** $P <$ 0.014) in the frontal and temporal lobes, primarily driven by high-intake outliers (>5 drink/day), while Western diet showed no significant effect. Women showed higher CBF compared to men. Vascular and metabolic covariates did not have a significant effect on perfusion. In a secondary aim, gut microbiome diversity metrics were analyzed in relation to CBF. Faith's Phylogenetic Diversity (PD), an alpha diversity metric reflecting within-individual evolutionary microbial richness, was positively

associated with CBF. Beta diversity analyses using Bray-Curtis dissimilarity index revealed significant sex-dependent differences in gut microbial community; however, this metric was not associated with CBF. **Conclusion:** While Western diet was not associated with perfusion, we observed that phylogenetic richness of gut microbiota may influence cerebrovascular function and brain blood flow. The results of the alcohol consumption analysis were limited given the few participants had high intake but suggested that alcohol may also impact cerebral perfusion. These results point toward potential pathways through which modifiable factors could impact dementia risk via cerebrovascular mechanisms.

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Neuroendovascular Outcomes in a Newly Established Comprehensive Stroke Center: Feasibility in a Resource-Limited Setting

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Introduction: Achieving Comprehensive Stroke Center (CSC) certification requires robust infrastructure, multidisciplinary expertise, and continuous performance improvement, criteria that pose substantial challenges in resource-limited regions. The establishment of a Stroke Center (SC) in Caguas, Puerto Rico (PR) was burdened by substantial costs, funding disparities, insurance bureaucracy, and geographic isolation. Providing the services of an SC in this context is further challenged by PR's large population and the ~2,400 Large Vessel Occlusions that occur annually on the island. Despite these barriers, the SC was awarded CSC certification in September 2025, the first on the island. The evolution of our center and its neuroendovascular outcomes are highlighted, demonstrating clinical efficacy and the feasibility of developing highlevel stroke care in an underserved, resource-limited setting. Methods: We performed a retrospective review of patients who underwent Mechanical Thrombectomy (MT) between October 2022 and August 2025. Data on demographics, interventions, and outcomes were collected. Procedural success was assessed using the Thrombolysis in Cerebral Infarction (TICI) grading system, and clinical outcome using the modified Rankin Scale (mRS) and NIH Stoke Scale (NIHSS). Results: 352 patients underwent MT from October 2022 to August 2025, 53% female (n=186) and 47% male (n=166). Mean patient age was 68 years. Average NIHSS was 16.3 at presentation, 14.9 within 24 hours, and 13.7 at discharge. TICI score distributions were: TICI 0(1.4%), TICI 1(0.5%), TICI 2a(9.8%), TICI 2b(21.6%), and TICI 3(69.9%). In-hospital mortality was 26%, with an additional 6.5% mortality at 90 days. mRS ≤2 was observed in 50% of patients at 90 days. Conclusion: This retrospective study demonstrates that the rapid establishment of a CSC in PR and similar settings is feasible, despite major obstacles. Our results are consistent with reported trials, despite multi-factorial delays in treatment. Maintaining initiatives such as protocol optimization and public outreach will further strengthen stroke care across the island. The achievement of CSC certification serves as a model for improving stroke care in underserved and geographically isolated settings.

Parental Discomfort When Facing Diagnosis and Treatment of Type 1 Diabetes Among Hispanic Adolescents

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Introduction/Objective: Most caregivers of children with type 1 diabetes (T1D) suffer intense discomfort at diagnosis and when facing its treatment complexities. However, the content of such discomfort has not been explored in depth among Hispanic caregivers. We examined the specific content of caregivers' discomfort/annoyance about T1D in their offspring and the relationship between the most common annoyance sources and diabetes-related variables. **Method:** Participants were 65 Hispanic caregivers (81.54% women) aged 32–58 (*M*=43.34) who completed eligibility assessments for an adolescent depression treatment study (IRB-approved). Caregivers answered an open-ended question about things that had bothered/annoyed them most about T1D in their children (aged 12-17 years) since diagnosis. We identified the main themes of parental annoyance and coded responses using categories defined based on their content. We used Chi-square tests, Welch ANOVA ($p \leq .05$), and Pearson correlation (one-tailed) to assess associations between reports of most common themes and diabetes-related criteria. **Results:** Caregivers' responses produced 83 codable units. Agreement between two coders was excellent (κ=.97). Sources of parental discomfort and their occurrence were: "Social or Structural Barriers" (SSB; 35.23%), "Self-Care Issues" (21.69%), "Impact on the Family" (IOF; 22.89%), "Youth's Cognitive/Emotional Aspects" (8.43%), and "No Discomfort/Recall of Discomfort" (14.46%). The proportion of caregivers' responses coded within these categories was 33.85%, 27.69%, 27.69%, 10.77%, and 18.46%, respectively. Compared to their counterparts, a higher proportion of caregivers annoyed by SSB had children who were non-compliant with insulin care (previous 3 months). Caregivers bothered with diabetes IOF had offspring with significantly lower scores on family support with insulin care. Coding within this category was associated with more severe adolescents' worries about T1D. **Conclusion:** Our findings suggest that parental interventions should include strategies to

address caregivers' most common sources of annoyance about T1D in their offspring, and examine the relationship between these sources, diabetes self-care, and diabetes-related family support.

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The New and evolving field of Neuroplastic Surgery: Integrating Reconstruction, Regeneration, and Implantable Neurotechnology

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Introduction: Neuroplastic surgery is an emerging interdisciplinary field that bridges plastic and neurosurgery to restore both form and function in patients undergoing central or peripheral nervous system procedures. Its goal is to ensure that neurosurgical patients achieve anatomical and aesthetic restoration while minimizing complications and the need for secondary operations. **Methods:** Relevant Neuroplastic Surgery literature was reviewed and included in this summary of neuroplastic surgery techniques. A retrospective review of 450 consecutive cranial reconstructions (2012–2017, IRB approved) was conducted, identifying adult patients who underwent implant-based cranioplasty with concomitant scalp and/or dural augmentation using autologous rectus fascia grafts (ARFGs). Data on outcomes, complications, and long-term follow-up were analyzed. **Results:** Twelve patients (average follow-up 10 months) received ARFGs for scalp augmentation with concurrent cranioplasty, all of whom had undergone multiple prior cranial operations and/or radiation therapy. Two major complications (17%) were related to recurrent intracranial infections, with no cases of scalp breakdown or implant extrusion. In a subset of six patients requiring dural reconstruction with ARFGs for active CSF leaks and concurrent cranioplasty, all demonstrated 100% leak closure with no recurrent CSF complications. Additional literature on novel neuroplastic techniques were reviewed and showed benefit. **Conclusion:** Neuroplastic surgery represents a rapidly advancing discipline dedicated to optimizing functional and aesthetic outcomes in neurosurgical patients requiring reconstruction. The integration of neuroplastic techniques into neurosurgical practice has been shown to enhance patient recovery, reduce complications, and improve overall quality of life following cranial and spinal procedures.

Stem Cell-Derived Neuron Replacement in Parkinson's Disease

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Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons, resulting in motor symptoms such as tremors, as well as mobility and balance impairment. To reduce these symptoms, numerous treatments have been explored, including clinical trials assessing novel therapeutic approaches. Stem cell-replacement therapy has emerged as an alternative to create safe and effective therapies that could alleviate PD symptoms. Induced pluripotent stem (iPS) and human embryonic stem (hES) cells have been evaluated due to their unique ability to differentiate into any cell type, offering a potential source for generating dopaminergic neurons lost in PD. Methods: A literature review was conducted to identify current clinical trials on stem cell-replacement therapy for PD, emphasizing on its effects on motor symptoms. In total, two clinical trials were included based on studies published in *Nature.* Key words used to carry out the search were "Parkinson's disease", "cell replacement", "hES cells", and "iPS cells". Recent findings indicate that stem cell-derived dopaminergic neuron transplantation can lead to measurable improvements in motor function. A phase I clinical trial tested bemdaneprocel, a hES cell-derived dopaminergic neuron progenitor, in patients with PD. Bilateral putaminal transplantation of bemdaneprocel was well tolerated over 18 months and one year of immunosuppression, with no reports of graft-induced dyskinesias. Participants, especially those who received a higher dose, demonstrated clinically meaningful improvements as evidenced by reductions in the MDS-UPDRS scores. The higher-dose treatment group showed a mean improvement of 23 points, while the low-dose group showed only a 8.6 point reduction. ^{18}F-DOPA PET imaging confirmed graft survival and dopaminergic activity. The second trial evaluated bilateral transplantation of dopaminergic progenitors derived from iPS cells that resulted in graft survival, dopamine production, and no tumor formation. Results: Four out of the six participants showed improvements in both the MDS-UPDRS part III OFF score (9.5 points) and five on the ON score (4.3 points) at 24 months after transplantation, with no serious adverse effects being reported. A significant increase in the ^{18}F-DOPA influx rate constant values (44.7%) within the putamen

was identified, indicating restoration of dopamine synthesis. Conclusion: Together, these findings highlight stem cell-derived dopaminergic neuron replacement as a promising regenerative approach for PD. Future research should focus on optimizing stem cell differentiation and delivery techniques to enhance dopaminergic neuron function. Moreover, additional randomized controlled trials will be essential to confirm long-term efficacy and safety of stem cell therapy.

Comparative Clinical Course and Relapse Severity in Two Seropositive Neuromyelitis Optica Spectrum Disorder (NMOSD) Cases

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Introduction. Neuromyelitis Optica Spectrum Disorder (NMOSD) is an uncommon yet impactful inflammatory and demyelinating disorder of the central nervous system in which relapse severity has a great impact on long-term disability. NMOSD primarily targets the optic nerves, brainstem, and spinal cord, presenting unique challenges for diagnosis and management. Despite similar serologic profiles, patients demonstrate wide variability in clinical course and treatment response. **Methods.** A retrospective analysis of two AQP4-IgG–positive NMOSD cases with markedly different clinical courses was conducted to illustrate the impact of disease duration, relapse severity, and treatment adherence on functional outcomes. **Results.** Comparative assessment demonstrated that prolonged disease duration and inconsistent adherence to immunotherapy correlated with higher relapse frequency and greater disability, whereas early and continuous treatment was associated with clinical stability and prevention of disease progression. **Conclusion.** These findings highlight the importance of early diagnosis and sustained immunotherapy adherence for relapse prevention and to preserve neurological function in NMOSD.

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Intraoperative 0.05% chlorhexidine gluconate lavage for the prevention of surgical site infections in lumbar instrumented fusions and ancillary machine learning identification of associated risk factors

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Introduction: Spinal surgical site infections (SSIs) remain a significant complication following spine surgery. Recent interest in surgical site wound decontamination, particularly in the context of SSI and reoperation prevention, has prompted research efforts. The objective of this work is to compare one commonly used intraoperative irrigation method, normal saline (NSS), with intraoperative 0.05% chlorhexidine (CHG) irrigation. As a secondary objective, we wanted to utilize a machine learning model to evaluate driving factors for early postoperative infection. Methods: We performed a retrospective cohort review of patients who underwent lumbar spine instrumentation from 2018-2024. We included patients that were irrigated intraoperatively with NSS (Group A) and sterile CHG (Group B). All patients were evaluated at the clinics at 2, 4, and 12 weeks postoperatively. SSIs, surgical characteristics, fusion at one year, and the need for reoperation were also reported. Additionally, we implemented a supervised machine learning workflow to explore multivariable patterns associated with early postoperative infection. Results: For our cohort, mean age for Group A (n=228) was 63.0 ± 10.6 years and in Group B (n=220), 61.2 ± 12.4 years. SSI at 4 weeks post-op was reported in 16 patients (7.0%) in Group A and in 3 patients (1.4%) in Group B (p-value= 0.006). During the study period, 10 patients had to be reoperated due to persistent infection: 9 patients (4%) in Group A and 1 patient in Group B (p-value= 0.02). At 1-year post-op, spinal fusion was achieved in 91.2% of patients in Group A and in 93.2% of patients in Group B. Upon machine learning analysis, perioperative patient factors (age, comorbidities) primarily drove discrimination. Conclusion: The primary findings of this study suggest that intraoperative lavage using CHG is associated to decreased incidence of SSI following lumbar spine instrumentation when compared to NSS and, more importantly, reduced incidence of reoperation. Additionally, the machine learning model determined multiple contributors to SSI development at 4 weeks postoperatively.

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Profile of Sedation for Brain MRI in Pediatric Patients

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Introduction: for pediatric patients requiring sedation for brain MRI, and other non-invasive procedures, it is essential to evaluate safety and efficacy to achieve optimal imaging conditions and ensure adequate recovery (American Academy of Pediatrics, 2016). This study aimed to determine the prevalence, age distribution, and primary diagnoses of patients undergoing brain MRI under sedation; identify the sedative agents used and their combinations associated with shorter recovery times; assess the incidence of adverse effects; and describe current sedation practices within our institution. **Methods:** Pediatric patients undergoing brain MRI and other non-invasive procedures at the imaging center between June and October 2024 were evaluated. Patients who required opioids were excluded. The use of dexmedetomidine (Dex) alone or in combination with other sedatives was analyzed. A Mann–Whitney U test was applied, with a pvalue < .05 considered statistically significant. IRB approval was obtained (Protocol #2504387985). **Results:** A total of 120 patients were included. MRI was the most common study (97.5%), specifically brain MRI (83.8%). Average age was 46 ± 42 months. Frequent diagnoses were seizures, congenital brain malformations, and autism spectrum disorder. Dex (61.7%) was most used, followed by Dex with ketamine or midazolam (34.2%). Sedation time was similar for both groups (median 49 vs. 47 minutes, p = .61). No differences were found in study time, and no adverse events were reported. **Conclusion:** Optimizing pediatric sedation for brain MRI depends on factors such as age, diagnosis, and individual response to sedative agents. No significant differences were observed between the use of dexmedetomidine alone or in combination with other agents. Younger and developmentally delayed patients were more likely to require sedation to complete the study successfully. Given that brain MRI is one of the most common noninvasive imaging procedures in pediatrics, these findings highlight the importance of standardized sedation protocols to

ensure safety, consistency, and efficiency across imaging centers. Individualized approaches, careful monitoring, and structured post-procedure care may further enhance patient outcomes and overall safety.

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Where the Dura Gives Way, Acetazolamide Steps In: Revisiting a Classic Drug for a Modern Problem

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Introduction: Cerebrospinal fluid (CSF) leak is a common risk of spinal surgery. Our study investigated the effectiveness of acetazolamide (ACZ) in treating intraoperative durotomies. **Methods:** We retrospectively reviewed all spinal surgeries performed at our tertiary care center from 2023- 2024 (N=955). Of these, we identified patients who developed an intraoperative CSF leak (N=94) and compared outcomes of patients who were treated postoperatively with ACZ 250 mg TID (N=17) to those who were not (N=77). Endpoints included wound-related complications, postoperative length of stay, readmission and reoperation rates, and flat bedrest requirements. **Results:** Wound dehiscence was observed in 0% of ACZ-treated patients vs 3.8% of non-ACZ-treated patients (p=0.411). The readmission rate was 0% for ACZ-treated patients vs 2.5% for the non ACZ-treated group (p=0.505); the reoperation rate was 0% vs 1.3% (p=0.625), respectively. 6% of the ACZ-treated group was placed on flat bedrest postoperatively vs 20.3% of the control group (p=0.154). Among patients who did not undergo primary durotomy repair, those treated with ACZ spent an average of 4 fewer days in the hospital postoperatively compared to patients not treated with ACZ (8.11 vs 12.03 days, respectively; p=0.149). **Conclusions:** Among patients presenting with CSF leaks following spine surgery, those treated with ACZ experienced a lower rate of wound-related complications, flat bedrest placement, readmission, and reoperation, as well as shorter postoperative LOS. The latter was especially true for patients in which a primary durotomy repair was not achievable. Even though these findings were not statistically significant due to small sample size, our study shows that ACZ could serve as an effective adjunct in the treatment of intraoperative CSF leaks, particularly in patients with complicated durotomy not amenable to primary repair.

Comparative Clinical Features of TBCK Syndrome in Two Puerto Rican Siblings: A Case-Based Analysis of Disease Progression

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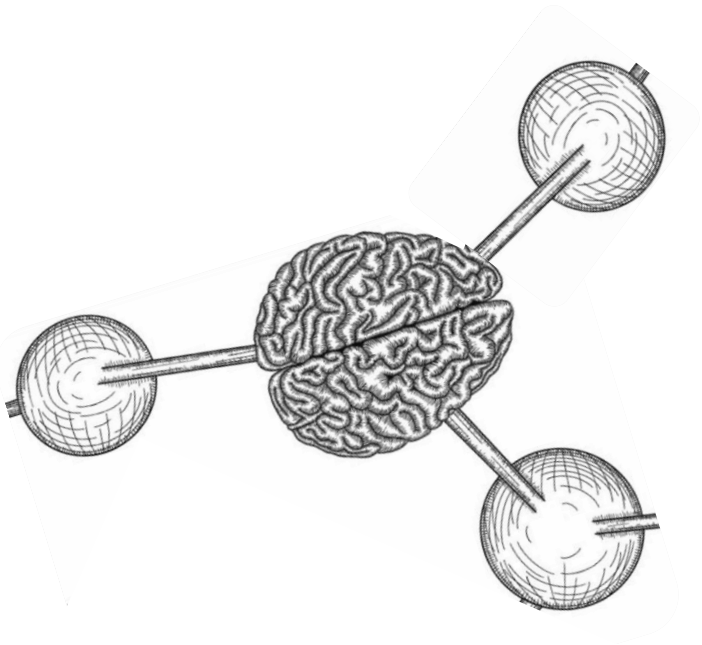
Introduction: TBC1 domain-containing kinase (TBCK) syndrome is a rare autosomal recessive neurodevelopmental disorder characterized by global developmental delay, severe hypotonia, epilepsy, and multisystem involvement affecting the respiratory, gastrointestinal, endocrine, and musculoskeletal systems. It results from biallelic pathogenic variants in the TBCK gene (OMIM: 616900), which encodes a Rab GTPase-activating protein involved in mTOR signaling and autophagy regulation. A homozygous founder mutation has been identified in individuals of Puerto Rican descent, underscoring the need for local diagnostic awareness. **Methods:** A retrospective case-based analysis was conducted on two Puerto Rican male siblings with genetically confirmed TBCK syndrome. Clinical data from medical records (2014–2025), including neurological, genetic, and systemic evaluations, were reviewed to compare disease onset, progression, and multisystemic involvement. **Results:** Both siblings presented in infancy with severe hypotonia, global developmental delay, and early-onset epilepsy. The older sibling, evaluated at 20 months, experienced profound hypotonia, intractable multifocal seizures, scoliosis (35°), cerebral salt-wasting syndrome, hypothyroidism, and progressive respiratory decline requiring CPAP support. Despite multidrug antiepileptic therapy, seizures remained refractory, and his condition led to recurrent hospitalizations and developmental regression. The younger sibling, evaluated at 4 months, exhibited early seizures, truncal hypotonia, and a congenital pulmonary arteriovenous malformation. Although he initially demonstrated developmental gains (crawling, babbling, two-word vocabulary), his condition later progressed to include scoliosis, hip subluxation, hypothyroidism, and sleep-related hypoventilation requiring oxygen therapy. He also developed complications such as MRSA pneumonia, tracheostomy, and gastrostomy for dysphagia. **Conclusion:** This comparative analysis illustrates the phenotypic variability and progressive multisystemic burden of TBCK syndrome, even among siblings sharing identical pathogenic variants. The older sibling showed more rapid neurological and musculoskeletal deterioration, while the younger exhibited slower decline with prolonged medical stability. These findings emphasize the unpredictable genotype–phenotype correlation, the importance of early genetic testing in infants with syndromic hypotonia and epilepsy, and the value of multidisciplinary management to address neurological, respiratory, endocrine, and gastrointestinal complications. Greater awareness of TBCK syndrome and its Puerto Rican founder variant may facilitate earlier diagnosis, comprehensive care, and improved family counseling in affected populations.

IRB Approval: EMSJBIRB- 21-2025



Category: Molecular Neuroscience

Lysosomal Characterization of Cathepsin B in HEK293 Lines

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Introduction: Impairment of protein degradation and turnover pathways through lysosomal dysfunction is believed to drive aberrant alpha-synuclein (αSyn) accumulation and Parkinson's disease (PD) pathology. Cathepsin B (CTSB) is a lysosomal cysteine protease shown to cleave αSyn in vitro at multiple sites, and has recently been nominated as a genetic risk factor for PD. Mutations in *GBA1*, a gene linked to lysosomal storage disorders, likewise increase PD risk. Additionally, CTSB has been identified as a modifier of disease penetrance in *GBA1*-associated PD, suggesting possible synergy between these genes. **Methods:** This study aims to characterize lysosomal function in *CTSB*- or *GBA1*-deficient HEK293 cell lines using high-content confocal imaging. We hypothesized that CTSB and *GBA1* deficiencies affect lysosomal biology in distinct but overlapping ways. To test this, we used wild-type (WT) and *GBA1* heterozygous-null lines that were then rendered *CTSB* knockout (KO) via CRISPR/Cas9 gene editing, or *CTSB* knockdown (KD) via shRNA-mediated gene-silencing. Lysosomal number and size were assessed using Lamp1-eGFP overexpression, LysoTracker dye, and immunocytochemistry (ICC) for endogenous Lamp1 and Lamp2. Lysosomal pH (LysoSensor) and protease activity (DQ-BSA) were also evaluated. **Results:** *CTSB* KO cells showed a significant increase in lysosomal counts by LysoTracker and a similar trend with Lamp1/Lamp2 ICC. Consistently, CTSB KO/*GBA1* het cells had higher lysosomal counts via Lamp1-eGFP and ICC. Notably, lysosomes were significantly smaller in *CTSB* KO/*GBA1* heterozygous double-mutant cells, reflecting a novel form of lysosomal impairment. Additional lab data showed that *CTSB* KO alters protein levels of other cathepsins and autophagic flux. Unbiased proteomic analysis revealed upregulation of factors involved in lysosomal acidification and membrane integrity, highlighting potential compensatory mechanisms. **Conclusion:** Together, our findings suggest that *CTSB* and *GBA1* deficiencies synergistically disrupt lysosomal homeostasis, implicating this pathway in the cellular dysfunction observed in PD.

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Poly-UG RNA Quadruplexes as Structural Scaffolds Linking Stress Granule Dynamics to TDP-43 Pathology in ALS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the progressive loss of motor neurons. Although more than 40 genes have been associated with ALS, over 90% of cases are sporadic and lack identifiable familial mutations. Regardless of etiology, the mislocalization and aggregation of the RNA-binding protein TDP-43 represents a central neuropathological hallmark in nearly all ALS subtypes. Normally nuclear, TDP-43 becomes depleted from the nucleus and accumulates in cytoplasmic inclusions, where it is often associated with stress granules (SGs). SGs are transient, membrane-less ribonucleoprotein condensates formed during cellular stress. Under conditions of chronic or unresolved stress, SGs can lose their dynamic, reversible nature, providing a nucleating environment for pathological protein aggregation, including TDP-43. Emerging evidence suggests that RNA secondary structures, particularly RNA G-quadruplexes (rG4s), contribute to the assembly and stability of SGs by modulating RNA–protein partitioning. Recently, a non-canonical poly-UG (pUG) RNA quadruplex was discovered in C. elegans, formed on long UG tracts that fold into a stable quadruplex configuration. Building on this, our preliminary data indicate that human UG12 tracts can similarly form pUG-like structures in vitro, and independent studies have shown that UG-rich motifs are among the most enriched sequences in SG-associated transcriptomes. Notably, TDP-43 displays high binding affinity for UG repeats of comparable length, suggesting a direct intersection between RNA structural elements and protein recognition. **Methods:** We applied DMS-BASH-MaPseq to probe pUG quadruplex formation in vitro and conducted a bioinformatic analysis of SG-enriched transcriptomes to identify UG repeat enrichment patterns. **Results:** DMS-BASH-MaPseq revealed that UG12 RNA forms a potassium-dependent quadruplex structure, displaying guanine protection signatures consistent with quadruplex folding. Bioinformatic analyses confirmed that UG12 is the most abundant di-nucleotide repeat among SG-enriched RNAs, supporting the idea that pUG structures are recurrent RNA features in granule biology. **Conclusion**: These findings suggest that pUG RNA quadruplexes may act as structural RNA scaffolds that both nucleate stress granules and recruit TDP-43, linking RNA folding to protein aggregation in ALS. Future work will test this model using

Electrophoretic Mobility Shift Assays (EMSA) to characterize pUG/TDP-43 interactions and circular dichroism (CD) spectroscopy to confirm quadruplex folding within TDP-43-binding RNAs. Collectively, this study introduces pUG RNA structures as potential drivers of SG formation and TDP-43 aggregation, providing new mechanistic insights and therapeutic entry points for ALS.

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Reward-Associated Cues Reduce H-Current Amplitude in Midbrain Dopamine Neurons

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INTRODUCTION: Cocaine is a psychoactive substance that targets brain regions involved in motivation and reward learning. Lateral ventral tegmental area (VTA) dopamine (DA) neurons play a critical role in these processes, yet their intrinsic modulations during drug and non-drug self-administration or reward-associated cue learning remain poorly understood. **METHODS:** This study employed an Intermittent Access (IntA) cocaine model, both contingent and non-contingent, to examine how reward delivery and associated cues modulate the hyperpolarization-activated cyclic nucleotide-gated (HCN) current (I_h), an intrinsic property regulating neuronal physiology. Male rats were divided into saline and cocaine IntA groups, with yoked controls receiving non-contingent cocaine infusions either paired (Yoked + cue) or unpaired (Yoked - cue) with a light cue to control for contingency and cue exposure. A parallel sucrose self-administration cohort served as a non-addictive reward control. **RESULTS**: Whole-cell patch-clamp recordings in lateral VTA DA neurons revealed reduced I_h amplitude in the cocaine IntA and Yoked-cocaine + cue groups, accompanied by a hyperpolarizing voltage shift in all cocaine-treated animals. Cocaine IntA enhanced input integration, whereas IntA animals also exhibited reduced membrane capacitance (C_m). Similar I_h reductions were observed in sucrose IntA and Yoked-sucrose + cue groups. **CONCLUSION:** These learning-associated changes may enhance DA neurons' ability to signal reward anticipation or saliency. We propose that I_h modulation in VTA DA neurons maintains intrinsic excitability, improves signal-to-noise ratio, and facilitates learning of reward-salient cues—processes essential for motivation toward drug and non-drug rewards. This hypothesis provides insight into how intrinsic plasticity in VTA DA neurons shapes reward learning.

Sex-Dependent Behavioral and Molecular Effects of Prenatal Stress in Mice

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Abstract

Introduction: Prenatal stress (PNS) has been associated with long-term neurobiological alterations that impact emotional regulation, motivation, and cognitive function. Approximately 40% of pregnant women experience significant stress during gestation, potentially disrupting fetal brain development through neuroinflammatory and oxidative stress mechanisms. This study aims to evaluate the behavioral and molecular consequences of PNS in offspring of those mothers exposed to PNS. We hypothetize that PNS exposure promotes changes at the behavioral level and at the molecular level, specifically in protein expression related to neuronal outgrowth, neuroinflammation and the Reactive Oxygen Species Defense Mechanism.

Methods: Pregnant dams underwent a two-week restraint stress protocol during late gestation and were divided into stressed (PNS) and non-stressed (control) groups. Adult offspring were evaluated through behavioral tests, including the open field, elevated plus maze, social interaction, sucrose preference, and platform-mediated avoidance tasks. Brain homogenates from the prefrontal cortex, amygdala, and ventral hippocampus were analyzed via Western blot to quantify expression of GAP-43, GFAP, Catalase, and Glutathione Peroxidase.

Results: Behavioral analyses revealed sex-dependent differences beteween groups. PNS-exposed males displayed reduced social interaction, with their mothers and opposite-sex individuals. Females on the experimental group (PNS- exposed) showed a significant reduction in locomotion when compared to controls.

Molecular analyses revealed no significant changes in GAP-43, GFAP, or Glutathione Peroxidase expression between groups (i.e Non PNS-exposed vs PNS-exposed) or between gender. However,

Catalase expression was significantly decreased in the prefrontal cortex of the PNS exposed females, suggesting increased vulnerability to buildup of oxidative stress in this region.

Conclusion: Therefore, our hypothesis is accepted, PNS exposure promotes changes at the behavioral level and at the molecular level. Furthermore, according to our results these changes are gender specific. Our results suggest that males from mothers exposed to PNS are prone to develop anti-social behavior towards animals from the opposite sex. On the other hand, females, from PNS mothers tended to exhibit greater anxiety or nervous behavior and to be more vulnerable than males to Reactive Oxygen Species Buildup.

Further studies are needed to elucidate potential molecular changes in other brain regions involved in decision-making, fear, and memory, such as the midbrain and dorsal hippocampus.

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Characterizing the IGF-Acid Labile Subunit During Intestinal Regeneration using *Holothuria glaberrima*

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Introduction: The Insulin-like Growth Factor Binding Protein Acid-Labile Subunit (IGF-ALS) is an integral component of the IGF system, which consists of IGF itself, a family of IGF-binding proteins, and the IGF-ALS. The IGF-ALS is a binding protein that stabilizes the IGF complex and is the focus of this study. This system is known for its role in promoting growth, development, and regulating cellular processes. The IGF-Acid labile subunit modulates IGF bioavailability, meaning that both its deficiency and excess can bring forth different problems. However, little is known of the role of IGF-ALS in regenerative processes. In this study we have used the sea cucumber Holothuria glaberrima, an excellent model organism to study the role of IGF-ALS in regeneration, due to its remarkable ability to eviscerate its internal organs and later regenerate them. **Methods:** Transcriptomic data were analyzed to examine IGF-ALS expression across different stages of intestinal regeneration. RNA was extracted from regenerating and normal intestinal tissues and performed PCR to analyze the expression of IGF-ALS and associated genes involved in the IGF pathway. **Results:** In this study we characterize the IGF system in *H. glaberrima* and analyzed its expression during intestinal regeneration. Our results show that IGF-ALS cannot be detected in normal (non-eviscerated) sea cucumbers or during the early stages of regeneration. Its expression is detected at later stages of regeneration. Interestingly, a second, distinct IGF-ALS protein was identified and included in our analyses. In contrast, only one IGF-ALS protein has been reported in mammals, suggesting a possible species-specific diversification of this protein in sea cucumbers. **Conclusion:** These findings suggest that IGF-ALS may play a regulatory role during the later stages of intestinal regeneration in *Holothuria glaberrima*. The identification of two distinct IGF-ALS proteins highlights a potential species-specific diversification of this component, offering new insights into the evolution and function of the IGF system in regeneration.

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Insights into the Insulin-like Growth Factor System During Echinoderm Regeneration: Lessons from *Holothuria glaberrima*

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Introduction: Mammals, including humans, exhibit a limited capacity to regenerate tissues, often relying instead on repair mechanisms that result in scarring rather than full restoration. However, nature offers remarkable examples of full-body regeneration, revealing biological processes that could transform regenerative medicine. Among these organisms, the sea cucumber *Holothuria glaberrima* stands out as a model system due to its ability to regenerate entire organs including its digestive tract and nervous system. A key element in these regenerative processes is the Insulin-like Growth Factor (IGF) system, which is essential for development and has been associated with regenerative events in various organisms. However, its precise role in organ regeneration remains poorly understood, and whether this system is conserved in this echinoderm is still unclear. **Methods**: To address this gap, we characterized and analyzed the expression dynamics of key IGF system components—IGF, IGF-binding proteins (IGFBP), the Acid Labile Subunit (ALS), and the IGF receptor (IGFR)—during intestinal regeneration. We combined genomic, transcriptomic, and PCR-based analyses to identify and quantify IGF system components and assessed their expression profiles across different intestinal regions and regeneration stages. **Results**:Genomic and transcriptomic analyses confirmed that the IGF system is conserved in *Holothuria glaberrima* and exhibits differential expression during intestinal regeneration. Moreover, IGF system components showed distinct expression patterns across different intestinal regions, indicating tissue-specific regulation. During the regeneration process IGF surged in the first three weeks of regeneration, IGFBP-ALS was consistently downregulated, and IGFBP initially decreased but rebounded with late-stage overexpression. Interestingly, IGFR expression remained stable in early regeneration, suggesting a nuanced regulatory mechanism. **Conclusion**: These findings illuminate how the IGF system orchestrates regeneration in echinoderms, laying critical groundwork for exploring its role in other tissues and potential applications in human regenerative medicine. By decoding these mechanisms, we edge closer to unlocking nature's blueprint for self-repair.

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High Glucose-Induced Activation of Microglia: Insights into Neuroinflammation in Diabetes

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Introduction: Type 2 diabetes mellitus is a chronic metabolic disorder that not only disrupts glucose regulation but also increases the risk of neurodegenerative diseases such as Alzheimer's disease. Accumulating evidence suggests that chronic hyperglycemia can trigger neuroinflammation by activating microglia, the resident immune cells of the central nervous system. Excessive microglial activation can lead to the release of pro-inflammatory cytokines, oxidative stress, and neuronal dysfunction. **Methods:** To evaluate the effects of elevated glucose on microglial activation, human microglial HMC3 cells were cultured under normal and highglucose conditions. Morphological changes were examined using light microscopy, while expression of inflammatory genes (*tumor necrosis factor-alpha* and *interleukin-6*) was quantified through quantitative polymerase chain reaction (qPCR). Cytokine secretion was evaluated using enzyme-linked immunosorbent assay (ELISA), oxidative stress levels were assessed via Muse flow cytometry with a reactive oxygen species-sensitive fluorescent dye, and activation of the NFκB signaling pathway was analyzed through protein expression and nuclear translocation studies. **Results:** Exposure to high glucose caused pronounced morphological alterations in microglia, characterized by reduced branching and enlarged somas indicative of an activated phenotype. Elevated reactive oxygen species levels pointed to oxidative stress as a central mediator. Expression and secretion of *tumor necrosis factor-alpha* and *interleukin-6* were upregulated, confirming an inflammatory response. Increased NF-κB expression and nuclear translocation confirmed a mechanistic link between high glucose and microglial inflammatory signaling. **Conclusions:**These findings demonstrate that high glucose directly induces microglial activation through oxidative stress and NF-κB–mediated pathways, contributing to the neuroinflammation observed in diabetes. This in vitro model characterizes the mechanisms linking hyperglycemia to neuroimmune activation and outlines potential therapeutic strategies to address diabetes-related neurodegeneration. Future work will extend these findings to animal models to assess translational relevance and therapeutic potential.

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Apurinic/apyrimidinic endonuclease 1 (APE1) activity in blood and postmortem striatum associates with somatic expansions in Huntington's Disease

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Introduction: Huntington's disease (HD) is a rare, autosomal dominant disorder caused by a CAG repeat expansion in the huntingtin (HTT) gene. The pathogenesis of HD involves ongoing somatic repeat expansion throughout the lifespan of neurons [1], a process that is closely associated with oxidative DNA damage [2]. APE1, the primary endonuclease in the base excision repair pathway, is essential for repairing apurinic/apyrimidinic (AP) sites [3] in mitochondrial and nuclear DNA [4], however, its function in the brain and the systemic circulation in HD remains unclear. Moreover, APE1 has been implicated in modulating somatic expansion in HD [5]. **Methods:** We hypothesize that APE1 activity decreases early during HD pathogenesis and that it may contribute to somatic CAG repeat expansions. To test this, we assess APE1 activity in peripheral blood mononuclear cells (PBMCs) and post-mortem brains from patients with HD using a fluorescent oligonucleotide containing an oxidative lesion [6]. **Results:** Our results show that APE1 activity is increased in PBMCs from younger individuals. Interestingly, APE1 activity showed a significant decline over time in blood samples from the same HD patients. APE1 activity is reduced in early-stage HD post-mortem brains. We also assessed somatic instability and found no significant changes in blood samples collected from the same patients at different times. In contrast, post-mortem HD striatal samples exhibited increased somatic instability with disease progression. **Conclusion:** These findings suggest that APE1 activity is impaired both in the brain and the periphery in HD patients and may precede the onset of somatic instability in the brain.
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BDNF/TrkB Signaling Pathways in the Extinction of Morphine Place Preference

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INTRODUCTION: Opioid overdose remains a major public health crisis in the U.S., and related deaths have spiked in recent years, with prescription opioid overdose deaths increasing drastically among women. This rise, and failure of current pharmacological treatments to prevent relapse, highlights the need for novel, sex-informed approaches. Exposure-based therapies in humans aim to reduce relapse by exposing individuals to drug-associated cues in the absence of the drug. Extinction training (ET), a behavioral model simulating exposure-based therapy, involves exposure to drug-associated cues without reinforcement and has shown promise in reducing drug-seeking in preclinical models. Previously, we showed that by using conditioned place preference (CPP), ET reduced preference for the drug-paired side (DPS) accompanied by an increase in brain-derived neurotrophic factor (BDNF) expression in the hippocampus (HPC) of male rats. Similarly, females also showed significant BDNF increase in the HPC, but also in the ventral striatum/nucleus accumbens (VS/NAc), a vital region to reward and relapse. This study aimed to characterize BDNF-related signaling pathways in the HPC and the VS/NAc of female rats following ET. METHODS: Adult female Sprague-Dawley rats were conditioned with morphine (5 mg/kg) for 8 days, followed by 4 days of forced extinction sessions. Our control group did not receive ET and spent this time in their home cage. Rats were euthanized post-ET, and VS/NAc and HPC tissue were analyzed by Western blot for the BDNF receptor, the phosphorylated TrkB (pTrkB). RESULTS: Our results showed TrkB upregulation in the VS/NAc of female rats that underwent ET and were classified in the extinction-resistant group. In the HPC, no significant changes in pTrkB were noticed although a tendency to increase in the extinction group was observed. CONCLUSION: Our results suggest that while ET activates BDNF pathways in females, TrkB activation may be regulated in a region-specific manner, highlighting the need for further research on sex-specific mechanisms underlying opioid abuse.

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Ionic modulation of radial nerve cord explants from *Holothuria glaberrima*

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Introduction: Nervous system regeneration remains one of the major unanswered questions in neuroscience. *Holothuria glaberrima*, a species of sea cucumber, is an echinoderm notable for its remarkable regenerative capacity, making it a valuable model for studying the regeneration of nervous tissue. Previous studies demonstrated that the radial nerve cords (RNCs) of *H. glaberrima* can remain morphologically stable *in vitro* for up to two weeks. In this study, we aimed to extend the viability and structural integrity of isolated RNCs beyond this time frame by modifying the ionic composition of the culture medium, specifically by adjusting sodium (Na^+) and potassium (K^+) concentrations. We hypothesized that increasing K^+ concentration would help preserve nerve activity and maintain neuronal structure and properties over time. **Methods:** To test this, we dissected sea cucumbers and extracted four of their five RNCs. The nerves were enzymatically treated with collagenase for 24 hours to remove surrounding tissues and were subsequently cultured in L-15 medium containing varying ionic concentrations: [1.5 K^+], [2.5 K^+], [1.5 Na^+], and [2.5 Na^+]. The RNCs were monitored periodically to assess morphological stability, cellular integrity, and onset of regenerative events during culture. **Results:** Preliminary results indicated that elevated K^+ levels prolonged delayed the onset of both regeneration and cellular dedifferentiation, suggesting that ionic composition plays a critical role in maintaining RNC stability and modulating regenerative responses. **Conclusions:** These findings highlight the importance of ionic changes in sustaining neural tissue viability *in vitro* and provide insight into how extracellular ionic environments influence the regenerative potential of echinoderm nervous tissue. This study advances our understanding of the physiological parameters that affect nervous system regeneration, offering a comparative basis for investigating similar processes in vertebrate systems.

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Molecular Lineage Markers in the regenerating nervous system of echinoderms

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Echinoderms carry out formidable regeneration of their intestinal and nervous system tissues. Their close phylogenetic relationship to humans makes them valuable models to understand the molecular mechanisms that drive the regeneration response. Radial Glia-Like Cells (RGLC) serve as precursors of regeneration in the nervous system of *Holothuria glaberrima*. Recently, molecular markers of RGLCs and neural stem cells (NSC) have been identified in the cells that constitute *H.glaberrima's* radial nerve cord (RNC). However, the search for other glial markers, including those from the ependymal lineage, has been neglected. **METHODS:** This study further characterizes the expression of gene markers in normal and regenerating nervous tissue of *H. glaberrima*. The studied genes include the ependymal marker FOXJ1, astrocytic marker SOX9, and three different Prominin sequences known to be expressed in NSC. Preliminary **results** show FOXJ1 and SOX9 expression in the surrounding areas of the RNC. FOXJ1 expression is mostly located in the peripheral fibers innervating the longitudinal and circular muscle, while SOX9 is mostly located in the cells within the RNC horns. Neither $Sox9^{+}$ nor $Foxj1^{+}$ cells were immunoreactive to ERG1, the traditional RGLCs marker. While PROM2 expression is attributed exclusively to RGLCs, PROM1 expression is more prominent in the lateral basal neuropile of the nerve cord, and low PROM3 expression is seen in RGLCs in the middle of the neuroepithelium. **CONCLUSIONS:** Taken together, this study characterizes the spatial expression of novel lineage markers and provides an amplified view of gene expression in the holothurian nervous system.

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Isolated Brain, Hungry Circuits: Targeting Synaptic Stability (SV2A) to Offset Sex-Dependent Changes in Stress-Related Eating

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Introduction: Binge eating (BE) linked to early-life adversity (ELA) is a common maladaptive coping mechanism in people with obesity. Social isolation (SI) during adolescence—a strong form of ELA—profoundly affects brain development and emotional regulation. This study investigated whether behavioral and synaptic changes caused by SI increase vulnerability to BE and examined the therapeutic potential of targeting synaptic vesicle glycoprotein 2A (SV2A), a key regulator of synaptic communication. **Methods:** First, we compared baseline SV2A levels between male and female Lewis rats using PET with F-18-SDM8 to measure hippocampal SV2A binding during adolescence (n = 8). A group of adolescent rats (n = 64; 50% female) was housed either in pairs (two per cage) or alone (one per cage) from postnatal day 28 to 63. Behavioral tests included assessments of emotionality and a three-week intermittent high-fat diet that mimics Western eating habits. In the final week, rats received either a vehicle or levetiracetam (LEV; 10 mg/kg), an SV2A-binding anticonvulsant known to stabilize synaptic function. A subgroup of rats (n = 24) was monitored over time in the PhenoTyper Home-Cage System to measure SI-driven changes in activity, exploration, and anxiety-like behaviors, summarized as overall z-scores. Hippocampal tissue was examined by fluorescent in situ hybridization, immunofluorescence, and flow cytometry to assess synaptic and microglial phagocytic markers. **Results:** Females showed greater hippocampal microglial density but lower CD68 expression, indicating decreased microglial engulfment capacity. Conversely, females exhibited higher SV2A immunoreactivity, which was not associated with microglial-pruning measures—highlighting a sex-specific decoupling of synaptic abundance from microglial activity. LEV treatment normalized BE behaviors in SI-exposed females and partially restored microglial–synaptic interactions. **Conclusion:** These findings provide compelling evidence for sex differences in synaptic integrity during adolescence. Further, this research offers strong preclinical support for SV2A-focused treatments for stress-related eating disorders and underscores the importance of considering sex as a biological variable in neuropsychiatric studies. **Acknowledgments:** IACUC #23-026. NIDDK 1R21DK124727-01, LLU-NIH IMSD R25GM060507, GM060507, and LLUH GRASP and SIMS Awards.

Molecular and Functional Characterization of TREM-Like Transcript 1 (TLT-1) in Microglia

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Neurodegenerative diseases, such as Epilepsy, Alzheimer's Disease and many others, are characterized by chronic neuroinflammation. Microglia, the resident immune cells of the central nervous system (CNS), express receptors that regulate these inflammatory responses, one of them being the family of receptors known as Triggering Receptor Expressed on Myeloid Cells (TREM). Part of this family are TREM1 and TREM2, which are known to mediate microglial inflammatory responses in the brain during pathophysiological conditions. Our study investigates TREM-Like Transcript 1, an abundant platelet receptor involved in immune hemostasis and coagulation processes. Despite its characterized role in platelets and hemostasis, the presence and function of TLT-1 in the CNS has not been investigated. Methods/Results: Preliminary data from our lab identified TLT-1 expression in brain tissue, neurons, astrocytes, and SIMA9 microglial cells by Western Blot and Immunofluorescence, suggesting a role beyond platelet biology. Our current studies are confirming the expression of this protein in microglia and other brain cells by qRT-PCR. To determine if TLT-1 responds to proinflammatory insults, our studies evaluated how microglial TLT-1 protein expression changes following exposure to TNF-a. We found no significant changes in TLT-1 expression after 1 hour and 24 hours of TNF-a exposure. Furthermore, we assessed the contribution of TLT-1 to microglial activation by examining Erk1/2, found to be implicated in microglial inflammatory signaling, and the general microglial activation marker IBA1. This was performed under different treatment conditions which include untreated, TNF-a, sTLT-1, and TNF-a + sTLT-1. We found that the exposure of sTLT-1 individually and accompanied TNF-a, increased total Erk1/2 expression and Erk1/2 phosphorylation. By characterizing TLT-1 expression and its influence on microglial activation, this study seeks to expand current knowledge of neuroimmune signaling and identify novel molecular players in neuroinflammation. Understanding how platelet-derived factors modulate microglial responses could uncover new therapeutic strategies for neurodegenerative diseases.

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Elucidating the role of Notch signaling in radial nerve cord and intestinal regeneration in the sea cucumber *Holothuria glaberrima*

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Introduction: Understanding the genetic regulation of tissue regeneration, particularly the involvement of signaling pathways, remains a crucial area of investigation. Notch signaling, a highly conserved pathway in multicellular organisms, plays a pivotal role in various cellular processes, including proliferation, dedifferentiation, and fate specification. However, its specific function in post-traumatic regeneration remains poorly characterized. **Methods:** In this study, we employ a small-molecule inhibitor of the Notch pathway, DAPT, to investigate its role in CNS and intestinal regeneration in a novel model system, the sea cucumber *Holothuria glaberrima*. We induce evisceration of the intestines, induce injury to the radial nerve cord (RNC), and study the subsequent regeneration. **Results:** Our observations reveal that DAPT inhibition of the Notch pathways causes a significant delay in cell dedifferentiation in both tissues. Additionally, DAPT treatment decreases cell proliferation in both tissues at 8 days post-injury, typically the peak period of proliferation. **Conclusion:** The observed delays in cell dedifferentiation, reduced proliferation, and altered tissue morphology suggest that Notch signaling regulates these aspects of regeneration. The fact that DAPT treatment similarly affects both CNS and intestinal regeneration suggests that Notch signaling plays a significant role in the amazing regenerative abilities of echinoderms and points to a process that could provide insights into the regeneration processes in other organisms. Ongoing experiments include TUNEL assays to further explore whether Notch signaling directly regulates cellular events or operates through upstream regulatory networks affecting the tissue environment as a whole during regeneration. Additional studies are needed to fully elucidate the mechanisms by which Notch signaling influences regeneration in sea cucumbers and to explore its potential as a target for therapeutic interventions in regenerative medicine.

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Longitudinal Machine Learning and snRNA-Seq Reveal Stroke-Specific Behavioral and Transcriptomic Signatures

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Introduction: Ischemic stroke is a leading cause of death and disability worldwide, with over 690,000 new cases each year. While cortical stroke (CS) patients typically develop symptoms such as hemiparesis and speech impairments, white matter stroke (WMS) often presents with more subtle cognitive deficits. Despite comprising 30% of all strokes and being a leading cause of vascular dementia that affects 90% of the population above age 65, WMS remains poorly understood. In preclinical studies, current rodent behavioral assessments lack comprehensive, unbiased methods to characterize these functional deficits. Additionally, little is known about the underlying molecular mechanisms, thereby hampering the development of effective treatment options. **Methods:** To address these limitations, we employed Motion Sequencing (MoSeq), an unsupervised machine learning approach, to longitudinally assess behavioral changes in young/aged male and female mouse models of CS and WMS. Moreover, we used single-nucleus RNA-sequencing (snRNA-seq) of ischemic tissue samples to identify key cellular and molecular responses that might accompany behavioral deficits during the subacute and chronic phases of ischemia. **Results:** MoSeq effectively captured distinct behavioral phenotypes, revealing that CS mice exhibited primarily motor impairments that partially recovered during the chronic phase, whereas WMS mice developed progressively worsening cognitive deficits. These changes were consistent across age and sex groups, though more pronounced in aged and male mice. Single-nucleus RNA sequencing revealed corresponding molecular signatures, with CS tissue exhibiting prominent inflammatory gene expression during the initial subacute phase, while WMS tissue showed sustained downregulation of myelination-related pathways. **Conclusion:** By characterizing unique features of these stroke subtypes across different time points, ages, and sexes, our study contributes to a more comprehensive understanding of stroke, providing insights into the development of therapeutic strategies for stroke patients. Our findings highlight the need for subtype-specific approaches, with CS potentially benefiting from interventions that support the transition to homeostatic states that could reduce motor deficits, while WMS may require therapies that specifically target myelination pathways to prevent progressive cognitive deterioration.

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Role of miR-9 in Alcohol-Induced Activation of the Wnt/β-Catenin Pathway

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Introduction: Molecular alcohol tolerance involves changes in gene expression and protein pathways that regulate large-conductance calcium-activated potassium (BK) channels, which influence neuronal excitability and behavior. This study focuses on the role of microRNA (miR-9) in alcohol tolerance. The two main components of molecular alcohol tolerance are the regulated expression and distribution of BK channels. MiR-9 is upregulated by ethanol and inhibits the alcohol-sensitive BK channel isoform Zero. Furthermore, miR-9 activates the Wnt/β-catenin signaling pathway, leading to the internalization and redistribution of BK channels, thus altering their surface distribution. Our findings demonstrate that ethanol-induced miR-9 upregulation is essential for Wnt/β-catenin activation and BK channel redistribution. Therefore, miR-9 regulates BK channel expression and localization, coordinating both components of alcohol molecular tolerance. The primary objective of this research was to examine the role of miR-9 in the second component of alcohol tolerance by assessing the activation of the Wnt/β-catenin signaling pathway through miR-9 upregulation in response to ethanol. **Methods:** To achieve this, cell culture, immunocytochemistry, and molecular techniques, including transfection, were used. Specifically, a HEK293 cell line stably expressing the BK channel Zero isoform was employed. These cells were cultured to 70% confluence, transfected with miR-9 inhibitors, and treated with 25 mM ethanol. Cells were stained for BK channel expression, β-catenin, and membrane and nuclear markers, and analyzed using confocal microscopy with NIS-Elements Viewer Software. The analysis included measurement of β-catenin and BK channel staining intensity, colocalization studies to determine Manders' and Pearson's coefficients, and statistical tests such as Student's *t*-test and one-way ANOVA. **Results:** Preliminary results confirm that miR-9 upregulation promotes β-catenin expression, indicating that miR-9 upregulation is required for ethanol-induced activation of the Wnt/β-catenin pathway. **Conclusion:** These results demonstrate miR-9's role in regulating BK channel isoform expression and modulating the Wnt/β-catenin pathway, which is essential for ethanol-induced BK channel redistribution.

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Role of WDR33 in Modulating Alpha-Synuclein Induced Cytotoxicity

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Introduction: Parkinson's disease (PD) is one of the most prevalent and impactful neurodegenerative disorders affecting aging populations worldwide. While the disease may arise through various genetic and environmental triggers, a consistent pathological hallmark is the accumulation of misfolded alpha-synuclein, canonically in dopaminergic neurons of the substantia nigra, a brain region that regulates motor function. To elucidate the molecular drivers of alpha-synuclein accumulation, gene-gene and protein-protein interaction screens centered on alpha-synuclein were conducted, coupled with bioinformatic analyses integrating in-house datasets and published genome-wide association studies. These efforts identified more than 2,000 candidate genes implicated in disease mechanisms. CRISPR-based knockdown screens demonstrated that silencing a subset of these genes induces cellular toxicity, with the effect most pronounced in cells overexpressing alpha-synuclein. Among the strongest candidates was WDR33, which encodes a canonical isoform (V1) that functions within the cleavage and polyadenylation specificity factor complex, as well as two noncanonical isoforms (V2 and V3) implicated in regulation of the cGAS-STING-dependent innate immune response. These isoforms are proposed to modulate neuroinflammatory signaling and autophagy, thereby influencing alpha-synuclein aggregation. **Methods**: To evaluate this hypothesis, WDR33 isoform expression was profiled in induced pluripotent stem cell (iPSC)-derived neurons overexpressing alpha-synuclein, compared with isogenic controls. Alpha-synuclein protein levels were quantified by Western blot, and isoform-specific expression was assessed by mRNA analysis. In addition, a CRISPR-based knockdown screen using a dead Cas9 system was conducted over 28 days to assess alpha-synuclein-induced cytotoxicity. **Results**: Preliminary results indicated no significant change in V1, V2, or V3 isoform expression across alpha-synuclein iPSC lines. However, WDR33 knockdown markedly increased cytotoxicity in neurons with high alphasynuclein expression by day 18. **Conclusion**: These findings suggest that WDR33 loss exacerbates alpha-synuclein-induced toxicity, underscoring a critical role for WDR33 in neuroinflammatory pathways and proteostasis in PD. Future work will focus on generating a WDR33 knockout model, testing isoform-specific rescue of alpha-synuclein toxicity, and quantifying cytokine

induction through the cGAS-STING pathway. Collectively, these studies aim to define the mechanistic role of WDR33 in PD pathogenesis and identify novel therapeutic targets for neuroprotection.

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Effects of voluntary exercise in PTEN deficient Purkinje cells and mouse motor coordination

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INTRODUCTION: Germline heterozygous mutations in Phosphatase and Tensin Homolog Deleted on Chromosome 10 gene (PTEN) are found in up to 20% of children with autism spectrum disorder (ASD) and macrocephaly. PTEN is a regulator of metabolic signaling that inhibits the mTORC1 pathway. The conditional deletion of the Pten gene (Pten-cKO) in cerebellar Purkinje cells (PCs) causes cellular hypertrophy and neurodegeneration. We have found that lack of PTEN in PCs leads to metabolic signaling impairment and mitochondria deficits in PC dendrites. It is known that physical exercise improves neuronal function by enhancing cellular metabolism and mitochondria biogenesis. **METHODS:** Currently, we are testing the effects of wheel running in mice conditionally deficient of PTEN in PCs (Pten-cKO). Particularly, we have been investigating the effects of wheel running in PCs metabolic signaling, dendritic neurofilament aggregation, and mouse motor coordination. **RESULTS:** Our preliminary results indicate that pS6R immunoreactivity, as a marker of mTORC1 activation, in PCs from Pten-cKO mice is increased in 8-week sedentary mice when compared to wild-type (WT) sedentary mice. However, a further increase in mTORC1 activation is measured by higher levels of pS6R immunoreactivity in PCs from running Pten-cKO mice. Interestingly, immunoreactivity of phosphorylated AMPK in PCs from sedentary 8-week Pten-cKO mice is significantly decreased when compared to 8-week sedentary WT but significantly increased to WT levels in PCs from running Pten-cKO mice. AMPK is a metabolic kinase that inhibits mTORC1-anabolic activity and increases mitochondria biogenesis. Thus, we analyzed the levels of PDHA1+ mitochondria and LAMP1+ lysosomes in the dendrites of PCs from 8-week WT, sedentary Pten-cKO, and runner Pten-cKO mice, and found that running prevented the decrease of these markers in PCs from Pten-cKO mice when compared to WT mice. However, running increased pathological aggregation of cytoskeletal neurofilaments and dendritic hypertrophy in 8-week Pten-cKO PCs when compared to 8-week Pten-cKO sedentary and WT mice. Still, we found a significant improvement in motor coordination as tested by the horizontal rung task. **CONCLUSIONS:** Overall, our preliminary data suggest that wheel running alters mTORC1 and AMPK metabolic signaling that improves mitochondria deficits but worsens dendritic pathology in mouse PCs that are deficient in PTEN. However, a significant improvement in motor coordination in Pten-cKO runner mice suggests either an improvement in PC function or a compensatory effect of other neuronal populations involved in motor control.IACUC Protocol #2025-04

Investigating the Cytotoxic Potential of Polysaccharide Peptide on A172 Glioblastoma Cells

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Background: Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor, known for its rapid progression and poor prognosis. Despite multimodal treatment with surgery, radiotherapy, and temozolomide (TMZ), patient outcomes remain dismal due to therapeutic resistance and significant toxicity. These challenges highlight the urgent need for alternative strategies that are both effective and less harmful. Polysaccharide peptide (PSP), a bioactive molecule derived from the medicinal mushroom Coriolus versicolor, has shown immunomodulatory and anticancer potential in various tumor models. This study investigated PSP's cytotoxic effects on A172 human glioblastoma cells in vitro. **Methodology**: A172 cells were cultured and treated with PSP (500–4000 µg/mL) for 6 and 9 days, and viability was assessed using the MTT assay to evaluate dose- and time-dependent responses. **Results**: PSP exposure resulted in a pronounced reduction in glioblastoma cell viability. At higher concentrations (3500– 4000 µg/mL), cell survival decreased to about 48% at day 6 and further to 30% by day 9, while lower doses showed moderate effects. These results indicate a clear, concentration-dependent cytotoxic response that intensifies with extended exposure. **Conclusion**: PSP markedly inhibits A172 glioblastoma cell growth in vitro, suggesting its potential as a natural adjunctive or alternative therapy. These findings warrant further exploration of PSP's underlying mechanisms and potential synergistic effects with standard chemotherapeutics, such as TMZ, for enhanced glioblastoma management.

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CDCH expression and retrograde nerve labeling of B*iomphalaria's* CNS, an intermediate snail host for human schistosomiasis

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Introduction: Schistosomiasis affects over 200 million people in tropical regions worldwide. The snail *Biomphalaria Glabrata* serves as a key host for *Schistosoma mansoni*, the parasite causing the most common form of human schistosomiasis. Infected snails show reduced egg production, suggesting energy is redirected toward parasite development. However, the impact of parasitism on *Biomphalaria*'s reproductive physiology remains unclear. Understanding the localization of the reproductive caudodorsal cell hormone (CDCH) will enhance knowledge of host-parasite interactions. **Methods:** The methods utilized in this study include standard histological procedures, *in situ* hybridization, and immunohistochemistry to localize the reproductive hormone CDCH in the CNS of *Biomphalaria*. Retrograde nerve labeling of the visceral ganglia nerve was performed to identify neurons involved in the function of the female reproductive system. Immunohistochemistry negative controls were included to confirm the integrity of the procedure. All protocols were approved by the UPS MSC IACUC (Protocol #3220119). **Results:** It was found that most CDCH cells were clustered in the right and left cerebral ganglia, with fewer in the left parietal and visceral ganglia. Prominent CDCH-like immunoreactive fibers were observed in the cerebral commissure, a neurosecretory region between the cerebral hemiganglia. This localization agrees with previous findings in other pulmonate snails, indicating CDCH is secreted into circulation to regulate reproductive behaviors. It was also found that there were fewer immunoreactive fiber expressions in the visceral ganglia. CDCH expression was also detected in the peripheral nervous system, particularly in female reproductive organs. Retrograde nerve labeling analysis revealed no colocalization of neurons involved in the control of the nerve going directly to the nidamental gland with CDCH protein. However, colocalization of fibers in the cerebral commissure was found. **Conclusion:** Future attempts will involve other nerves to further analyze CDCH action on the CNS and peripheral tissues, particularly to study whether CDCH in the parietal nerves is correlated to the female reproductive organs. General findings indicate that CDCH regulates female reproduction in *Biomphalaria*. As there was no cell colocalization with the retrograde nerve labeling performed in the

visceral ganglion, CDCH transport to the periphery might involve other ganglia, such as the parietal. Future studies will investigate whether CDCH expression changes during *Schistosoma mansoni* infection.

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Myomodulin expression & retrograde nerve labeling analysis for the central nervous system of *Biomphalaria glabrata*

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Background and Objectives: Schistosomiasis, or snail fever, is a parasitic disease caused by *Schistosoma* blood flukes that affect millions of people in tropical and subtropical regions with poor sanitation. *Schistosoma mansoni* relies on the freshwater snail *Biomphalaria glabrata* as an intermediate host, where it develops into the infectious cercarial stage. Therefore, understanding the biology of this snail is crucial for controlling disease transmission. In this study, we investigated the expression of the neuropeptide myomodulin in the central nervous system (CNS) of *B. glabrata*, a key vector of *S. mansoni*. Myomodulin is known in gastropods to regulate muscle contraction and behaviors such as feeding and locomotion. However, our findings suggest it may also play a role in male reproductive functions. **Methods:** To explore this, we examined myomodulin expression in the CNS and peripheral tissues of *B. glabrata* using whole-mount immunohistochemistry (antigen: PMNMLRL-NH_2), hybridization chain reaction (HCR) in situ hybridization, and retrograde nerve labeling, following IACUC-approved protocols. **Results:** The results showed myomodulin immunoreactivity in several central ganglia, with clusters of positive cells in the buccal and pedal ganglia and abundant expression in the cerebral ganglia. Additionally, some peripheral tissues displayed myomodulin labeling. Importantly, retrograde labeling of the penis nerve revealed cells expressing myomodulin-like peptide, indicating that myomodulin may be involved in the contraction or functional regulation of the penis and other male-associated structures. **Conclusions:** Overall, mapping myomodulin localization in *B. glabrata* enhances our understanding of host–parasite interactions in this biomedical model and may contribute to the development of new vector control strategies for schistosomiasis. **Acknowledgements:** Supported by the National Institutes of Health: MD007600 (RCMI), P30GM149367 (COBRE); National Science Foundation: IOS-2217657 (OSIB), HRD-1137725 (CREST), OISE-1545803 (PIRE), and DBI-1337284. Imaging support was provided by the UPR COBRE Center for Neuroplasticity, Neuroimaging and Electrophysiology Facility (NIEF).

Discrimination of Autism Mouse Models via Machine Learning Methods

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Introduction: Autism Spectrum Disorder affects millions worldwide, yet its neural underpinnings remain poorly understood. Multiple autism-associated genes converge on similar behavioral phenotypes, but whether convergent neural phenotypes exist remains unclear. **Methods**: We investigated three high-confidence autism genes: GRIN2A, GRIN2B (NMDA receptor subunits) and MECP2 (transcriptional regulator). We recorded spontaneous brain activity from 129 mouse sessions (57 control, 72 knockdown) using widefield calcium imaging with GCaMP6f. Adult mice were awake and head-fixed during 20-30 minute recordings. Neural dynamics from 82 cortical regions were captured at 10Hz and processed into 3-second windows. We developed a Brain Vision Transformer that treats spatiotemporal brain activity as images, processing 246 patches through 6 transformer layers. **Results:** Our model achieved 82% test accuracy distinguishing autism models from controls across all three genes (sensitivity: 84.53%, specificity: 78.64%). Gene-specific connectivity analyses revealed distinct patterns: MECP2 knockdowns showed decreased connectivity, GRIN2B increased connectivity, and GRIN2A displayed mixed effects. Despite different molecular mechanisms, all genetic models produced AI-detectable neural signatures. **Conclusions:** Vision Transformers successfully identify autism-related neural patterns invisible to traditional analysis. These findings demonstrate convergent neural disruptions across different genetic causes, suggesting common circuit-level mechanisms in ASD despite distinct molecular pathologies. This machine learning approach opens new avenues for understanding autism's neural basis and potential therapeutic targets.

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RVG peptide functionalized gold-liposomes enhance miRNA-based therapy internalization in Glioblastoma cells.

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Introduction: Glioblastoma (GBM) is the most lethal primary brain tumor, with a median survival of just 12–15 months after diagnosis. Despite decades of research, the Stupp Protocol which involves surgery followed by radiotherapy and chemotherapy has been the gold-standard in over 40 years. This stagnation, coupled with consistently poor prognosis, emphasizes the urgent need for novel therapeutic strategies. MicroRNAs (miRNAs), small non-coding RNAs that regulate gene expression post-transcriptionally, have emerged as potential targets in GBM. Specifically, miR-92b is consistently upregulated in GBM tissues and cell lines, with a contributing role in proliferation, drug resistance, and aggressiveness. Inhibition of miR-92b using Oligonucleotide MicroRNA Inhibitors (OMIs) has shown promising results in reducing tumor burden in preclinical models. However, therapeutic delivery across the blood-brain barrier (BBB) remains the major challenge when treating GBM. To overcome this challenge, we utilized a modified Rabies Virus Glycoprotein (RVG) peptide, which targets nicotinic acetylcholine receptors expressed on both BBB and GBM cells. **Methods:** The RVG peptide sequence was modified and conjugated to liposomes nanoparticles for targeted delivery. OMIs against miR-92b were bind to 15 nm gold nanoparticles to form spherical nucleic acids (SNAs), which were then encapsulated within the RVG-functionalized liposomes to create the gold-liposome nanocarrier system. **Results:** GBM cells incubated with the modified RVG-functionalized liposomes demonstrated significantly enhanced uptake of the nanoparticle formulation compared to those treated with unmodified RVG. These findings suggest that RVG peptide modification enhance internalization efficiency, a critical step toward effective therapeutic delivery. **Conclusion**: Our study presents a novel and targeted delivery system capable of delivering miR-92b inhibitors directly to GBM cells. The modified RVG-functionalized gold liposomes represent a promising platform for RNA-based therapeutics in glioblastoma, potentially addressing the development of effective treatments for this devastating disease.

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Erinacines Enhance Antiviral Immunity Through PKR and Cofilin Regulation: Implications for HAND and NeuroAIDS

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Introduction: HIV-associated neurocognitive disorders (HAND) remain a major challenge despite advances in antiretroviral therapy, highlighting the need for novel approaches that strengthen neuroimmune resilience. Erinacines, bioactive diterpenoids isolated from Hericium erinaceus (Lion's Mane mushroom), are recognized for their neuroprotective and anti-inflammatory actions, but their immunomodulatory potential against HIV-1 has not been defined. This study examined whether erinacines enhance antiviral immunity by regulating key molecular mediators, with a focus on Protein Kinase R (PKR), an antiviral kinase that restricts viral fusion through cofilin-1 phosphorylation. **Methods**: T lymphocytes were treated with erinacines (50–1000 µg/mL) for three days. Protein expression of PKR, Toll-Like Receptor 4 (TLR4), Signal Transducer and Activator of Transcription 1 (STAT1), Nuclear Factor Kappa B (NFκB), and cofilin-1 were analyzed by Western blot, while cell viability was determined by MTT assay. **Results**: Erinacine exposure significantly increased PKR, TLR4, STAT1, and NFκB levels in a dose-dependent manner, accompanied by reduced total cofilin-1 and elevated phosphorylated cofilin-1, indicating activation of an antiviral signaling cascade without cytotoxicity. These results suggest that erinacines enhance innate antiviral pathways and modulate actin dynamics through PKR–cofilin-1 signaling. **Conclusion**: Erinacines exhibit potent immunostimulatory effects in T-cells, supporting their potential as natural modulators of antiviral and neuroprotective responses. These findings lay the groundwork for future studies in microglial models to evaluate erinacines as candidates for mitigating HAND by reinforcing CNS defenses against HIV-1.
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Modulating Macrophage Interactions with the Blood Brain Barrier: Edelfosine as a Potential Therapeutic in Epilepsy-Associated Inflammation

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Seizures and epilepsy can disrupt the blood-brain barrier (BBB), which is normally a highly selective barrier that protects the brain, maintains homeostasis, and controls the entry of peripheral immune cells under inflammatory conditions. BBB disruption in conditions such as epilepsy promotes the recruitment of immune cells, including macrophages, into the brain. The infiltration of macrophages into the brain and their activation can lead to the release of pro-inflammatory cytokines, which contribute to the development of seizures in epilepsy. Thus, our studies search for novel alternative therapies against epilepsy with fewer or no side effects. The ether lipid edelfosine has been shown to decrease the expression of proinflammatory protein markers in macrophages and proinflammatory mRNA markers in microglia. **As a drug with anti-inflammatory effects in central and peripheral immune cells, we hypothesize that edelfosine will decrease the adhesion of macrophages to BBB cells.** To address this question, our **methods** included performing a macrophage adhesion assay using murine blood-brain barrier (BBB) endothelial cells (bEND.3 cell line, ATCC) and murine macrophage cells (RAW 264.7 cell line, ATCC). In this assay, to simulate a seizure-like insult, we will expose cells to pilocarpine, a muscarinic receptor agonist, and determine if macrophages attach to the endothelial BBB cells in the presence and absence of our drug edelfosine. Preliminary **results** show that edelfosine decreased the adhesion of macrophages to the BBB cells, similar to the classical anti-inflammatory drug dexamethasone. In **conclusion**, these results suggest that one of edelfosine's mechanisms as an anti-inflammatory molecule under seizure-like conditions could be by decreasing macrophage adhesion to the BBB. Since edelfosine is a drug that does not cross the BBB, ongoing experiments are being carried out where a protein-based drug delivery system is being used for the effective delivery of edelfosine in vivo. To start establishing edelfosine-loaded nanoparticle safety, viability assays with drug concentration curves will be performed with the endothelial and macrophage cell lines. Once safety doses are calculated, the same adhesion assays will be performed with the nanoparticles in the presence and absence of pilocarpine. We expect the edelfosine-loaded nanoparticles to decrease macrophage adhesion, as well as the classic anti-inflammatory drug dexamethasone. These findings

highlight the relevance of our in vitro BBB–macrophage adhesion model as a valuable platform for studying neuroinflammatory mechanisms in epilepsy and underscore the novel therapeutic potential of edelfosine as an anti-inflammatory agent in seizure-related pathophysiology. **Funding:** COBRE3 Neuroplasticity- P30-GM149367 (YFA), COBRE Center for Microbiome Studies- 1P20156713-01 (YFA), and Neuro ID: 5R25NS080687 (PGA).

Ethanol-Induced Wnt/β-Catenin-Dependent Internalization of the ZERO Variant but not STREX, BK Channel Isoform

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INTRODUCTION Large-conductance Ca^{2+}-activated K^+ (BK) channels are key regulators of neuronal excitability and implicated in alcohol-induced tolerance, as a form of neuronal plasticity. In particular, the canonical Wnt/β-catenin signaling pathway has been identified as a crucial mediator of persistent BK channel downregulation during ethanol exposure. Prolonged ethanol exposure increases β-catenin level, and blocking β-catenin accumulation prevents the ethanol-induced internalization of BK channel. Direct activation of Wnt/β-catenin signaling reduces BK current density, indicating that this pathway negatively regulates BK channel surface expression. This background has established Wnt/β-catenin signaling and new protein synthesis as critical factors in a persistent form of BK molecular tolerance, paralleling other long-term synaptic plasticity mechanism. **METHODS** Here, we investigated whether this Wnt/β-catenin-mediated regulatory mechanism is isoform-specific by comparing two prevalent BK channel splice variants, ZERO (insertless) and STREX (stress-regulated exon), in a heterologous HEK293 cell model. Both isoforms were stably expressed and monoclonally selected in HEK293 cells to isolate their individual responses to ethanol. **RESULTS** We found that acute 25 mM ethanol exposure, which is physiologically releveant, robustly activated Wnt/β-catenin signaling in cells expressing the ZERO isoform, leading to internalization of ZERO BK channels as evidenced by confocal microscopy (a hallmark of molecular tolerance). In striking contrast, cells expressing the STREX-containing BK channels showed no activation of the Wnt/β-catenin pathway and no significant loss of BK expression after ethanol treatment. Thus, the STREX variant appears resistant to ethanol-induced endocytic downregulation, consistent with prior observations that STREX-containing BK channels are largely insensitive to alcohol's effects. Our results indicate that the presence of the STREX insert alters the channel's regulatory interface, preventing the Wnt/β-catenin–triggered internalization that occurs with the ZERO isoform. This can partly be explained by the retained exon containing more β-catenin sites, favoring stronger anchor points compared to its Zero variant. This isoform-specific difference outlines a novel mechanism of neuronal adaptation: ethanol selectively removes alcohol-sensitive BK channels (ZERO) from the membrane via Wnt/β-catenin signaling, while sparing or even favoring the retention of alcohol-insensitive channels (STREX). Such dynamic reconfiguration of BK

channel isoform expression represents a form of plasticity and molecular tolerance, whereby neurons adapt to prolonged alcohol exposure by diminishing the functional impact of ethanol on their potassium conductance. **CONCLUSION** In summary, our findings demonstrate that alternative splicing of the BK channel (ZERO vs. STREX) governs the engagement of Wnt/β-catenin signaling in response to ethanol, providing a mechanism for BK channel–dependent plasticity underlying alcohol tolerance.

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Sex-Specific Synaptic Plasticity in the Posterior Hypothalamic Nucleus Following Acute Stress

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Introduction: Adaptation to stress involves autonomic and neuroendocrine responses orchestrated by brain regions such as the infralimbic cortex (IL) and posterior hypothalamic nucleus (PH). These brain regions are involved in regulating emotions and the control of stress responses, respectively. Animal studies show that PH neurons receive excitatory innervation from IL and that IL stimulation increases activity in the PH. Likewise, both brain regions are simultaneously activated in response to stress. However, whether synaptic plasticity in the IL-to-PH pathway contributes to stress adaptation remains unclear. Method: In this study, we examined whether stress adaptation involves the IL-to-PH pathway. We hypothesized that restraint stress will decrease IL excitation of PH neurons if this pathway contributes to stress adaptation. We injected a channelrhodopsin-expressing virus into the IL of male and female adult rats for optogenetic stimulation of IL terminals in the PH. Eight weeks after surgery, the animals were divided into non-stress and stress groups. Stressed animals were restrained for 30 minutes. Afterward, rats were sacrificed, and we analyzed synaptic inputs to PH neurons using whole-cell patch-clamp recordings. Results: We found that IL neurons form functional excitatory synapses onto PH neurons in both males and females. However, compared to male rats, acute stress reduced N-methyl-D-aspartic acid (NMDA) receptor-mediated synaptic transmission in the IL-to-PH pathway in female rats. We also measured spontaneous brain activity in the PH to see if stress affected NMDA-mediated synaptic transmission in this region. We did not observe an effect of stress on NMDA-mediated synaptic transmission within the PH. Conclusion: These results suggest that the impact of stress on synaptic plasticity in the PH is specific to the IL-to-PH pathway. Together, these findings show that males and females respond to stress differently at the cellular level. Understanding these differences could help explain why some stress-related disorders are more common in one sex than the other and may guide future treatments.

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Polyamine Accumulation in Glial Cells Without the Synthesis

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Introduction: Polyamines (PAs) such as spermine (SPM) and spermidine (SPD) are small cationic molecules essential for cellular homeostasis and signaling in the central nervous system (CNS). Attributed to participate in neuronal metabolism, recent evidence indicates that PAs are localized in glial cells and not neurons, in healthy adult brain and retina. The source of this glial accumulation, synthesis or uptake, remains unknown. **Methods:** In this study, we investigated the cellular localization of PAs and their biosynthetic enzymes across developmental and aging stages in rat retina using immunohistochemistry and confocal microscopy. **Results:** We detected SPM and SPD labeling in Müller glial cells, while spermine synthase (SpmS) and spermidine synthase (SpdS) were restricted to neurons, suggesting that mature Müller glia accumulate PAs, but do not synthesize. Observed decline in neuronal expression of SpmS and SpdS, dependent on age progression, suggests a reduced capacity for PA biosynthesis with aging. **Conclusion:** These findings support a model in which glial cells act as repository and regulators of extracellular PA levels, possibly through connexin-43 gap junctions, hemichannels, and organic cation transporters (OCTs, SLC22A3, SLC18B1) or lysosomal transporter ATP13A4. PAs are colocalized in tiny glial processes together with key glial channels such as Kir4.1 (and Cx43). Since such channels are extremely sensitive to SPD and SPM we conclude that the colocalization of polyamines and their targets is evolutionary established and functionally designed vital necessity. Given their reported antioxidant, anti-inflammatory, neuroprotective, and memory enhancing properties, glial accumulation and redistribution of PAs may represent a key mechanism underlying neuroprotection, neuronal excitability, and age related decline in CNS functions. Our results

highlight the role of PAs as novel gliotransmitters and propose glial polyamine metabolism as a therapeutic target for neurodegenerative and neurodevelopmental disorders.

Institutional Review Board Statement: All experiments were conducted following the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research and in compliance with NIH requirements. All procedures received approval from the Universidad Central del Caribe Institutional Animal Care and Use Committee under protocol #018-2024-01-00, adhering to the National Institutes of Health guidelines for the humane treatment of laboratory animals.

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Reducing Neurogranin and Tau Expression in the Infralimbic Cortex Enhances Fear Extinction Memory in Stress-Exposed Female Rats

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Introduction: Traumatic experiences can induce long-lasting changes in the brain, that impair fear extinction and increase vulnerability to trauma-related disorders. Although these disorders are more prevalent in females, the molecular mechanisms underlying impaired fear extinction in this population remain poorly understood. In our previous work, we found that adult female rats exposed to single prolonged stress (SPS) and classified as stress-susceptible, based on extinction performance showed a distinct infralimbic cortex (IL) proteomic signature compared to resilient rats. Among the differentially expressed proteins, neurogranin and microtubule associated protein tau (MAPT), two proteins associated with learning and memory we upregulated in susceptible animals suggesting a possible contribution to extinction impairments. **Methods:** To assess the potential contribution of these proteins to extinction impairments, we infused an AAV9 vector expressing 3 different shRNAs targeting either neurogranin or MAPT, along with a fluorescent reporter (mCherry) into the IL of adult female rats. One month later, female rats were exposed to SPS, followed by open field test, auditory fear conditioning and extinction training one week after stress exposure. Brain tissue was collected for immunofluorescence analysis to confirm infusion site and knockdown efficiency. **Results:** IL knockdown of either protein did not alter locomotor activity, exploratory behavior, or fear acquisition and extinction learning. During extinction recall, both shRNA-NRGN and shRNA-MAPT groups displayed significantly reduced freezing compared to controls, suggesting a role for these proteins in mediating stress-induced extinction deficits. **Conclusion:** This suggests that elevated NRGN and MAPT in the IL may underlie stress-induced extinction impairments and highlights these proteins as potential molecular targets for improving extinction-based therapies in females.

IACUC approval: The Ponce Health Sciences University Institutional Animal Care and Use Committee approved all the animal work (IACUC No. 2203000877 and IACUC No. 2411144063).

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Olfaction Modulates Dopaminergic Survival in *C. elegans* Model of Traumatic Brain Injury

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Introduction: Olfactory modulation can shape both neural and metabolic responses across species. In mammals, scent perception from food sources influences appetite regulation and fat storage, highlighting a link between sensory input and physiological outcomes. Reducing mitochondrial respiration protects neurons from trauma, using 2,3-pentanedione (Dishart, G. J., 2024). Our study investigates whether exposure to volatile compounds prior to ingestion can modulate dopaminergic neuroprotection and metabolic responses in *C. elegans*. **Methods:** Methods included a series of chemotaxis assays to evaluate olfactory circuits and variabilities on neurodegeneration according to the compound used; volatile exposure assays to demonstrate which compound offered dopaminergic protection, and lastly, a neuroprotection assay, to quantify our model of trauma. **Results:** Findings supported the neuroprotection efficiency of 2,3-pentanedione in presence of an injury, almost equalizing control group levels. **Conclusion:** Mentioned chemotactic attraction suggested that olfaction influences neuronal function and survival to mechanical stress, which modulation might represent a new therapeutic avenue for neurodegenerative diseases. For future directions, it will be crucial to identify molecular mechanism involved in overall demonstrated neuroprotection, such as genomics, and continuing screening for volatile compounds that may modulate neuronal survival in other models of neurodegeneration.

Impact of neuromast size variation across development on hair cells quantification in Surface and Cave Populations of the Mexican Tetra*, Astyanax mexicanus*

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Introduction: Hearing loss is a persistent healthcare problem associated with hair cell damage. While significant strides have been made towards understanding hair cell regeneration in non-mammalian models, less is known about the plasticity of this system in natural models with sensory variation. Non-mammalian species, such as amphibians and fish, possess a remarkable superficial organ, the lateral line, that is evolutionarily linked to the vertebrate inner ear and is used to detect water currents, predators, and prey, which is crucial for survival in extreme environments such as perpetually dark caves. This is the case of the Mexican tetra, *Astyanax mexicanus*, a species that exists as surface fish and blind cavefish, with cavefish populations compensating for vision loss by expanding their lateral lines and increasing neurotransmission. However, less is known about the development of their mechanoreceptors and whether they differ from surface fish at cellular and subcellular levels across larval development. **Methods:** To answer this, we collected surface fish and cavefish larvae at various larval stages and performed immunohistochemistry and live-dye labeling to identify hair cells of the lateral line across development. **Results:** We expected the diameter of the neuromast and the number of hair cells to differ between cavefish and surface fish morphotypes. **Conclusion:** This work will shed light on the mechanisms underlying sensory system compensation across development in a fish species with adapted mechanosensory systems.

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Macro and Microscopic Changes During Regeneration and Aestivation in *H. glaberrima*

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Introduction: Echinoderms, members of the deuterostome group, exhibit remarkable capabilities in physiological processes such as regeneration and aestivation. These types of mechanisms are characteristic in members of the class Holothuroidea, also known as sea cucumbers. Following injury or autotomy, these animals can regenerate many of their external or internal organs. In addition, they can undergo aestivation, a dormant state, to survive under stressful conditions, such as a lack of nutrients. In this study, we focus on exploring the cellular and anatomical changes behind aestivation and regeneration processes in the sea cucumber *Holothuria glaberrima*. **Methods:** Four animals were eviscerated and left to regenerate for two months and compared with 4 non-eviscerated controls. None of the animals were fed during the experimentation period. We conducted weekly macroscopic and microscopic measurements in both regenerating and control groups. These included: length, width, weight, volume measurements and the analysis of the different cell populations in the coelomic fluid. **Results:** Preliminary experiments show a small downward trend in the overall body size of both holothurian groups but no significant difference between eviscerated and non-eviscerated groups. At a cellular level, differences in cell populations were found between eviscerated and non-eviscerated animals. The observed macroscopic and microscopic changes shed light into the ongoing processes that mediate aestivation and regeneration. **Conclusion:** Therefore, this will assist in establishing a route into further understanding of the echinoderm remarkable properties.

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MARF manipulation in *Drosophila*: Role of Glial Mitochondria in Neuronal Function and Neuroplasticity

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Introduction: Neuroplastic processes, aside from neuronal function, support communication and adaptability within the brain, thus requiring a substantial amount of energy. The energy required for these activities originates from mitochondrial oxidative phosphorylation (OXPHOS). It is suspected that neuronal metabolism receives critical support from glial cells by specifically shuttling lactate, a result of glycolysis. As it is widely recognized, glial cells primarily depend on glycolysis and the mitochondrial function within the cells is less relevant. Findings by the Barnhart lab, show that there is a higher rate of large and branched mitochondria in glial cells. The mitochondrial morphology and functional significance within these glial cells, however, are unresolved and poorly understood. This study focuses on understanding how the differing mitochondrial morphologies in glial cells may affect neuronal function and neuroplasticity in *Drosophila melanogaster*. **Methods:** To comprehend this, GAL4/UAS genetic manipulation will be implemented to adjust the expression of MARF, a mitochondrial pro-fusion factor. MARF will be amplified to stimulate the elongation of the mitochondria, while RNAi will promote the more punctate and shorter mitochondria. **Results:** As a result, these assays demonstrated that the overexpression of MARF results in longer mitochondria, with minimal impact on fly viability, while MARF knockdown leads to fragmented mitochondria, decreasing fly viability. **Conclusions:** Understanding the structural aftermath within the *Drosophila* brains, these alterations will be analyzed utilizing confocal microscopy. Additionally, behavioral experiments, such as a geotaxis climbing assay, will be performed to further evaluate the neuronal function. Another variable that will be introduced to measure the effects of the glial mitochondrial perturbation on neuronal plasticity is alcohol, as it has a direct impact on glial cells and their oxidative processes. This project pretends to shed a light on the comprehension of glial cells' mitochondrial morphology, their role and impact within neuronal function and neuroplastic processes, as well as understanding the contributing factors for overall health and adaptability of glial cells in the nervous system.

Chronic Glyphosate Exposure at a Dose Considered Safe Induces Neuroinflammation and Metabolic Alterations in Adult Male Rats

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Introduction: Glyphosate is a widely used herbicide whose agricultural benefits are well recognized; however, growing evidence suggests that it increases anxiety-like behaviors even at levels considered safe by the Environmental Protection Agency (2.0 mg/kg/day), though the molecular and metabolic pathways underlying these effects remain largely unknown. **Methods:** Adult male rats were given ad libitum access to either glyphosate-contaminated drinking water (2.0 mg/kg/day) or filtered water (control) for 12 weeks. Behavioral assessments were performed in week 10 using the Elevated Plus Maze to evaluate anxiety-like behaviors, measured by time spent in the open arms. At the end of the exposure period, blood and brain samples were collected for ELISA-based quantification of inflammatory markers and metabolomic analyses. **Results:** Glyphosate-exposed rats exhibited a significant increase in anxiety-like behaviors compared to controls (Ctrl: 13 sec; Glyph: 8 sec; t28 = 2.157, p = 0.0397; T-test). ELISA findings showed elevated IL-6, IL-10, and CXCL in serum and brain, indicating activation of systemic and neuroinflammatory pathways. Metabolomic profiling further revealed alterations in metabolites associated with neuroprotection and energy balance, including hydracrylic acid, hydroxybutyric acid, L-serine, and L-threonine. **Conclusion:** Chronic oral exposure to glyphosate, even at doses considered safe, induced significant inflammatory responses in blood and brain and disrupted essential metabolites critical for neuronal health. These findings provide novel evidence that glyphosate may impact metabolic and neuroinflammation pathways, offering mechanistic insight into how environmental herbicide exposure could contribute to the development of anxiety-related phenotypes.

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Neuroplasticity and Niche Interactions: Modulating Hematopoietic Engraftment and Neural Circuit Maturation

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Introduction: Hematopoietic stem and progenitor cells (HSPC) can develop into all blood cell types and are used in transplants to treat blood disorders, but their interaction with the nervous system is poorly understood. Recent studies suggest that cholinergic signaling pathways are essential for regulating HSPC behavior and may influence transplant success. This research hypothesizes that neuroplasticity plays a role during early stem cell engraftment in developing zebrafish and affects sensory responses and neural circuit organization. Methods: Using zebrafish, the study will employ pharmacological modulation, imaging, and protein expression to investigate how cholinergic signaling impacts the engraftment of hematopoietic stem cells. Conclusion: The expected outcome is to identify cholinergic fibers forming and establishing connections near hematopoietic niches in the kidney marrow. Over the coming months, we will analyze zebrafish tissues treated with cholinergic signaling compounds to assess if modifications to neural plasticity and the relevance of these mechanisms for the study engraftment of hematopoietic stem cells.

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Tachykinin expression in the central nervous system of *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni*

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Introduction: The snail *Biomphalaria glabrata* serves as the intermediate host for *Schistosoma mansoni*, the parasite responsible for the most common form of human schistosomiasis. Despite its biomedical significance, little is known about the neuropeptidergic systems of *B. glabrata*. Tachykinins are a highly conserved family of neuropeptides in vertebrates, often linked to gut–brain axis signaling and modulation of digestive and reproductive activity. However, studies on tachykinins in gastropods, particularly *B. glabrata*, are limited. Understanding their distribution could provide insights into the neural regulation of visceral processes and host–parasite interactions. **Methods:** Dissections were performed to isolate the central nervous system (CNS) of adult *B. glabrata*. Specific probes and antibodies were designed against *B. glabrata* tachykinin transcript and peptide (YRPSGFQGSR-NH_2). In situ hybridization chain reaction (HCR) and immunofluorescence were used to assess expression at the mRNA and protein levels, respectively. Whole-mount CNS samples were imaged with confocal microscopy, and images were analyzed using ImageJ. All protocols were approved by the UPS MSC IACUC. **Results:** Tachykinin mRNA and peptide expression were detected in multiple CNS ganglia. In the visceral ganglion, approximately eight peptide-positive and seven mRNA-positive cells were identified, six of which co-expressed both markers. The left parietal ganglion showed twelve peptide-positive and nine mRNA-positive cells, while the right parietal ganglion contained a single co-expressing cell. Bilateral symmetry was observed in the cerebral ganglia, with twelve peptide-positive and seven mRNA-positive cells, all mRNA-positive cells showing co-expression. In the buccal ganglia, expression was asymmetrical, and additional peptide-positive cells and fibers were observed beneath the crop in a gastric gland region. **Conclusion:** The expression of tachykinin mRNA and peptide within specific central ganglia, particularly the visceral and parietal regions, suggests a conserved role in modulating visceral motor and digestive functions in *B. glabrata*. The presence of tachykinin-positive fibers and cells in peripheral digestive tissues indicates possible neuromodulatory roles beyond the CNS. These

findings point to a functional link between tachykinin signaling and visceral physiology, potentially influencing host–parasite interactions. Future studies should investigate receptor localization, physiological effects on gut motility, and changes in tachykinin expression following *S. mansoni* infection to better understand the functional significance of this neuropeptide system in the snail host.

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Exploring epigenetic mechanisms of alcohol tolerance: the role of Tip60 via CRISPR editing

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Introduction: Sustained alcohol consumption alters neural circuitry through adaptive neurobiological processes that drive tolerance and dependence, ultimately contributing to the progression of Alcohol Use Disorder (AUD). This chronic brain condition affects nearly 56% of Puerto Ricans both on the island and in the U.S., underscoring its major public health burden. Tolerance reflects maladaptive plasticity within circuits governing reward, stress, and executive control, yet the molecular regulators of these neural changes remain understood. Epigenetic remodeling has emerged as a key mechanism shaping alcohol-induced neural plasticity, with histone acetyltransferases such as Tip60 playing critical roles in regulating synaptic gene expression and neuronal function. In this study, we investigate Tip60 as a neuroepigenetic modulator of alcohol tolerance using the Drosophila melanogaster nervous system as a genetically tractable model. Methods: We developed a transgenic construct for targeted epigenetic editing that fuses the histone acetyltransferase (HAT) domain of Tip60 to a catalytically inactive CRISPR-dCas9 platform. Results: Using Gibson Assembly, we successfully cloned the HAT domain into a dCas9 construct, validated its genomic incorporation, and established the framework for precise acetylation of alcohol responsive genes within neuronal circuits. This approach enables us to manipulate chromatin states in a locus specific manner, directly linking Tip60-mediated acetylation to neural gene regulation underlying alcohol tolerance. Conclusion: Future work will involve crossing the validated construct with gRNA-expressing Drosophila lines, followed by behavioral alcohol tolerance assays in the F1 generation. These experiments will test whether targeted epigenetic editing of neuronal genes alters the trajectory of alcohol tolerance, providing insight into how Tip60-driven chromatin remodeling contributes to maladaptive neuroplasticity and the pathogenesis of chronic alcoholism.

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Testing the Necessity of LB23 Neurons in *Drosophila* Grooming Behavior

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Abnormally repetitive behaviors are hallmark symptoms of autism and obsessive-compulsive disorder (OCD). These behaviors have been linked to disruptions in brain regions that select and execute movement sequences. Therefore, elucidating the neural pathways involved in sequential actions is crucial for understanding some of the challenging physical symptoms of autism and OCD. To this end, *Drosophila melanogaster* is a well-established model for studying neural organization of sequential actions, as they groom in a predictable sequence of leg movements when sensory stimuli such as dust contact bristles on the body. In fact, previous work in *Drosophila* has shown that mutations in genes implicated in human repetitive behaviors can also produce repetitive grooming in flies. Using the FlyWire 3D electron microscopy dataset, we previously identified a hemilineage of brain neurons (LB23) that shows high connectivity and receives input from bristle mechanosensory neurons. Optogenetic activation of LB23 subsets via CsChrimson elicits grooming of specific head regions, whereas simultaneous inhibition of most LB23s causes head-grooming defects. We therefore hypothesized that LB23 subsets are not only sufficient but also necessary for proper head grooming. **METHODS:** To test this, we expressed *hid* in specific LB23 subsets using five split-GAL4 lines—the same used for activation—to induce cell death, along with a split-GAL4 control. We also used R77C10-GAL4 for broad LB23 inhibition with a corresponding control. Isolated flies were coated with dust and left undisturbed for either 1.5 or 6 minutes to evaluate how well they groomed over time. Impaired grooming resulted in dust accumulation on specific head regions, which was imaged via light microscopy to generate heat maps of dust patterns in FIJI. Our **results** show that inhibition of LB23 subsets via the split-GAL4 lines resulted in no grooming defects relative to controls, suggesting they are not necessary for head grooming. In contrast, broad LB23 inhibition via R77C10-GAL4 resulted in head grooming defects. These results indicate that LB23 neurons as a population are required for proper head grooming, but the LB23 subsets could be compensating for each other when inhibited alone. Indeed, it is also possible

that other interneurons besides LB23s could be involved in grooming and are yet to be discovered. **CONCLUSIONS:** Identifying the neurons involved in the grooming circuit could help uncover general principles of how neural networks generate sequential actions and complex behaviors, with potential relevance to symptoms of autism and OCD.

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Design and Validation of a High-Throughput Screening Platform to Identify Compounds that Promote Healthspan in *Drosophila melanogaster*

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Introduction: Human aging is influenced by oxidative stress, which causes cellular damage and contributes to the development of chronic diseases. In Puerto Rico (PR), where the average population age is 65 compared to 77 in the continental United States, the aging population faces growing health challenges. Extending healthspan, the portion of life spent in good health, has become a central goal in aging research, focusing on genetic, pharmacological, and dietary interventions. The fruit fly *Drosophila melanogaster*, due to its genetic similarity to humans, short lifespan (60–80 days), and well-characterized aging phenotypes, serves as a valuable model organism for investigating mechanisms of aging. One assay commonly used in this context, the Smurf Assay (SA), assesses gut integrity by feeding flies blue dye; when the dye leaks into the hemolymph, the flies turn blue, signaling loss of gut barrier function and impending death. Because gut integrity is closely tied to both lifespan and healthspan, we are developing a high-throughput (HT) screening platform that automates the SA to enable rapid testing of compounds that may promote gut health and delay aging-related diseases. **Methods:** The platform involves feeding flies food infused with blue dye #1 and test compounds, followed by quantification of the “Smurf” phenotype using spectrophotometry. This design provides a scalable and reproducible alternative to traditional assays, facilitating efficient compound evaluation. **Results:** As a proof of concept, we are currently optimizing assay parameters and testing curcumin, a bioactive compound found in turmeric known for its antioxidant and lifespan-extending properties, to assess its effect on gut barrier function in $Sod1^{n1}$ heterozygote mutants—a model of accelerated aging due to oxidative stress. **Conclusion:** Future work will focus on validating the HT platform and expanding the screening to include additional natural compounds, such as sesamin and resveratrol, as well as potentially detrimental agents like allopurinol. By leveraging Drosophila genetics and automated screening technology, this work aims to establish a robust tool for identifying compounds that protect against oxidative stress and promote healthier aging, with potential translational relevance to human healthspan research.

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TFEB Regulates DVL2 via CLEAR Motifs to Modulate Wnt Signaling

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Introduction: Autophagy and Wnt signaling are evolutionarily conserved pathways that maintain cellular homeostasis, differentiation, and stress responses. Although both are implicated in neurodegeneration and cancer, and are critical developmentally, their mechanistic crosstalk remains poorly defined. The transcription factor EB (TFEB) is a master regulator of lysosomal biogenesis and autophagy, activating target genes through CLEAR (Coordinated Lysosomal Expression and Regulation) motifs. In silico motif analysis of TFEB binding targets, revealed three CLEAR motifs within the promoter of Dishevelled 2 (DVL2), a critical component of the Wnt pathway, suggesting that TFEB may directly regulate Wnt signaling. **Methods:** We conducted chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) to test TFEB binding to the DVL2 promoter and performed TOPFlash/FOPFlash luciferase assays to assess how TFEB overexpression impacts Wnt pathway activation. ChIP-qPCR confirmed specific, CLEAR-motif-dependent binding of TFEB to the DVL2 promoter. **Results**: Functional assays demonstrated that TFEB overexpression significantly alters canonical Wnt signaling activity, consistent with transcriptional regulation of DVL2. These findings reveal that TFEB directly influences Wnt pathway dynamics by binding to defined CLEAR elements. **Conclusions**: These findings suggest a previously unrecognized TFEB–DVL2 regulatory axis linking autophagy and Wnt signaling. Ongoing and future studies will aim to validate this interaction through additional mechanistic assays, including site-directed mutagenesis of CLEAR motifs, qRT-PCR and western blot analyses of downstream Wnt targets, and disease-model experiments assessing autophagy–Wnt crosstalk. We will also explore the role of TFEB post-translational modifications, such as TFEB PARsylation, in modulating this interaction. This work may uncover new regulatory nodes at the interface of autophagy and Wnt signaling, with implications for pathologies such as cancer, aging and neurodegeneration.

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Smurf Bees: an assay to determine intestinal integrity as a measure of health decline with age or other factors in honey bees (*Apis mellifera*)

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Introduction: Aging affects overall health, including gut integrity. Intestinal permeability, the passage of materials from the gut lumen into circulation due to epithelial damage, is observed with reduced gut integrity in various species. The Smurf Assay (SA), developed in *Drosophila melanogaster*, assesses gut integrity using non-absorbable blue dye. In healthy individuals, the dye remains in the gut, but in compromised individuals, it spreads throughout the body, indicating reduced gut health and proximity to death. **Methods:** We adapted SA to *Apis mellifera* to assess gut integrity changes due to age or stressors. First, we tested dye toxicity in forager bees at 0%, 0.1%, 0.5%, and 2% dye concentrations in 2 m (molal) sucrose. With 40–50 bees per box, survival probability and Smurf phenotype frequency were recorded over eight days. **Results:** Smurf bees appeared in <1% of cases after 24 hours. Mortality did not increase in blue-fed bees for the first four days but rose thereafter, correlating with age. When paraquat, an oxidative stressor, was added, Smurf frequency and mortality increased with paraquat dose. **Conclusion:** This study is the first to adapt SA in bees, demonstrating its effectiveness in assessing gut permeability, similar to other species. We discuss the significance of increased Smurf phenotype frequency with age and stress exposure.

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Elucidating the Genomic Modulation of the H-current in Ventral Tegmental Area Dopamine Neurons following Cocaine Sensitization

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Introduction: Substance Use Disorder (SUD) is considered one of the leading causes of premature death worldwide, with cocaine ranking as the second most commonly used illicit substance in the United States. Despite its widespread use and serious health consequences, effective treatments for cocaine dependence remain absent. Drugs of abuse such as cocaine induce long-lasting plastic changes in the mesolimbic dopamine (DA) system, which plays a central role in reward processing, and epigenetic alterations may mediate these functional modifications. Previous studies show that cocaine sensitization alters intrinsic properties of DA neurons in the ventral tegmental area (VTA), including reductions in the hyperpolarization-activated cation current (Ih), mediated by HCN channels, yet the epigenetic regulation of HCN channels or the Ih current remains unknown. **Methods:** Using whole-cell patch-clamp recordings, we investigated the effects of the HDAC inhibitor SAHA on cocaine-induced changes in Ih, and evaluated SAHA's influence on evoked excitability in brain slices from cocaine-sensitized rats. Evoked action potentials were recorded in current-clamp mode and quantified as spikes elicited by thirteen consecutive depolarizing current steps (50 pA increments, 1-second pulses). Midbrain slices were incubated in vitro with SAHA (3 μM, two hours). **Results:** SAHA treatment restored Ih amplitude to baseline levels, reversing the cocaine-induced reduction. Current-clamp analyses further showed that cocaine exposure enhances DA neuron responsivity to depolarizing current injections, whereas SAHA-treated tissue exhibited baseline levels of evoked excitability. **Conclusion:** These results support the hypothesis that cocaine-induced reductions in Ih amplitude in VTA DA neurons are epigenetically regulated, and the normalization of excitability following SAHA treatment suggests that HDAC inhibitors may reverse cocaine-induced neuroadaptations in the mesolimbic dopamine system.

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Regeneration of sensory hair cells in late-larval stages of the Mexican tetra

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Hair cells are mechanoreceptors located in the center of the neuromast, the functional units of the lateral line. They play a key role in enabling fish to sense water movement and the locations of prey and predators. These sensory cells are homologous to those found in the inner ear, responsible for translating auditory and vestibular information into electrical signals that our central nervous system can interpret. While non-mammalian vertebrates, like zebrafish, can readily regenerate hair cells, mammals, including humans, are not able to regenerate these mechanoreceptors. Our goal is to explore whether natural models with hair cell compensations due to lack of other senses, like vision, also have enhanced regeneration mechanisms, or whether regeneration in these natural models is impaired. Here, we ablated hair cells of 10 to 13-day post-fertilization (dpf) surface fish and cavefish larvae using ototoxic antibiotics (neomycin). We imaged hair cells as they regenerated in live-dye labeled larval surface fish specimens, at 24-, 48-, and 72 hours post-treatment (hpt). Next, we quantified hair cells from fluorescence images labeled with FM-143 (hair cell live-dye). Based on preliminary data, we are expecting cavefish to regenerate slower compared to surface fish morphotypes. This could be associated to changes in their immune response, which is a critical and preceding step to regeneration. This work will reveal whether regeneration is unchanged, reduced, or enhanced in adapted lateral lines, offering insights into the evolution of sensory repair mechanisms.

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Understanding the Interaction Between the Extracellular Domain (ECD) of the α10 nAChR Subunit with the Cone Snail Toxin α-RgIA

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Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels that play essential roles in presynaptic neurotransmission and are postulated to be involved in several mechanisms, such as locomotion, cognition, and auditory functions. nAChRs have also been identified as potential pharmacological targets for schizophrenia and substance abuse, including nicotine and amphetamine. However, to develop specialized pharmacotherapies targeting nAChRs, it is necessary to understand how they interact with ligands. This study focuses on determining the interaction between the extracellular domain (ECD) of the α10 nAChR subunit with the cone snail toxin α-RgIA. We designed an α10 nAChR ECD–acetylcholine binding protein chimera (α10–AChBP) using the α7–AChBP chimera as a template. The α10–AChBP was expressed using the baculovirus/insect cell expression system, purified by standard recombinant techniques, and the structure will be solved by crystallography and x-ray diffraction.

Matrilin-3 Gene Expression is Associated with Glial Remodeling and Poor Prognosis in Glioblastoma

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INTRODUCTION: Glioblastoma (GBM) is the most common primary brain malignancy, characterized by rapid progression and poor survival despite multimodal therapy. **METHODS:** To identify prognostic molecular features, RNA-seq data from 110 newly diagnosed GBMs (IDH-wildtype grade 4) in the GLASS consortium were analyzed. A transcriptome-wide univariate log-rank screen was performed to construct an initial gene signature. Subsequent multivariable Cox regression was conducted to assess independent prognostic value, adjusting for age, sex, radiotherapy, temozolomide treatment, and ESTIMATE score. Weighted gene co-expression network analysis (WGCNA) was performed based on expression quartiles, followed by functional enrichment analysis for modules of interest. Lastly, preranked gene set enrichment analysis (GSEA) using Spearman correlation coefficients was visualized with Cytoscape EnrichmentMap. **RESULTS:** High quartile MATN3 expression correlated with shorter survival in the univariate screen ($p = 0.01$) and remained an independent predictor in multivariable Cox regression (likelihood ratio = 7.16, $p = 0.007$). WGCNA identified a significant co-expression module with eigengene expression paralleling survival differences across MATN3 quartiles. Functional enrichment for biological processes of this module implicated glial cell differentiation, gliogenesis, and various myelination processes (FDR < 0.001). An additional module, enriched in MATN3-low tumors, suggested low-expression tumors are associated with upregulated mitochondrial anti-apoptotic signaling pathways, primarily through MTRNR2L family genes (FDR < 0.001). Preranked GSEA with a stringent threshold (FDR ≤ 0.001, $p \leq 0.001$, overlap coefficient = 1) revealed prominent clusters of gene sets linked to ion regulation, deoxyribonucleotide biosynthesis, and protein membrane targeting. **CONCLUSIONS:** These results suggest MATN3 expression is associated with poor survival in GBM and a transcriptional network enriched for glial differentiation and myelination pathways.

Monocarboxylate transporter 2, lactate dehydrogenase A & B protein expression in the brain and peripheral tissues in an Angelman syndrome mouse model

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Introduction: Angelman syndrome (AS) is a neurodevelopmental disorder characterized by intellectual disability, muscle and movement defects, seizures, and a happy demeanor. Alterations in the maternal chromosomal region 15q11.2-q13.1 cause Angelman syndrome (AS) by deleting the maternal *UBE3A* gene, which encodes a ubiquitin-protein ligase E3 that plays a key role in protein degradation and nervous system development. In the Angelman syndrome mouse model, altered metabolism has been observed, characterized by elevated levels of lactate, acetate, and succinate, as well as increased lactate dehydrogenase A (*Ldha*) gene expression in mouse embryonic fibroblasts. We hypothesize that the loss of the maternal *Ube3a* gene expression affects the protein expression of monocarboxylate transporter 2 (MCT2), lactate dehydrogenase A (LDHA), and lactate dehydrogenase B (LDHB) in the AS mice model, which could contribute to the high lactate levels. **Methods:** Western Blot analysis measured the protein expression of MCT2, LDHA, and LDHB in the hippocampus, cerebellum, lung, heart, pancreas, liver, and kidneys of adult *Ube3a* $^{m-/p+}$ AS and *Ube3a* $^{m+/p+}$ wild-type mice. **Results:** The results reveal significant differences in LDHB in the cerebellum, LDHA in the kidneys, and
LDHB, LDHA, and MCT2 in the liver, between genders and genotypes. **Conclusion:** Loss of Ube3a can increase protein expression of LDHA, LDHB, and MCT2 in various brain and peripheral tissues. However, this effect is not observed in all cases and is also gender-specific.
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Bmal1 regulates daily Ca2+ amplitude rhythms in motor cortex.

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Introduction: Circadian rhythms regulate daily behaviors such as locomotion, primarily via the suprachiasmatic nucleus (SCN). Other brain regions, including the primary motor cortex (M1), may contain local circadian clocks important to modulate circadian expression of locomotor behaviors. Here, we tested whether the core clock gene *Bmal1* regulates circadian patterns of Ca2+ activity in M1. **Methods:** We performed bilateral viral knock-out of *Bmal1* in M1 neurons of *Bmal1* $^{f/f}$ mice by injecting an AAV8-Syn-Cre virus and recorded neuronal calcium activity via fiber photometry by injecting in the same site an AAV8-Syn-GCaMP8m virus. Locomotor behavior was simultaneously tracked using ATOMs (Automated Tracking of Mouse behavior). Data were collected over four consecutive days under a 12:12 light-dark cycle. **Results:** Preliminary results indicate that *Bmal1* $^{f/f}$ mice exhibited calcium circadian rhythms with higher amplitude compared to wildtype controls. Moreover, both genotypes displayed increased calcium activity and locomotion during the dark phase when mice show higher locomotor activity, suggesting that circadian modulation of M1 neurons may be preserved despite local *Bmal1* knock-out. Histological analysis showed that in fact BMAL1 signal was reduced in the Cre-expressing neurons. **Conclusions:** These findings suggest that M1 calcium rhythms may persist independently of local *Bmal1*, possibly driven by extrinsic circadian input.

Characterization of Metabolomic and Behavioral Changes in Young and Old *Drosophila* Adults Mono-Associated with Probiotic *Lactiplantibacillus plantarum*

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Introduction: The gut microbiota-brain axis is a bidirectional communication between the resident microbes, the gut, and the brain; it plays a pivotal role in aging and age-related diseases. Probiotics have emerged as a promising avenue for interventions targeting age-related diseases by modulating this axis. Of particular interest is how certain probiotics can influence the host metabolome, a mechanism that could further our understanding of bacterial effects on the gut-brain axis. **Methods:** To study this we are using *Drosophila melanogaster* as a genetically amenable model that displays age-related phenotypes and has a simple microbiota that is easy to manipulate. To explore the effects of the commensal bacteria and probiotic *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) on the metabolome of young and old *Drosophila*. First we aged flies to 5 days (young) or 45 days (old), then we treated them for 5 days with an antibiotic cocktail to generate Antibiotic-Induced Microbiome-Depleted (AIMD) flies. Then, we mono-associate them with *L. plantarum* for 1 day. Flies were flash-frozen and separated by heads (brain) and bodies (gut) and collected separately. From a total of 855 biochemicals, we identified changes in metabolites that are age-, tissue- and treatment-specific. PCA analysis revealed as expected there was a distinct separation between age and body parts. **Results:** Although the influence of *L. plantarum* supplementation on the metabolome appeared relatively modest, there were significant differences in neurotransmitters, specifically acetylcholine which is increased in the bodies of old flies treated with *L. plantarum*. Next, we explored the effects of *L. plantarum* on locomotion and sleep in conventional and AIMD-raised flies. Flies were treated for 3 days with either a 5% sucrose solution as a mock control, one of two *L.plantarum* strains (wild-type fly-derived LpWF or cabbage-derived Lp39), or *E.coli* as a negative control in 5% sucrose. Locomotor and sleep behaviors were assessed using the Trikinetics DAM2 monitoring system. Results indicated that AIMD flies treated with *L. plantarum* exhibited an increase in overall sleep duration and a decrease in sleep latency but the latter is not specific to *L. plantarum* as *E.coli* had the same effect. In terms of locomotion, *L. plantarum* seems to rescue a hyperactive phenotype exhibited by AIMD flies. **Conclusions:** Our findings suggest that *L. plantarum* exerts subtle but specific effects on neurotransmitter metabolism and behavior in aged *Drosophila*. Ongoing experiments aim to determine

whether these phenotypes are mediated by neurotransmitters such as acetylcholine.

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Optimization Protocol for Obtaining Organic Extracts from Tropical Marine Macroalgae with Potential Anxiolytic Effects

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Introduction: Marine macroalgae represent a promising source of natural compounds with neuropharmacological potential. This study focuses on optimizing extraction and preparation protocols for bioactive compounds with anxiolytic effects derived from *Stypopodium zonale*, a brown macroalga collected from coastal waters of Puerto Rico. The main objectives included the comparison of extraction methods, the evaluation of solvent systems with varying polarities, and the development of a preparation protocol for administration in *Drosophila melanogaster*. Method: Three extraction techniques were compared: passive extraction, Soxhlet extraction, and ultrasonic bath. Results: The ultrasonic bath method demonstrated the highest recovery yield and operational efficiency. Various solvent systems were evaluated, with the dichloromethane: methanol (2:1) mixture proving most effective for obtaining the crude extract. The preparation protocol optimized the dissolution of the crude extract in 95% ethanol, establishing an ideal concentration of 1 µg/µL to ensure safe and low-toxicity administration in *D. melanogaster*. Previous behavioral assays in *D. melanogaster* showed a reduction in anxiety-related behaviors following exposure to the extract, suggesting the presence of potential anxiety-related agents. Complementary analyses were initiated, including flash chromatography and H-NMR spectroscopy, for chemical characterization of the obtained fractions. Conclusion: This work consolidates various future methodologies to isolate and identify the compounds responsible for the observed effects, thereby contributing to the potential development of a macroalgae metabolite database in Puerto Rico and the exploration of marine-derived therapeutic agents.

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Neural Downregulation of Serotonergic Signaling During Aestivation in Biomphalaria glabrata

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Introduction: Aestivation is an ecological phenomenon in which snails and other animals enter a state of dormancy in response to drought. This behavior enables species like the freshwater Pulmonata snail *Biomphalaria glabrata* to survive prolonged periods of environmental stress. Notably, B. glabrata serves as the intermediate host for Schistosoma mansoni, the trematode responsible for schistosomiasis, a disease affecting over 200 million people worldwide, particularly in rural areas of developing countries. Understanding how B. glabrata aestivates is of epidemiological interest, as this dormancy may facilitate long-term parasite survival and hinder disease containment efforts. While prior studies have focused on the metabolic consequences of aestivation, little is known about its effects on the central nervous system (CNS). Method: In this study, we examined CNS adaptations in uninfected, aestivated B. glabrata using immunohistochemistry. Snails were maintained in a moist incubator (90 ± 1% humidity; 23.5 ± 1°C) for 30 days to induce aestivation. Whole-mount preparations were processed using standard IACUC approved protocols and labeled with antibodies against serotonin. Results: Confocal microscopy revealed that all major serotonergic neuron clusters identified in active snails, including those in the cerebral, pedal, parietal, and visceral ganglia, were anatomically preserved during aestivation. However, serotonin-like immunoreactivity was markedly reduced across all ganglia, with diminished staining intensity and fewer detectable fibers. Conclusion: Given serotonin's key role in locomotion, arousal, reproduction, and sensory processing, these results suggest that downregulation of serotonergic signaling contributes to the CNS suppression and behavioral inactivity observed during aestivation. Therefore, this work provides novel insight into neurochemical adaptations that may support survival in extreme environments.

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Metabolomic brain and blood profiling after glyphosate exposure in adult male rats

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Introduction: The herbicide glyphosate may harm behaviors related to mental health. Preliminary work from our group suggests that safe levels of glyphosate (2.0 mg/kg/day, E.P.A.) increase anxiety-like behaviors in rats. Increased anxiety is detrimental and may affect other defensive behaviors such as avoidance. Our exploratory work is focusing on avoidance behaviors and determining the biological mechanisms by which glyphosate influences avoidance. Here, we focus on metabolites that participate in cellular homeostasis to regulate physiological processes, and metabolite levels associated with anxiety-like behaviors. There are two key brain regions implicated in avoidance: 1) medial prefrontal cortex and 2) amygdala. Thus, we hypothesized that exposure to glyphosate disrupts metabolites in both brain regions. We also compared systemic effects of glyphosate in the blood.

Methods: We tested our hypothesis by assessing a subset of male rats (Sprague-Dawley, 3 months of age at commencement of experimetns) that were trained on platform mediated avoidance. Next, rats were exposed to glyphosate (2.0 mg/kg/day) or filtered water for controls for 12 weeks. Upon completion of the behavioral experiments, for brain samples (Glyph: n=6; Ctrl: n=6) and blood (Glyph: n=13 ; Ctrl: n=13) were collected. Brain tissue and blood samples underwent metabolomic analysis by researchers blinded to the experimental groups using gas chromatography-mass spectrometry.

Results: We observed that glyphosate exposure altered the concentrations of 27 metabolites in brain tissue and 10 metabolites in blood. Specifically, 4-hydroxybutanoic acid, a critical GABA metabolite essential for anxiety control, was decreased in blood ($p = 0.0004$), indicating compromised inhibitory neurotransmission. Additionally, L-serine showed decreased blood levels ($p = 0.0005$) but increased brain accumulation ($p = 0.014$), suggesting impaired neurotransmitter synthesis pathways. Concurrently, azelaic acid was elevated in brain tissue ($p = 0.033$), indicating active neuroinflammation and oxidative stress, while ethanolamine increased in brain ($p = 0.012$), reflecting membrane damage.

Conclusions: These metabolic alterations demonstrate that glyphosate exposure disrupts GABAergic neurotransmission and induces neuroinflammation, providing molecular evidence for the observed anxiety-like behavioral phenotype.

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High Glucose-Induced Activation of Microglia: Insights into Neuroinflammation in Diabetes

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Background: Type 2 diabetes mellitus (T2DM) is associated with an increased risk of neurodegenerative diseases, including Alzheimer's disease. Chronic hyperglycemia can induce systemic inflammation, which may extend to the central nervous system and trigger microglial activation, a hallmark of neuroinflammation. However, the molecular mechanisms linking high glucose to microglial inflammatory responses remain incompletely understood. **Objective**: To evaluate the effects of high-glucose exposure on microglial activation, oxidative stress, and inflammatory signaling pathways. **Methods**: Human microglial (HMC3) cells were cultured under normal or high-glucose conditions. Morphological changes were assessed microscopically. The expression and secretion of pro-inflammatory cytokines (TNF-α and IL-6) were quantified using qPCR and ELISA, respectively. Reactive oxygen species (ROS) production was measured by flow cytometry, and NF-κB activation was evaluated by expression and nuclear translocation assays. **Results**: High-glucose exposure induced morphological changes consistent with microglial activation, characterized by reduced branching and enlarged soma. ROS production significantly increased under high-glucose conditions, indicating elevated oxidative stress. Furthermore, TNF-α and IL-6 expression and secretion were markedly upregulated. NF-κB activation and nuclear translocation were also enhanced, confirming the involvement of this pathway in glucose-mediated neuroinflammation. **Conclusion**: Our findings demonstrate that high-glucose conditions promote microglial activation through oxidative stress and NF-κB-dependent inflammatory signaling. These results provide mechanistic insight into the link between diabetes and neuroinflammation, highlighting NF-κB and ROS pathways as potential therapeutic targets to mitigate diabetes-associated neuropathology.

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Astroglial Overexpression of Tissue Non-Specific Alkaline Phosphatase Induces Hyperphosphorylation of Tau, Possible Implication in Alzheimer's

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Introduction: Alzheimer's Disease (AD) is a progressive neurodegenerative disease and the leading cause of dementia worldwide, with no cure and available treatments offering modest benefits. As a tauopathy, AD is characterized by abnormal agglomerations of hyperphosphorylated Tau that spread in a prion-like manner through the brain. Extracellular Tau (eTau) plays an important role in this propagation and promotes neurotoxicity. Tissue non-specific alkaline phosphatase (TNAP), an ectonucleotidase and ectophosphatase, dephosphorylates eTau, favoring its neurotoxic effects. Accordingly, TNAP levels have been seen to increase in AD patient brains and overexpression of neuronal TNAP increases hyperphosphorylation of intracellular Tau, while TNAP blockade ameliorates neurotoxicity in tauopathy mouse models. Previous studies have focused on systemic cerebral TNAP or neuronal TNAP, and the role of astroglial TNAP specifically has not been previously examined. **Methods:** In this study, the function of astrocyte-specific TNAP in the context of AD was investigated using a murine model of astroglial TNAP overexpression, histologically and molecularly characterized via immunohistochemistry, immunofluorescence and Western Blotting. **Results**: Our preliminary findings indicate that astroglial TNAP overexpression favors glial reactivity, reduces neuronal density, and promotes Tau hyperphosphorylation in the hippocampal region of the mice. **Conclusion**: These findings suggest a relevant role for astroglial TNAP in AD and other tauopathies. More comprehensive investigations are ongoing to confirm and further elucidate this role.

The effects of Angiotensin-Converting Enzyme inhibitor in Caenorhabditis elegans Parkinson's Disease model

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Background: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the aggregation of α-synuclein in presynaptic terminals. Under normal conditions, α-synuclein regulates synaptic vesicle trafficking and release; however, in PD it misfolds and forms insoluble fibrils. The brain's renin-angiotensin system (RAS) modulates sensory processing, learning, and memory, and its activation in the nigrostriatal dopaminergic circuit can increase neuronal vulnerability through NADPH-derived reactive oxygen species (ROS). Reducing Ang II activity at AT1R has been associated with decreased oxidative stress, neuronal loss, and neuroinflammation. This study hypothesizes that the ACE inhibitor captopril can decrease α-synuclein aggregation in Caenorhabditis elegans.Methods: C. elegans strain NL5901 was used to perform fluorescence assays and Nose Touch Avoidance (NTA) tests following exposure to 2.54 mM captopril. Strain BZ555 was exposed to 6-OHDA to induce dopaminergic neuron degeneration. Fluorescence imaging quantified α-synuclein aggregation in synchronized NL5901 worms, while NTA assays assessed locomotor response (backward movement) following nose touch stimulation. Results: Preliminary NTA results showed a significant difference between the control group (M9 buffer) and the experimental group exposed to 2.54 mM captopril, indicating improved behavioral response. Initial fluorescence data suggest that α-synuclein aggregation intensity decreases in NL5901 worms at the L4+7 days stage after captopril exposure. Conclusions: Early findings indicate that captopril may reduce α-synuclein aggregation and improve sensory-motor behavior in C. elegans. Ongoing fluorescence assays using strain BZ555 will further determine whether captopril can protect dopaminergic neurons from degeneration.

Evaluating automated brain cell detection performance against manual counting in DAB-stained non-human primate tissue

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Introduction: Accurate identification of neurons in brain tissue is critical for evaluating the effectiveness of viral transfection techniques, particularly in non-human primates (NHPs), where transfection success can vary due to differences in promoters, tags, and other factors. This project aimed to assess transfection outcomes—for example cell density and cell type distribution—across different brain regions, experimental conditions, and transfection strategies. Although manual cell counting is highly accurate, it is time-consuming and impractical for large-scale analyses. To address this, we validated an automated neuron detection system developed in QuPath for identifying DAB-stained neurons in macaque brain slices. Method: The system's performance was evaluated by comparing automated detections to manual annotations using spatial (Euclidean distance) and morphological (intersection over union) criteria. Detections were classified as true positives (TP), false positives (FP), or false negatives (FN), and standard performance metrics—including sensitivity, precision, and F-score—were calculated. Results: The results demonstrate that the automated system reliably detects and quantifies neurons, ensuring that observed differences in cell density or morphology across conditions reflect true biological variation rather than detection artifacts. Conclusion: This approach enables efficient evaluation of transfection outcomes and supports broader implementation in both neuroscience research and clinical applications.

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Liposome-Based Therapeutics: A Novel Strategy to Mitigate Microglial Activation and Neuroinflammation in Neurodegenerative Diseases

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Background: Microglia, the resident immune cells of the central nervous system (CNS), play a key role in immune defense and inflammatory responses in the brain. As primary modulators of neuroinflammation, microglia release proinflammatory cytokines, nitric oxide (NO), and reactive oxygen species (ROS) in response to various stimuli. While essential for immune response, these inflammatory processes contribute to the progression of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Effective treatments for these conditions are limited, partly due to the challenge of crossing the blood-brain barrier (BBB). Encapsulation of therapeutic agents in liposomal vesicles has shown potential to improve drug delivery across the BBB and enable targeted delivery to brain cells. **Objective:** Evaluate the safety, cellular uptake, and anti-inflammatory effects of liposome formulations on activated microglial cells, with the aim of developing liposome-based therapeutic strategies capable of crossing the blood-brain barrier to mitigate neuroinflammation. **Methods**: In this study, we synthesized six liposome formulations incorporating DOPC, cholesterol, PEG-2000, t-butanol and an anti-inflammatory compound to evaluate their effects on microglial cell health, ROS production, and IL-6 cytokine release in IFN-gamma-activated human microglial (HMC3) cells. Cytotoxicity was assessed using MTT assays, ROS production by flow cytometry, gene expression by real-time PCR, and cytokine release by ELISA. **Results**: Our results indicate that all liposome formulations were safe for microglial cells at concentrations below 100 µg/mL, with no significant cytotoxicity. Notably, Liposome 2 effectively reduced ROS levels and proinflammatory cytokine release in activated microglia ($p < 0.05$, $n = 4$), suggesting an anti-inflammatory effect. Additionally, Liposomes 2, 4, and 6 demonstrated efficient internalization within microglial cells, indicating their suitability for CNS drug delivery. **Conclusion**: These findings suggest that Liposome 2 may inhibit microglial activation without toxicity, supporting the potential of liposome-based therapies for managing neuroinflammation in neurodegenerative diseases. This work provides a foundation for developing novel treatments aimed at AD and PD.

A Targeted Blood-Brain Barrier Drug Delivery System for the Treatment of Epilepsy

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Introduction: Epilepsy is a chronic neurological disorder characterized by recurrent seizures, often linked to neuroinflammation and neuronal hyperexcitability. The muscarinic receptor agonist pilocarpine induces seizures that progress to chronic epilepsy, providing a wellestablished model to study this disorder. Previous *ex vivo* studies from our group demonstrated that edelfosine, a lysophospholipid and phospholipase C-β (PLC-β) inhibitor, protects hippocampal neurons from pilocarpine-induced hyperstimulation. We hypothesized that edelfosine-loaded nanoparticles (NPs) can cross the blood-brain barrier (BBB), reduce seizure activity, and exert neuroprotective and anti-inflammatory effects. **Methods:** To test this, we induced epilepsy in mice using pilocarpine and administered edelfosine-loaded NPs designed for BBB targeting via transferrin and formulated as protein-based carriers using bovine serum albumin (BSA). Seizure severity was assessed over three days post-insult using the Racine scale. To examine the anti-inflammatory effects of edelfosine, RT-PCR was performed in cultured microglial cells to quantify the expression of TNFα, IL6, and NFKB1. Infrared (IR) imaging of labeled NPs was performed to assess their ability to cross the BBB and to confirm their localization within the brain parenchyma. **Results:** Treatment with edelfosine-loaded NPs significantly reduced seizure-like behaviors from days 1 to 3 after pilocarpine exposure in both male and female mice. RT-PCR analysis showed a marked downregulation of TNFα, IL6, and NFKB1 expression in microglial cells 24 hours after treatment. Preliminary IR-labeled NP imaging further indicated successful BBB crossing and delivery of edelfosine into brain tissue. Ongoing histological studies are assessing whether this treatment preserves hippocampal granule cells— identified by NeuN immunostaining—which are typically compromised following pilocarpineinduced injury. **Conclusion**: these findings suggest that edelfosine-loaded, brain-targeted nanoparticles can reduce seizure severity in a pilocarpine mouse model and suppress proinflammatory signaling in microglial cells exposed to pilocarpine. As an FDA-

approved investigational drug for cancer, edelfosine represents a promising candidate for repurposing in epilepsy therapy, offering both neuroprotective and anti-inflammatory benefits.

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Association of One-Carbon Metabolism with Neurogenesis in the Hippocampus of Running Mice

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Introduction: One-carbon metabolism integrates the folate and methionine cycles to generate one-carbon units essential for nucleotide and protein synthesis, methylation reactions, and redox balance—processes fundamental to brain function and plasticity. Exercise alters brain metabolism, yet the link between one-carbon metabolism and neurogenesis remains underexplored. **Methods:** Male C57BL/6J mice (20 weeks old) were assigned to sedentary (SED) or running (RUN) groups, with RUN mice performing voluntary wheel exercise for 8 weeks. Study was approved by the UPR-MSC under protocol number A660121.Hippocampal metabolites were profiled using GC–MS, followed by univariate, multivariate, and pathway enrichment analyses in MetaboAnalyst 6.0. Metabolic correlation networks were constructed in Python from MetaboAnalyst correlation matrices ($|r| \geq 0.3$) and visualized in NetworkX using the Fruchterman–Reingold layout (red = positive; blue = negative correlations). A differential network ($\Delta r = r\_RUN - r\_SED$) was generated to identify metabolite pairs with exercise-induced changes in correlation strength. **Results:** Metabolomic analysis revealed that one-carbon metabolism was among the most significantly enriched pathways in RUN compared to SED mice (enrichment ratio = 11; FDR-adjusted $p = 9.4 \times 10^{-8}$). Methionine, a key metabolite in the methionine cycle, was significantly upregulated following exercise, suggesting enhanced production of S-adenosylmethionine (SAM), the universal methyl donor involved in epigenetic and transcriptional regulation. Serine and glycine, intermediates of the folate cycle, were also elevated in RUN mice. Serine supports sphingolipid and phospholipid biosynthesis essential for neuronal differentiation, axonal growth, and myelination, while glycine functions as both a co-agonist at NMDA receptors to facilitate synaptic plasticity and memory and as an inhibitory neurotransmitter regulating excitability. Threonine, which contributes to glycine synthesis, further linked exercise metabolism to one-carbon pathways.

Collectively, these findings indicate enhanced structural and functional support for neurogenesis and synaptic remodeling in response to exercise. **Discussion:** This study identifies one-carbon metabolism as a key mediator of hippocampal adaptation to exercise. Coordinated upregulation of methionine, serine, glycine, and threonine suggests increased methylation potential and biosynthetic activity supporting neuronal growth and plasticity. These metabolic shifts provide a mechanistic framework linking voluntary running to epigenetic regulation, neurotransmission, and neurogenesis. **Conclusion:** Voluntary running enriches hippocampal one-carbon metabolism, increasing availability of methionine, serine, and glycine - metabolites central to methylation, membrane biosynthesis, and synaptic signaling. These results highlight one-carbon metabolism as a critical metabolic pathway through which exercise promotes brain health and cognitive resilience. **Acknowledgement:** Study supported by the PRINBRE P20 GM103475.

4R-cembranoid attenuates anxiety-like behavior and improves spatial and recognition memory in a mouse model of Gulf War Illness

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Introduction: Persistent anxiety and cognitive dysfunction are the primary symptoms in Gulf War Illness (GWI) veterans. Animal studies have confirmed that CNS dysfunction in GWI is associated with glutamate excitotoxicity, neuroinflammation, and neuronal death. Despite advances in research, effective treatment for GWI has not been established. This work investigated the efficacy of an α7nAChR modulator, 4R-cembranoid (4R), in a mouse model of GWI. 4R is a natural product known to easily cross the blood-brain barrier (BBB) and protect the brain cells from inflammation and apoptosis.

Methods: To recreate GW conditions, we administered PB and PER with DEET, traces of DFP, and moderate stress for 12 consecutive days in C57BL/J6 male mice. One month later, mice were treated with vehicle or 4R (6 mg/kg) for four weeks.

Results: Behavioral assessments revealed anxiety-like behavior, impaired spatial learning, and short- and long-term memory deficits in GWI mice receiving vehicle, which were accompanied by loss of CD45-mediated inhibitory microglial control and cytoskeletal disruption, marked by increased MAP2 phosphorylation in the hippocampus. Treatment with 4R alleviated the presented behavioral deficits and restored microglial regulatory function and cytoskeletal integrity in GWI mice. Phosphoproteomic profiling revealed that 4R acts as a multi-target regulator normalizing MAP2 phosphorylation and rebalancing presynaptic (PCLO, RIMS1/2, STX4), postsynaptic (CAMK2A, PLCB1), and cytoskeletal (MAP1B, PACSIN1) protein markers.

Conclusion: These findings suggest that 4R restores coordinated signaling underlying synaptic plasticity, neuronal structure, and behavioral recovery in GWI. Collectively, this study provides the first

evidence that 4R therapy mitigates neuroinflammation and improves cognitive and mood function in a preclinical model of chronic GWI, supporting its translational potential for affected veterans.

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Defining the Drosophila NMJ as a model system to study Synaptic Maladaptation to Alcohol Exposure

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Introduction: Alcohol tolerance is at the center of addiction and is achieved through the nervous system's adaptation to alcohol. Understanding this maladaptation is essential to tackle the devastating effects of alcohol use disorders (AUDs). The *Drosophila* larval system has been used to shed light on AUDs. A seminal study showed that associative learning is inhibited in animals subjected to rapid exposure and withdrawal; however, it is restored in chronically exposed animals. This demonstrated the nervous system's adaptation to alcohol exposure and validated *Drosophila* as a model of choice to study this adaptation. Within the nervous system, studies describing the effects of alcohol on postsynaptic targets are numerous, but those on the presynaptic release machinery are less well studied. **Methods:** In order to shed light on the synaptic changes underlying this adaptation, we assessed 4 different alcohol exposures: (1) acute exposure, (2) rapid/binge exposure, (3) chronic exposure, and (4) exposure provoking withdrawal. We also asked whether activity-dependent synaptic activity (ADSP), the cellular correlate of learning and memory, was affected by the different alcohol exposures. To do so, we quantified the *de novo* synaptic boutons that are formed after repeated stimulation and are a hallmark of ADSP. **Results:** We found that the modulator of synaptic vesicle dynamics, Synapsin, is unaffected by alcohol. In contrast, the abundance of Synaptotagmin 1, a transmembrane synaptic vesicle protein and calcium sensor for exocytosis, decreases by 29% in animals subjected to rapid alcohol exposure. In contrast, Syntaxin 1A (a SNARE protein mediating the docking and fusion of vesicles with the presynaptic membrane) increases by 34 % and 56 % after acute and chronic alcohol exposure, respectively. In ADSP experiments, we found that the appearance of ghost boutons is reduced in animals submitted to rapid alcohol exposure but unchanged in animals chronically exposed. **Conclusion:** These results show that the synapse is subjected to significant remodeling at different stages of alcohol exposure, suggesting that synaptic adaptation is occurring in this system. In addition, the plasticity at the NMJ mirrors the results obtained with the associative learning paradigm and suggests that the synaptic modifications taking place during alcohol exposure might be part of the maladaptation leading to AUDs. **Acknowledgments:** We would like to acknowledge the following support: NIH NIGMS COBRE P20GM103642, and NIH-RCMI U54MD007600.

Characterizing Curcumin as a natural compound that promotes longevity and healthspan in *Drosophila melanogaster*

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Aging is a natural, progressive decline in tissue, organs, and organismal function. It is the greatest risk factor for many chronic diseases. As the global population continues to age rapidly, this presents a public health challenge, necessitating innovative strategies for the prevention and treatment of age-related diseases. Our goal is to identify new interventions that can promote the healthspan (the disease-free period of life) and/or lifespan (maximum longevity) extension by exploring the world of natural products (NPs) as a source of novel compounds. To achieve this, we have developed an affordable, high-throughput screening platform that enables the rapid identification of compounds promoting healthspan extension in *Drosophila melanogaster*. This platform utilizes the 'Smurf assay' as a readout, which quickly measures gut barrier dysfunction by feeding flies food mixed with blue dye No.1; if the gut is leaky, the flies turn blue, indicating imminent death. We are currently validating this platform by testing published NPs known to have either beneficial or detrimental effects on organismal health. Curcumin is a polyphenolic compound extracted from the turmeric root that has been reported to extend the lifespan of adult *Drosophila* via an unknown mechanism. I am particularly interested in characterizing the molecular mechanisms underlying curcumin's effects on longevity and healthspan. **METHODS:** To investigate this, we used the aging-accelerated fly model (18d) and fed the flies curcumin at concentrations of 0 µM, 62.5 µM, 125 µM, and 500 µM in 0.147% DMSO for 5 to 6 weeks. During this period, we performed several assays on flies treated with or without curcumin. These assays are the Smurf assay, IHC for the pHH3 mitotic cell marker, colony-forming units (CFU), and full-length 16S rRNA sequencing. Our **results** show that curcumin exerts a dose-dependent effect on leaky gut and the gut microbiome, with no observed impact on the excessive proliferation of mitotic cells. **CONCLUSIONS**: These findings provide insight into the mechanisms underlying the beneficial properties of this compound.

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Pharmacologic Modulation on Intestinal and Radial Nerve Cord Regeneration in *Holothuria glaberrima* by HNF4.

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INTRODUCTION: Understanding how transcription factors (TFs) regulate tissue regeneration is crucial. The highly conserved hepatocyte nuclear factor 4 (HNF4) is critical for proliferation, dedifferentiation, homeostasis, and lipid metabolism in the gastrointestinal tract, yet its function in the central nervous system (CNS) and post-traumatic regeneration remains largely unexplored. **METHODS:** We used the sea cucumber *Holothuria glaberrima* to study HNF4 activity during CNS and intestinal regeneration. To do this, we pharmacologically modulated HNF4 activity using the agonist NCT and antagonist BIM5078 after evisceration and after injury to the radial nerve cord (RNC) and assessed the subsequent regeneration of these organs. Intestinal outcomes included blastema size (transverse sections, 8 days post-injury), cell proliferation (BrdU across three blastema regions), cell dedifferentiation (phalloidin), and extracellular matrix (ECM) remodeling (collagen immunostaining). Neural outcomes included RNC stump width and area (longitudinal sections) and proliferation at the stump and lesion site (BrdU). Quantitative measures were analyzed using two-way ANOVA with Tukey's post hoc test. **RESULTS:** NCT-mediated activation of HNF4 altered tissue morphology and reduced proliferation at 8 days post-injury, a window that typically coincides with peak proliferation, consistent with delayed regeneration, while BIM5078 appears to promote regeneration. No significant differences in ECM remodeling were detected across groups, and a preliminary analysis of SLS localization suggests comparable patterns of dedifferentiation between treatments. **CONCLUSIONS:** These results indicate that HNF4's effects are selective rather than global during early stages of regeneration. Further studies are needed to fully elucidate the mechanisms by which HNF4 regulates regeneration in sea cucumbers and to explore its potential as a therapeutic target in regenerative medicine.

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Identification and Characterization of Notch Signaling Components in the sea cucumber *Holothuria glaberrima*

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Introduction: The Notch signaling pathway is a highly conserved mechanism that regulates essential cellular processes, including proliferation, differentiation, and cell fate determination. Despite its critical role in development and regeneration across metazoans, the molecular components of the Notch pathway remain largely unexplored in echinoderm models renowned for their regenerative capacity. In this study, we sought to identify and characterize the putative Notch receptor gene in the sea cucumber *Holothuria glaberrima* using bioinformatic approaches. Methods: Eighteen transcriptomic sequences were retrieved from transcriptomic databases and analyzed through BLAST searches to determine sequence homology. Multiple sequence alignments were conducted in ClustalW and Geneious, alongside Notch receptor sequences from other echinoderms, *Ophioderma brevispina*, *Anneissia japonica*, *Strongylocentrotus purpuratus*, and *Holothuria leucospilota*, to infer evolutionary relationships. Although several *H. glaberrima* sequences exhibited moderate to high similarity with known Notch proteins, conserved domain analysis revealed that most lacked the complete set of characteristic Notch domains but were rich in EGF-like domains. These sequences were downregulated during the early stages of intestinal regeneration, suggesting a temporary suppression. Results: A subsequent local BLAST using the *H. leucospilota* Notch receptor sequence identified one *H. glaberrima* transcript with 88.9% pairwise identity, containing 24 EGF-like repeats, an LNR domain, NOD and NODP regions, Ankyrin repeats, and a PAT1 domain, a domain architecture consistent with canonical Notch receptors in other echinoderms. These findings suggest that this candidate represents *H. glaberrima's* Notch receptor. To further validate this discovery, ongoing experiments include RNA extraction from regenerating intestinal and radial nerve cord tissues followed by cDNA synthesis and PCR amplification to confirm the sequence identity of the candidate gene and determine expression profiles during regeneration. Conclusion: Overall, this study provides a foundational step toward understanding the molecular architecture of the Notch signaling pathway in *H. glaberrima* and reveals its potential mechanistic role during echinoderm regeneration.

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The Power of Mild Stress: Running-Induced Hormesis and Brain Adaptation in the Hippocampus

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Introduction: Low-level environmental stressors, including health-enhancing challenges such as voluntary running exercise, promote neuronal resilience - a phenomenon known as hormesis. This idea provides a useful framework for understanding how exercise-driven metabolic changes in the hippocampus may support neurogenesis and improve cognitive function. However, the precise metabolic mechanisms remain unclear. Methods: Male C57BL/6J mice (20 weeks old) were assigned to sedentary (SED) or running (RUN) groups, with RUN mice performing 8 weeks of voluntary wheel exercise IACUC protocol A660121. Hippocampal metabolites were analyzed by GC–MS and LC-MS followed by univariate, chemometric, and pathway enrichment analyses. Metabolic correlation networks were generated in Python from MetaboAnalyst correlation matrices ($|r| \geq 0.03$) and visualized in NetworkX using the Fruchterman–Reingold layout (red = positive, blue = negative). A differential network ($\Delta r = rRUN - rSED$) identified metabolite pairs with altered correlations, while the Barnes maze evaluated spatial learning and memory. Results: Univariate, chemometric, and pathway enrichment analyses, together with metabolic correlation networks, revealed that running significantly altered neurotransmitter-related metabolites, decreasing glutamic acid and aspartic acid while increasing serotonin and glutamine. GABA levels remained stable, preserving excitatory–inhibitory balance. The RUN group showed enhanced performance in the Barnes maze, linking improved cognition to hippocampal metabolic remodeling. Discussion: Metabolomics analysis revealed strengthened coupling among glutamate, glutamine, GABA, aspartate, and asparagine in the RUN group, indicating a reorganization of excitatory–inhibitory amino acid metabolism. The observed coordinated shift suggests an adaptive, regulated control over glutamatergic signaling. This mechanism aligns with metabolic hormesis, promoting both neuroprotection and synaptic plasticity in response to physical exertion. Conclusion: Hippocampal glutamatergic signaling after exercise appears dynamically regulated rather than depleted. We hypothesize that exercise intensity determines whether these metabolic shifts promote or hinder neuroplasticity. Our findings support a dose-dependent hormetic model, where metabolic compartmentation and balanced neurotransmitter cycling drive long-term neuronal adaptation. Future work should clarify the role of glutamine–glutamate coupling in sustaining cognitive resilience through glial–neuronal metabolic integration.

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Impact of Angiotensin II on Tau Phosphorylation in vitro and Cognitive Function in vivo

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Introduction: Hypertension (>130/90mmHg), affects over half of the adult population in the United States and is the highest modifiable risk factor for age-related cognitive decline including Alzheimer's disease (AD). Previous research has shown that hypertension is associated with increased tau phosphorylation in amyloid negative elderly adults over a 5 year follow up. Thus, this project aims to elucidate the molecular basis of how hypertension contributes to tau phosphorylation. The Renin-Angiotensin System (RAS) is important in the pathophysiology of hypertension. The RAS involves renin-mediated cleavage of angiotensinogen to Angiotensin I, which is then converted to Angiotensin II (Ang II) by Angiotensin-Converting Enzyme (ACE). Ang II has many physiological functions, including regulation of blood pressure, thirst, and various neurobehaviors. In this study, we hypothesized Ang II promotes tau phosphorylation in vitro and affects cognitive function in vivo. **Methods:** Engineered human induced pluripotent stem cells derived neurons (iNeurons) were treated with Ang II (300nM or 1uM) and were collected at varying time points to assess the effect on various tau phosphorylation sites using Western blots. We also assessed whether GSK3β inhibition using the inhibitor CHIR99021 modifies the phosphorylation of tau. The cognitive effects of RAS hyperactivity were studied in vivo leveraging the sRA mouse model, a doubletransgenic model of brain-specific RAS characterized by elevated expression of human angiotensinogen and neuronal specific expression of human renin. Blood pressure measurements were taken prior to neurobehavioral testing involving Y-maze, Novel object recognition, Barnes maze, and Nest building. **Results:** In vitro, Ang II treatment did not significantly increase tau phosphorylation at the tested timepoints. In vivo, only Nest building behavior indicated possible cognitive deficits in daily living activities in sRA mice at 20 weeks. **Conclusion:** By understanding the mechanisms of how Ang II contributes to tau phosphorylation, we seek to unravel the involvement of the RAS in AD pathology and its impact on cognitive health, thus identifying potential targets for therapeutic intervention.

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Single-nuclei characterization of enteric and mesenterial neurons of the sea cucumber *H. glaberrima*

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Introduction: The process by which regenerated organs in adult animals are re-innervated is not well understood. The sea cucumber *Holothuria glaberrima* serves as a model to study the re-innervation by the nervous system of a regenerated organ. The present study is the first step to characterize the neuronal phenotypes in normal and early regeneration stages of intestinal regeneration. **Methods:** For this, we performed single-nuclei RNA sequencing in normal and regenerating intestines, analyzed the clustering, characterized cell types and performed downstream analyses. Trained Natural Language Processing models were used to classify the type of cell found in each cluster using the top 50 most overexpressed genes and their log2fold values. The cells were mainly characterized by the expression or neurotransmitters, motility/adhesion, hormonal signaling pathways. Gene mining techniques and cross-species dataset comparisons identified holothurian orthologues. **Results:** Two clusters best represented the neurons found in the mesentery mesothelium. These cell types were highly present in normal and both 1- and 3-days post-evisceration mesentery, but not in the normal intestine. **Conclusions/Discussion:** Our results provide a strong base for follow-up studies that aim at determining whether nerve-dependent regeneration, neuronal motility and growth gradients occur in the intestinal regenerative process.

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Special topics on Fentanyl

Harm Reduction and Overdose Prevention in Puerto Rico: Evaluating Strategies to Address the Opioid Crisis

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Introduction: Puerto Rico has faced an increase in drug harm and overdose due to a growing opioid crisis that worsened during the COVID-19 pandemic, as deaths related to opioid overdose, now often associated with synthetic opioids such as fentanyl or xylazine, continue to increase. Also, improper injection practices contribute to cases of viral infectious diseases like HIV, Hepatitis C, or both. This study explores issues related to the use of opioids, discussing pharmacology, with a focus on looking for measures that help mitigating overdose deaths as a means of reducing consequences associated with opioids. **Methods:** An extensive review of experimental studies was conducted to examine drug overdose cases and deaths, as well as to identify the most effective strategies for overdose prevention and harm reduction. **Results:** It was seen that, as of 2020-2022, men between 25-44 are most at risk, with towns such as San Juan, Caguas, and Ponce reporting a high rate of overdose cases. Harm reduction measures like needle exchange programs, distribution of naloxone, or a form of poppy string or paper lab test for fentanyl or xylazine distribution at community levels were seen to decrease risk of overdose, as they work as a safer form of taking drugs. Issues like social stigma, criminalization, lack of resources, or a lack of professionals with a caring demeanor continue to work as a barrier. Increasing education, training, and community-based programs will help address the overdose crisis as well as improve public healthcare outcomes in Puerto Rico. **Conclusion:** Overall, the findings highlight the need to expand evidence-based harm reduction and drug overdose with education and hands on protocols to improve health outcomes among people who use opioids in Puerto Rico.

Adulterants and Polysubstance Use in Fentanyl

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Introduction: Fentanyl is a synthetic opioid approximately 100 times more potent than morphine and 50 times more potent than heroin. Due to its extreme potency and low production cost, it is often adulterated with other substances or used in combination with different drugs, greatly increasing overdose risk. Common adulterants such as xylazine, benzodiazepines, and stimulants alter fentanyl's pharmacological profile, producing unpredictable and synergistic effects on the central nervous system. **Methods:** A comprehensive literature review was conducted using databases such as PubMed and ScienceDirect. Keywords included "fentanyl," "adulterants," "polysubstance use," "xylazine," "overdose," and "harm reduction." Over 20 primary and supplementary research articles were analyzed, including epidemiological studies and experimental reports published between 2020 and 2025. **Results:** Findings indicate that approximately 80% of synthetic opioid overdose deaths involve polysubstance use. In Puerto Rico, fentanyl-related overdoses have risen sharply, with most deaths involving multiple substances. **Conclusion:** Barriers to treatment include limited methadone access, stigmatization, and lack of long-term mental health coverage. Harm reduction tools such as naloxone distribution, fentanyl test strips, and medication-assisted treatment are essential for reducing mortality. Future research should continue to examine the prevalence of fentanyl in adulterated and polysubstance contexts, as its frequent presence in such mixtures underscores the urgency of understanding and addressing this public health threat.

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Fentanyl: Synthesis, Chemistry, and the Science Behind its Potency

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Introduction: Fentanyl is a synthetic opioid that works as a powerful and quick-acting substitute for morphine. Its chemical structure belongs to the 4-anilidopiperidine series of synthetic opioids which gives it characteristics that include high lipophilicity and extensive binding to μ-opioid receptors, which give fentanyl its rapid analgesic effect and high potency. Methods: This project analyzes the synthesis, pharmacology, metabolism, and detection of fentanyl to understand its aspects in medicine and public health crises. The methods used to achieve it consist of a comprehensive research and analysis of literature on fentanyl's chemical properties and role. Over the years, modification of the initial multi-step synthetic pathways into shorter and simpler pathways has enhanced production efficiency but facilitated illegal production resulting in the manufacture of lethal analogs such as carfentanil. Pharmacokinetic study emphasizes the extensive plasma protein binding of fentanyl, quick distribution, and hepatic biotransformation to norfentanyl by CYP3A4. Fentanyl is now available in a broad spectrum of administration routes, adding to its accessibility and uncontrollability, but most significantly, increasing the rate of overdose risk. Fentanyl's high potency also accounts for its severe toxicity due to its depressive effects on the respiratory system. This produces symptoms such as slowed rate of breathing, blue nails, and deep sedation. Analytical methods like LC-MS, colorimetry, and lateral flow devices have not only improved the detection accuracy with high sensitivity but also given options for rapid testing. Rapid testing for fentanyl can be lifesaving when the substance is cut into other illicit drugs in unmonitored amounts. Results: Fentanyl's lipophilicity (logP = 4.28), is significantly higher than morphine's (logP = 1.07), enhancing its ability to cross the blood-brain barrier and therefore increasing its potency. Fentanyl is estimated to be around 50 to 100 times more potent than morphine. Policy responses like the HALT Fentanyl Act now classifies fentanyl as a Schedule I under the Controlled Substances Act because of its extremely high abuse tendency. Conclusion: Understanding the chemistry and pharmacology of fentanyl will be crucial in developing safer opioid drugs, improving analytical detection methods for emergencies, and enforcing control regulation to alleviate its global impact on health.
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Epidemiology of Fentanyl in Puerto Rico

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Abstract:
INTRODUCTION: Fentanyl is a synthetic opioid clinically used to treat severe pain; however, its illicit use in Puerto Rico has increased due to its relaxing and stimulating effects. Although its pharmacological effects are well known, further research is needed to understand the social, economic, and environmental factors driving its spread across the island. **METHODS:** This study analyzes epidemiological data, reports from the Puerto Rico Department of Health, and scientific literature published between 2018 and 2024, with the objective of identifying the distribution, prevalence, and risk factors associated with fentanyl use and overdoses given its high abuse potential. **RESULTS:** The results show a significant increase in overdose deaths, with 2,838 reported cases among adult men aged 20 to 65, and the highest incidence occurring in the metropolitan and south regions. Moreover, additional risk factors include unemployment, lack of health insurance, drug contamination, and external stressors such as natural disasters like hurricanes and the COVID-19 pandemic. **CONCLUSION**: Therefore, this study highlights the importance of expanding access to preventive strategies such as Law No. 35 of 2021, strengthening epidemiological surveillance, and increasing naloxone distribution to mitigate the impact of fentanyl and improve the public health response in Puerto Rico.

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The sexual dimorphism of fentanyl responses and development of addiction on the motivational and reward systems of the brain

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Introduction: Fentanyl is a high-affinity μ-opioid receptor (MOR) agonist that acts within the central nervous system (CNS). It is exceptionally potent due to its elevated lipid solubility, which allows it to cross the blood-brain barrier (BBB) and produce intense feelings of pleasure and analgesia. Once this drug reaches the CNS, it inhibits pain-signaling pathways and activates the brain's reward circuit as well as the cortico-striatal-thalamo-cortical (CSTC) circuit. Some of Fentanyl's target sites include the Ventral Tegmental Area (VTA), the Nucleus Accumbens (NAc), the Anterior Cingulate Cortex (ACC), the striatum, and thalamic regions. **Methods:** This literature review was conducted using the PubMed database and synthesizes the findings from peer-reviewed studies published between 2021 and 2024. The key words used to conduct the search were "fentanyl" and "sex-linked differences". **Results:** Recent experimental studies on this drug have revealed sexdependent effects, including the upregulation and downregulation of multiple genes and the effectivity of reversal response. Gene expression and neurocircuit activity were measured using RNA sequencing and imaging techniques, along with pharmacological reversal outcomes in animal models. Data analyses were performed throughout the studies to identify sexual dimorphic trends in MOR signaling, dopaminergic abnormalities, and reversal drug responsiveness. Studies have shown comparable withdrawal symptoms between male and female rats, with males exhibiting greater fentanyl-seeking behavior. Results indicate an increase in the number of genes expressed in the NAc and VTA of females, suggesting enhanced neuroplasticity in the mesolimbic pathway. Males exhibited stronger dopaminergic activation within the VTA and more pronounced withdrawal-related behaviors; whereas females demonstrated increased expression of genes associated with synaptic remodeling, stress regulation, and neuroinflammatory pathways. Methadone administration reversed withdrawal in males but did not significantly alter fentanyl preference in females, which may relate to the influence of ovarian hormone on opioid response. Fluctuations in estradiol levels have been linked to changes in fentanyl preference and reward pathway activation, with higher estradiol associated with enhanced drug-seeking behavior. This may explain why females display persistent drug-seeking in contrast to males. **Conclusion:** The evaluation of sex-linked differences in response to fentanyl has primarily relied on rodent models, limiting the applicability to human physiology and leaving a gap in preclinical research. Implementing representative primate models is crucial to characterize both neurobiological and physiological sex-linked differences and is pivotal for

therapeutic innovation. Furthermore, the lack of studies characterizing the neurobiological effects of fentanyl addiction in females compared to males is notable.

Harm Reduction and Overdose Prevention in Puerto Rico: Evaluating Strategies to Address the Opioid Crisis

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Introduction: Puerto Rico has faced an increase in drug harm and overdose due to a growing opioid crisis that worsened during the COVID-19 pandemic, as deaths related to opioid overdose, now often associated with synthetic opioids such as fentanyl or xylazine, continue to increase. Also, improper injection practices contribute to cases of viral infectious diseases like HIV, Hepatitis C, or both. This study explores issues related to the use of opioids, discussing pharmacology, with a focus on looking for measures that help mitigating overdose deaths as a means of reducing consequences associated with opioids. **Methods:** An extensive review of experimental studies was conducted to examine drug overdose cases and deaths, as well as to identify the most effective strategies for overdose prevention and harm reduction. **Results:** It was seen that, as of 2020-2022, men between 25-44 are most at risk, with towns such as San Juan, Caguas, and Ponce reporting a high rate of overdose cases. Harm reduction measures like needle exchange programs, distribution of naloxone, or a form of poppy string or paper lab test for fentanyl or xylazine distribution at community levels were seen to decrease risk of overdose, as they work as a safer form of taking drugs. Issues like social stigma, criminalization, lack of resources, or a lack of professionals with a caring demeanor continue to work as a barrier. Increasing education, training, and community-based programs will help address the overdose crisis as well as improve public healthcare outcomes in Puerto Rico. **Conclusion:** Overall, the findings highlight the need to expand evidence-based harm reduction and drug overdose with education and hands on protocols to improve health outcomes among people who use opioids in Puerto Rico.

Medication-Assisted Treatments for Fentanyl Use Disorder: Antagonists and Novel Approaches

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Fentanyl is a synthetic piperidine opioid known for its analgesic and anesthetic properties in clinical settings. This opioid is often mixed with other illicit drugs and distributed for recreational use. Upon administration, fentanyl crosses the Blood-Brain Barrier and binds to μ-opioid receptors in the Central Nervous System, where it deploys its main effects in regions associated with respiratory control, pain perception and reward. Given its rapid onset, high potency, and tendency to be blended with illicit drugs, fentanyl has become a major factor of the current opioid crisis. This has aided in the increased rate of overdose, and opioid use disorder (OUD). Currently, there are various clinical and emergency interventions used to treat overdose, as well as long term therapies that manage OUD. However, some of these long-term medications face limitations related to short duration of action, affinity, and side effects. Recent research has suggested two new potential medications that may address these limitations, nalmefene and methocinnamox (MCAM). An extensive literature review was conducted on namelfene and methocinnamox as possible novel treatments for fentanyl overdose and opioid use disorder. A total of 12 scientific papers, sourced from high impact factor journals like *Neuropsychofarmacology* and *Biological Psychiatry* were used. Nalmefene, an FDA approved drug, has shown longer lasting binding effects when compared to other overdose reversal agents approved by the FDA, such as naloxone. In addition, studies report a significant reduction in cardiac and fentanyl-induced respiratory depression incidents following nalmefene administration. Its longer duration has also shown to help prevent withdrawal symptoms. In contrast, studies surrounding MCAM administration have demonstrated it reduces the rewarding effects of fentanyl and thus prevents withdrawal symptoms. In addition, reports suggest MCAM only blocks the effects of opioids and leaves other drug responses unchanged. This profile highlights the drug's high receptor selectivity for opioid pathways. In summary, both nalmefene and methocinnamox have shown great promise as possible novel treatments for fentanyl overdose and OUD. Their positive effects on withdrawal, decrease of rewarding symptoms and reduction of cardiac and respiratory incidents, provide an optimistic outlook on their implementation. Further studies and trials must be conducted to fully characterize MCAM’s safety profile and minimize its potential side effects.

Neurobiological & Cognitive Consequences of Fentanyl Abuse

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Fentanyl is a potent synthetic agonist of μ-opioid receptors (MORs) that is highly lipophilic, which crosses the blood-brain barrier rapidly and results in analgesic and anesthetic effects. Its neuronal targets are mainly dopaminergic neurons across the mesolimbic system, from the Ventral Tegmental Area (VTA) to the Nucleus Accumbens (NAc), yet GABAergic and glutamatergic neurons also modulate this process. Concerningly, fentanyl use causes multiple neurobiological and cognitive consequences, and its abuse is considered a public health crisis with increasing overdose deaths each year. In this review, we examined various studies reporting cellular and molecular changes, anatomical and physiological side effects, as well as cognitive consequences related to fentanyl exposure. We performed a focused narrative review, selecting and screening studies primarily from 2020–2025 and organizing findings into a themed outline. A small number of landmark pre-2020 papers were purposefully included to contextualize current evidence. Our review showed that fentanyl administration induced intense levels of apoptosis, oxidative stress, neuroinflammation, and a downregulation of D1, D2, and NMDA receptors. Moreover, chronic fentanyl use was associated with severe leukoencephalopathy, which could cause symptoms of inattention, forgetfulness, personality changes, and dementia. Studies also highlighted a relation to hippocampal volume reduction and respiratory depression. These findings suggest fentanyl use causes severe neurological and physiological alterations. Future studies should focus on further identifying specific targets for treatment and strategies for neuroprotection and rehabilitation from fentanyl use.

Methadone and Buprenorphine in the Fentanyl Crisis: Effectiveness, Challenges, and Clinical Implications

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Opioids are widely used to control severe pain, but in the past decade, the illegal use of fentanyl—originally developed for chronic pain management—has risen sharply. Fentanyl is 30–50 times more potent than heroin and 50–100 times more potent than morphine; as little as 2 mg can be fatal. Its widespread use and presence in other illicit drugs have driven a dramatic increase in overdose deaths. Pharmacological studies indicate that fentanyl's high affinity for μ-opioid receptors contributes to its potency and lethality. Excessive opioid exposure desensitizes these receptors and promotes tolerance, reducing cells' ability to respond to the same drug doses, thereby increasing the risk of dose escalation, dependence, and overdose. To treat opioid use disorder (OUD), μ-opioid receptor agonists such as methadone and partial agonists such as buprenorphine are considered the gold standard; the latter is also available in combination with the μ-opioid receptor antagonist naloxone. Although these medications have long been effective in treating OUD, the fentanyl crisis has raised new questions regarding optimal dosing, administration, and treatment retention. **METHODS:** We compiled recent findings on the effectiveness of these medications in the context of fentanyl use and identified gaps to guide future research. **RESULTS:** Overall, current evidence indicates that both methadone and buprenorphine remain effective for fentanyl-exposed patients. Methadone maintenance treatment protects against overdose and achieves one-year retention and abstinence rates similar to those of other opioid users. Higher buprenorphine doses (24 mg vs. 16 mg) improve retention, while weekly or monthly subcutaneous extended-release buprenorphine results in more fentanyl-negative urine samples than daily sublingual buprenorphine-naloxone. Large-scale analyses also show that buprenorphine-naloxone reduces mortality by 34% and nearly doubles abstinence rates compared to patients not receiving buprenorphine. Patients with μ-opioid receptor tolerance from fentanyl use may not experience the full therapeutic effects of methadone or buprenorphine. In such cases, controlled administration of short-acting full agonist opioids (e.g., morphine) can help further alleviate withdrawal symptoms and support patient stabilization during standard treatments. **CONCLUSIONS**: Beyond pharmacological considerations, treatment access remains limited by cost and stigma—even within healthcare settings. These findings suggest that while methadone and buprenorphine remain effective in the fentanyl era, optimizing dosing, delivery, and access is critical to improving outcomes. **Acknowledgements:** We thank Dr. Carmen S. Maldonado-Vlaar and the course Neuropharmacology of Use and Abuse of Drugs (BIOL3576-0U1, UPR-RP) for guidance in abstract and poster preparation, editing, and valuable feedback.

The Pharmacology of Fentanyl: Structure, Kinetics, and Systems Effects

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Introduction: Fentanyl, a highly potent μ-opioid receptor (MOR) agonist, drives disproportionate morbidity and mortality in Puerto Rico and around the world. To explain its risk profile requires integrating structural binding determinants, receptor kinetics, analog-specific toxicology, neural circuits for reinforcement and withdrawal, and emergent overdose countermeasures. Methods: We reviewed and combined results from recent peer-reviewed studies spanning: structureactivity relationships (SAR) and cryo-EM structures of MOR with fentanyl versus morphine; molecular dynamics and quantitative systems pharmacology (QSP) modeling of fentanyl-MOR residence/dissociation and naloxone competition; in vivo respiratory toxicology of illicit fentanyl analogs; preclinical evaluation of a fully human anti-fentanyl monoclonal antibody (CSX-1004); and circuit-level and behavioral studies of dependence, withdrawal, and decision making under adverse consequences. Findings: Fentanyl shows stronger and longer-lasting effects at opioid receptors compared to morphine, which helps explain its higher potency and risk. Small chemical changes in fentanyl's structure can alter both its strength and safety. Because of the way fentanyl interacts with the receptor, higher or repeated doses of naloxone are sometimes needed to reverse overdoses. Animal studies confirm that fentanyl and its analogs cause much stronger breathing problems than morphine. Modeling suggests that the usual community doses of naloxone may not always be enough, but higher doses are more effective. A new drug, CSX-1004, binds fentanyl with very high selectivity, reverses both pain relief and breathing suppression, and provides weeks of protection in animal studies without interfering with other opioids. On a brain-circuit level, fentanyl reinforces reward through dopamine pathways, while withdrawal symptoms involve stress circuits in the amygdala. Conclusion: Converging structural, kinetic, toxicologic, and circuit evidence explains fentanyl's outsized danger and informs practice: prioritize analog surveillance, anticipate severe respiratory depression, consider higher/repeat-dose antagonist protocols, explore antibody-based prophylaxis/therapy, and target circuit and behavioral strategies to reduce relapse. This guidance is directly applicable to current responses in Puerto Rico and globally.

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