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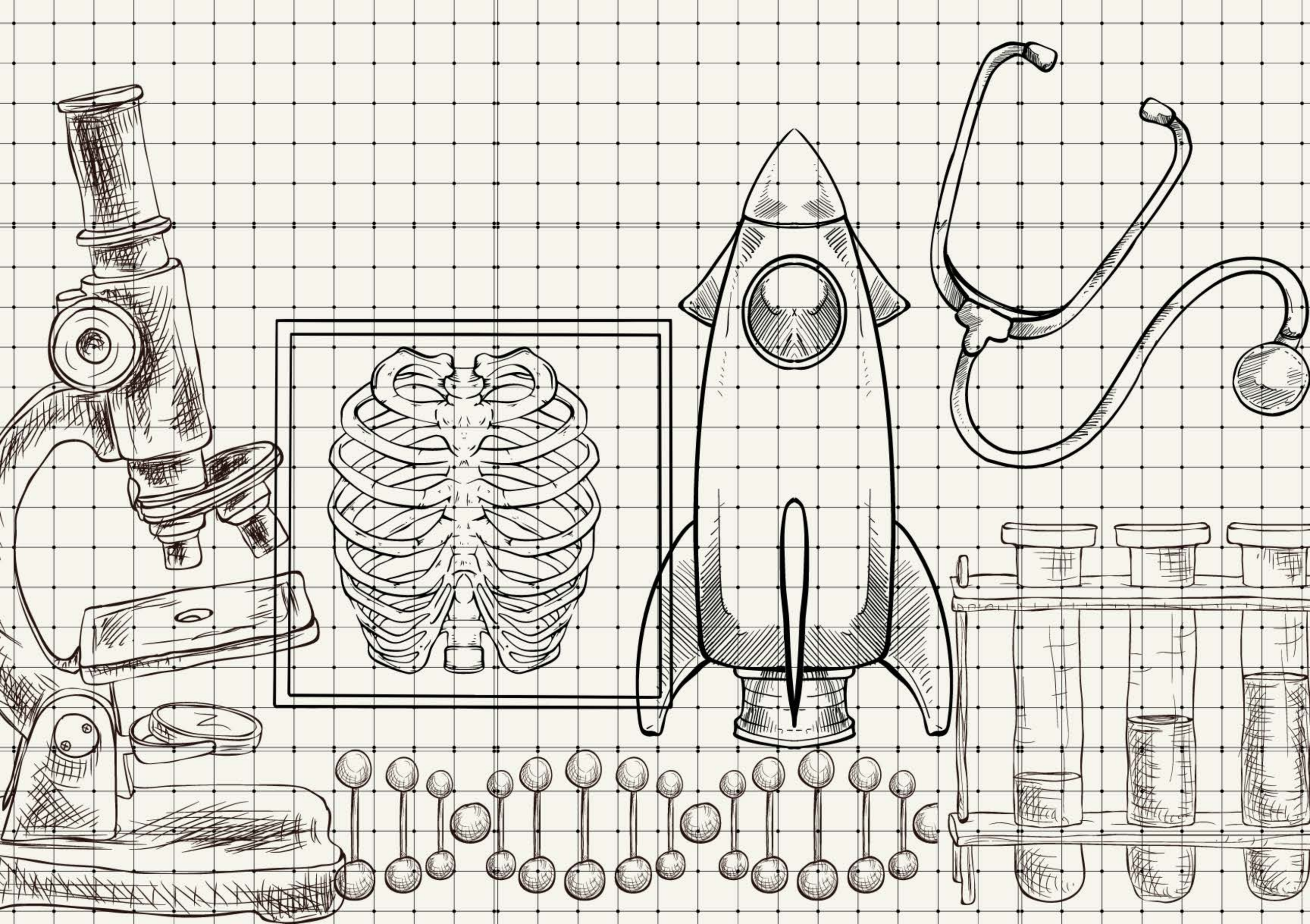

Welcome to the 2024 Graduate Research Annual Forum

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I would like to personally welcome you to the 2024 Graduate Research Annual Forum (GRAF 2024)! The Council of Biomedical Graduate Students (CBGS) has been organizing GRAF in collaboration with the College of Pharmacy and Pharmaceutical Sciences over the last four decades. This event is great time for students to present their work and get invaluable feedback from expert post-docs and faculty. GRAF also gives all of our research tracks a chance to learn about the other research projects taking place at the University of Toledo, helping foster interdisciplinary collaborations. Our students are very excited for this year's forum, including 39 poster and oral presentation and 15 volunteer judges from faculty and postdoctoral fellows. We are also thankful for the moderators who will be leading each poster and oral session. We want GRAF to benefit scientists and trainees at all levels, whether that is in the form of presenting to a new audience, organizing the event, or evaluating the presentations. We are excited to have this year's keynote speech delivered by Dr. Joshua Starmer. Dr. Starmer was an Assistant Professor at the University of North Carolina Chapel Hill when he founded the StatQuest YouTube channel. The channel's original purpose was to help educate his colleagues on statistics, but has since evolved to be a massive, freely available educational resource on math and statistics that has garnered over 1 million subscribers. Beyond being the Founder and CEO of StatQuest, Dr. Starmer also holds the title of Lead AI Educator at Lightning AI. I would like to thank all the Council members who helped plan this event, with a special thanks to those on the executive committee for their diligent planning and effort. I would also like to thank Drs. Kandace Williams and David Giovannucci for guiding the Council and providing us with their advice. This event would not be possible without the support of the UT COMLS Foundation, the COMLS Alumni Affiliation, and ThermoFisher, and we are very grateful for their contributions. Further, we cannot thank our departments enough for their support, with their donations to GRAF and all the resources they provide that allow us to host this event year after year. I hope all attendees enjoy the forum and walk away richer for the experience!

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Social transmission of negative valence in prairie voles using a new behavioral assay that assesses social learning.

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Keywords: Prairie Voles, Empathy, Autism, Social Learning

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Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental condition marked by notable deficiencies in social interaction, social communication, and repetitive behaviors. The intricate nature of these behaviors and the underlying molecular basis of ASD remain challenging to comprehend. A lack of behavioral assays that assess social learning deficits in animal models is one of the biggest challenges in the field.

Objectives: The study aims at developing a new behavioral assay in prairie voles, an animal model that exhibits prosocial behaviors, that aims to measure social learning aptitudes based on the observation of subtle social cues in their stressed partners following a fear conditioning paradigm.

Methods: Same-sex adult pairs of prairie voles (housed together since weaning, ntotal = 50, two groups) were tested on the newly developed paradigm called social transmission of negative valence in voles (STNV), which is an adaptation of a previous paradigm (1). STNV is a two-day behavioral paradigm that consists of fear conditioning demonstrators on day 1 to the tone, by associating 15 tones (30s, 6KHz) to 15-foot shocks (1s, 1mA) or no shocks (in the control group). Next, the partner (observer) is brought to the experimental cage to observe demonstrators (through a clear barrier) freeze to the tone during a fear memory recall task. On day 2, we measured freezing behavior in observers during the re-introduction to the experimental cage. Social learning was assessed by the percentage of observers' freezing, rearing, self-grooming, and by ultrasonic vocalizations they exhibit in both groups (fear conditioned demonstrators and control demonstrators). Rodent ultrasonic vocalization serves as an indicator of social communication and conveys their emotional state (2).

Results: We found a significant increase in freezing behavior in observers in the experimental group as compared to the control group ($p<0.05$). Also, observers showed a significant increase in self-grooming, and rearing behaviors in the experimental group as compared to the control group ($p<0.05$). Ultrasonic vocalizations also show a significant increase in frequency of calls in experimental observers (56-95 kHz) compared to controls (20-45 kHz, $p<0.05$).

Conclusion: STNV yields promising outcomes as a social learning paradigm, offering insights into how these rodent species learn through social transmission of subtle cues. Future directions consist of investigating the neural correlates of social learning and the effects of targeted drugs on social behavior.

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Developmental pyrethroid exposure disrupts molecular pathways for circadian rhythms and MAP kinase in mouse brain.

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Keywords: Pyrethroid, Deltamethrin, Neurodevelopmental Disorders

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Introduction: Neurodevelopmental disorders (NDDs) are a category of pervasive disorders of the developing nervous system with few or no recognized biomarkers. A significant portion of the risk for NDDs, including attention deficit hyperactivity disorder (ADHD), is contributed by the environment, and exposure to pyrethroid pesticides during pregnancy has been identified as a potential risk factor for NDD in the unborn child. We recently showed that low-dose developmental exposure to the pyrethroid pesticide deltamethrin in mice causes male-biased changes to ADHD- and NDD-relevant behaviors as well as the striatal dopamine system.

Objective: Here, we used an integrated multiomics approach to determine the broadest possible set of biological changes in the mouse brain caused by developmental pyrethroid exposure (DPE).

Methods: Using a litter-based, split-sample design, we exposed mouse dams during pregnancy and lactation to deltamethrin (3 mg/kg or vehicle every 3 days) at a concentration well below the EPA-determined benchmark dose used for regulatory guidance. We raised male offspring to adulthood, euthanized them, and pulverized and divided whole brain samples for split-sample transcriptomics, kinomics and multiomics integration.

Results: Transcriptome analysis revealed alterations to multiple canonical clock genes, and kinome analysis revealed changes in the activity of multiple kinases involved in synaptic plasticity. Multiomics integration revealed a dysregulated protein-protein interaction network containing primary clusters for mitogen-activated protein (MAP) kinase cascades, regulation of apoptosis, and synaptic function.

Conclusion: These results demonstrate that DPE causes a multi-modal biophenotype in the brain relevant to ADHD and identifies new potential mechanisms of action.

Uncovering biologically relevant Autism subtypes using advanced machine learning techniques

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Keywords: Autism, Machine Learning, Bioinformatics, Neuroimaging

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Background: Autism spectrum disorder (ASD) is characterized by main deficits in social interaction and social communication. This vague definition of ASD does not encompass the wide heterogeneity of its phenotypical presentation. It is critical to identify ASD subtypes that are biologically relevant and that respond to personalized treatment.

Objectives: The objective of this study is to use a multimodal approach to create biologically relevant subtypes.

Results: 114 adult men (18-45 years old), including 74 neurotypical (NT) and 40 ASD were recruited and completed a series of behavioral tests such the NEO-PI-R, reading the mind in the eyes (RMET), Symptom Checklist 90-revised questionnaire (SCL-90), intelligence quotient (IQ), and Broader Autism Phenotype Questionnaire (BAPQ). Clinical measurements and fMRI data was collected from ASD subjects for validation. We used a random forest tree algorithm to classify ASD and NT. We included NEO-PI-R and RMET in the main classifier. The random forest tree model classified ASD and NT with an average accuracy of 80%. Top features included personality domains such as extraversion and neuroticism. K-means clustering was used to derive ASD subtypes based on the shapely values; this created 3 subtypes (Subtypes 1, 2, 3). T-tests indicated significant differences in the following measures: ADI-R repetitive behaviors, BAPQ, IQ, SCL-90, neuroticism, extraversion, RMET and rs-FC between the superior temporal sulcus, anterior cingulate, and insula.

Conclusions: Our results suggest that ASD subtype 1 is characterized by high neuroticism, lower warmth, higher scores on RMET, higher IQ, and higher rsFC between STS and salience network. Subtype 2 was found to be close to neurotypicals. Subtype 3 is characterized by high neuroticism, high repetitive behaviors, and lower rsFC between STS and salience network. These results are very promising, and the next step is to examine whether these putative subtypes are biologically relevant and whether specific subtypes respond better to certain pharmacological treatments.

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Purinergic System Perturbations in Schizophrenia

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Keywords: Adenosine; Schizophrenia; Anterior Cingulate Cortex; Transcript Expression

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Background: Schizophrenia is a devastating neuropsychiatric disorder characterized by hallucinations, delusions, and disordered thought processes. Dysregulation of the glutamate and dopamine neurotransmitter systems are implicated in the pathophysiology of schizophrenia. The adenosine system is an important neuroregulatory system in the brain that modulates glutamate and dopamine signaling via adenosine receptors; however, the gene expression of the high affinity adenosine A1 and A2A receptors (A1R and A2AR) is not well characterized in neurons in frontal cortical brain regions implicated in this disorder.

Methods: In the present study, we analyzed A1R and A2AR mRNA expression via qPCR in enriched populations of pyramidal neurons, isolated from postmortem anterior cingulate cortex (ACC) tissue from schizophrenia (n=20) and age and sex-matched non-psychiatrically ill control (n=20) subjects, using laser capture microdissection.

Results: A2AR mRNA expression was significantly increased in schizophrenia subjects who were off antipsychotic medication (ANCOVA: $F(1,12)=6.444$, $p=0.026$), suggesting that A2AR expression may be normalized by chronic antipsychotic treatment. A1R expression was significantly increased in female schizophrenia subjects compared to female control subjects ($t(13)=-4.008$, $p=0.001$). A1R expression was also significantly decreased in female controls compared to male control subjects ($t(17)=2.137$, $p=0.047$). We also identified a significant positive association between dementia severity and A2AR mRNA expression (Spearman's $r=0.424$, $p=0.009$).

Conclusion: Overall, these results provide novel insights into the pyramidal neuron specific expression of adenosine receptors in the ACC in schizophrenia and suggest that changes in receptor mRNA expression may be sex-dependent and associated with dementia in these subjects.

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Protective immune role of platelets during respiratory viral infection

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Keywords: Platelets, Respiratory Virus

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Background: Platelets are small, anucleate cells derived from megakaryocytes. Conventionally, they are known for an indispensable role in hemostasis. Additionally, research in the past decade has now established platelets as orchestrators of immune response. At a molecular level, platelets express receptors that allow them to interact with viruses leading to platelet activation. Activated platelets can directly affect viral replication and modulate leukocyte behavior. Interestingly, thrombocytopenia is commonly observed during viral infections and is associated with worse disease outcomes. Most studies on platelets and viral infections are focused on severe viremic infections. However, the role of platelets and their impact on pulmonary infections such as those caused by Respiratory Syncytial Virus (RSV) is not clear.

Methods: Sendai virus (SeV) was used as a model pathogen. Flow cytometry was used to show in vitro platelet activation (P-selectin expression – CD62P) and internalization of SeV. Survival experiments post intranasal SeV infections were conducted using a mouse model of platelet depletion developed in our laboratory. Tissue damage was assessed by histology and immunohistochemistry. Viral loads were measured using qRT-PCR.

Results: Upon intranasal challenge with virus, control mice with normal platelet counts exhibited mild symptoms with no mortality. However, platelet depleted mice were highly susceptible to infection, had severe weight loss and high mortality rates. Detailed analysis of infected lungs showed that platelets modulate neutrophil accumulation in the lungs without affecting viral loads significantly. Histological analysis also revealed high levels of myeloperoxidase positive cells and severe tissue damage post infection in absence of platelets.

Conclusion: This study identified a significant protective role of platelets in immune response against respiratory viruses. The outcome of this study reinforces platelets as a therapeutic target to combat severe pulmonary viral infection.

Profound Non-Randomness in Dinucleotide Arrangements within Ultra-Conserved Non-Coding Elements and the Human Genome.

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Keywords: Ultra conserved non-coding elements, DNA structure, Bioinformatics, Inhomogeneity

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Introduction: Ultra-conserved non-coding elements (UCNEs) remain fascinating genomic fragments that have maintained almost perfect sequence identity for millions of years. UCNEs are defined as regions of DNA that are longer than two hundred nucleotides in length and at least 95% conserved between humans and chickens. Previous research found that UCNEs have an excess of GpC dinucleotides but a decrease in GpG/CpC dinucleotides compared to the human genome. However, similarities between UCNES or other characteristics that make them unalterable have yet to be unveiled.

Objectives: Based on these findings, we hypothesize that UCNEs have a distinct dinucleotide composition that may contribute to a unique DNA structure. We calculate the distance between all dinucleotide pairs within UCNEs and the human genome to identify patterns in dinucleotide arrangements.

Methods: We purified a publicly available UCNE database, which includes a total of 4,272 sequences, and human genome sequences with masked repetitive elements. Randomly generated UCNE and human genome sequences were created using the dinucleotide frequencies from the real perspective sequences. Statistical analysis was performed to assess the non-randomness in dinucleotide spacing arrangements using relative percentage difference (RPD).

Results: Remarkable non-randomness in dinucleotide spacing arrangements was observed within the entire human genome and UCNEs. Approximately 83% of all dinucleotide pairs within UCNEs showed significant (>10% RPD) non-random genomic arrangements when compared to the rest of the human genome. Most non-random arrangement of dinucleotide pairs occurred at short distances, 2-6

nucleotides. Non-randomness in dinucleotide spacing distances deviated up to 40% from the expected values and were frequently associated with GpC, CpG, ApT, GpG, and CpC dinucleotides.

Conclusion: The described peculiarities in dinucleotide arrangements have persisted for hundreds of millions of years within vertebrates. These distinctive patterns may suggest that UCNEs form a unique DNA structure with distinct properties that contribute to their extraordinary conservation.

Cysteinyl leukotriene receptors promote melanoma progression and metastasis

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Keywords: Melanoma, Cysteinyl Leukotrienes

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Background: Cysteinyl leukotrienes (cys-LTs; LTC4, LTD4, LTE4) are pro-inflammatory mediators mainly produced by hematopoietic cells, which enhance inflammation through their receptors, CysLT1R and CysLT2R. They are crucial in causing chronic asthma in humans. The link between inflammation and cancer has sparked interest in the role of cys-LTs in cancer progression and metastasis. Yet, the precise molecular mechanisms through which these inflammatory mediators regulate tumor proliferation and metastasis are still unexplored. A thorough investigation of these molecular mechanisms, and identification of the specific receptor/s responsible, can aid in understanding their therapeutic potential in various cancers like melanoma. Since the inhibitors of these receptors are already FDA approved for asthma treatment, we aim to repurpose these drugs to treat melanoma progression, thereby achieving maximum efficacy with minimal side effects.

Objectives: To determine the mechanistic aspects of how CysLTRs regulate melanoma tumor initiation, progression, and metastasis.

Methods: Protein expression by western blotting and ELISA, transcript expression by qPCR, viability and proliferation by XTT and BrDU, migration using trans-well assay, tumor growth and metastasis examined through in vivo experiments.

Results: B16F10 melanoma cells express high CysLT1R compared to CysLT2R. Further, cys-LTs mediated the activation of major signaling proteins such as ERK and p38 that are important for melanoma survival and proliferation. Moreover, treatment with CysLTR antagonists significantly reduced melanoma cell proliferation, survival, and migration *in vitro*. Accordingly, we observed a significant reduction in the melanoma tumor volume *in vivo* in both *Cysltr1-/-* and *Cysltr2-/-* mice compared to the WT mice. Interestingly, angiogenesis was significantly reduced in *Cysltr2-/-* mice but not in *Cysltr1-/-*.

Conclusion: Therefore, we speculate that while both receptors play a crucial role in tumor proliferation in vivo, CysLT2R is the main driver of angiogenesis and metastasis. Therefore, targeting both these receptors using their specific antagonists can offer effective therapy for melanoma progression.

The Role of Cysteinyl Leukotrienes and Their Receptors in EC-Macrophage Interaction and Therapeutic Implications for Atherosclerosis

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Keywords: Cardiovascular Disease, Atherosclerosis, Cysteinyl Leukotrienes, Endothelial cells, Macrophage

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Background: Cardiovascular disease (CVD) remains a global health threat with atherosclerosis at the forefront. Atherosclerosis involves low density lipoprotein oxidation, monocyte infiltration through endothelial cells (EC), smooth muscle cell (SMC) proliferation leading to plaque formation and chronic inflammation. During inflammation, cysteinyl leukotrienes (cys-LTs; LTC4, LTD4, LTE4) are released from the membrane via the 5-lipoxygenase pathway and exert their effects via cysteinyl leukotriene receptors (CysLTR) 1 and 2. Since these receptors transduce inflammatory signals and regulate EC and macrophage dysfunction, we hypothesized that they play vital role in macrophage-EC interactions in a co-culture and contribute to atherosclerosis.

Methods: Mouse dermal ECs were co-cultured with bone marrow-derived macrophages (BMDM) in the presence or absence of CysLT1R and CysLT2R antagonists MK571 and BayCysLT2 respectively for 6-hours. Culture supernatant and cells were collected for ELISA and qPCR analysis, respectively. EC contraction was determined by F-actin staining.

Results: We observed a significant increase in pro-inflammatory cytokine Interleukin (IL)-6 in the EC-BMDM co-culture. Further, BMDM in co-culture upregulated IL-6, IL-1β, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Oxidized Low-Density Lipoprotein Receptor-1 (OLR-1) transcripts with a simultaneous reduction in the Vascular Endothelial Growth Factor (VEGF) transcript. ECs in co-culture exhibited an increase in IL-6, and upregulation of adhesion molecules like Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1) transcripts and a contractile response revealed by gap formation. Importantly, IL-6 was significantly inhibited by both MK571 and BayCysLT2 suggesting that CysLTR signaling mediate EC-macrophage interactions.

Conclusion: Our results suggest that blocking CysLTR may offer a promising therapy to prevent plaque initiation during atherosclerosis.

Streamlining High Throughput Kinome Analysis: Introducing KADL, a Comprehensive Kinome Analysis Description Language

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Keywords: Kinome, Rust, Analysis Platform, PamChip

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Background: We deployed the PamGene kinome array platform for use with postmortem brain samples and iPSCs from schizophrenia (SZ) and Alzheimer's dementia (AD). The kinome array platform provides a read-out of protein kinase activity across hundreds of peptide substrates, measuring global protein kinase activity across serine/threonine and tyrosine subkinomes. We used this omics-based platform to generate novel hypotheses for the pathophysiology of severe neuropsychiatric disorders with cognitive dysfunction.

Methods: We used the PamGene kinome array platform to assess protein kinase activity in disease (AD, MDD, and SZ) and control (SZ) samples. We also evaluated protein kinase activity in stem cell cultures for these disorders. We used R programs (KRSA and UKA) to deconvolve the generated kinome array datasets to identify specific protein kinases altered across these disorders. Information for deconvolution of datasets was supplemented with recombinant kinase and kinase perturbation studies.

Results: A joint hit for AD and SZ was adenosine monophosphate kinase (AMPK), a master regulator of insulin signaling pathways. Subsequent studies of AMPK in AD and SZ reveal subunit-specific deficits in the frontal cortex in AD, with changes in the regulatory subunits for AMPK. Bioinformatics analyses revealed several novel pathways and several candidate drugs that might be repurposed for treating cognitive deficits in these disorders.

Conclusions: We used a hypothesis-free, kinome-based approach to extend our understanding of the pathophysiology of SZ and AD and provide novel leads to advance the diagnosis and treatment of these often-devastating illnesses.

An Integrative Analysis of Kinomic and Proteomic Profiling in Chronic Mild Stress Mice

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Keywords: Depression, Bioinformatics

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Objectives: Major Depressive Disorder (MDD) is a severe mental health condition characterized by the DSM-5 as persistent feelings of sadness, hopelessness, and a lack of interest or pleasure in daily activities. n this study, we focus on modeling MDD in mice through Chronic Mild Stress (CMS), a well-established paradigm that mimics the chronic stressors contributing to the development and exacerbation of depressive symptoms. Kinomics, the study of protein kinases and their signaling pathways, and proteomics, the comprehensive analysis of proteins expressed in a biological system, offer a holistic perspective on the molecular alterations associated with MDD. By combining these two high-throughput techniques, we aim to unravel the intricate molecular landscape underlying depressive phenotypes induced by chronic stress.

Methods:We induced Chronic Mild Stress (CMS) in a mouse model to mimic Major Depressive Disorder (MDD) and collected brain tissue samples for analysis. Bioinformatic tools were employed to interpret the functional significance of differentially expressed proteins and identify kinase targets. The integrative analysis of kinomic and proteomic data unveiled intricate molecular changes associated with CMS-induced depressive phenotypes.

Results: Proteomic analysis revealed significant changes in protein expression patterns, indicating a broad impact on cellular processes in response to the experimental conditions. Kinomic profiling identified alterations in kinase activity, suggesting potential modulation of signaling pathways. Integrative analysis and the observed overlap between proteomic and kinomic changes hinted at complex regulatory networks affected by the experimental manipulations.

Conclusions: In summary, our study employing a mouse model subjected to Chronic Mild Stress (CMS) successfully illuminated significant alterations in protein expression and kinase activity, providing valuable insights into the molecular landscape associated with Major Depressive Disorder (MDD). The integrative analysis of kinomic and proteomic data unveiled intricate regulatory networks, underscoring the complexity of molecular changes induced by chronic stress and offering potential avenues for understanding and addressing depressive phenotypes.

Klebsiella pneumoniae sugar import suppresses hypermucoviscosity

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Keywords: Klebsiella, Hypermucoviscosity, Capsule, Hypervirulent

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Background: *Klebsiella pneumoniae* is significant cause of community- and hospital-acquired infections, impacting both immunocompromised and immunocompetent individuals. The co-emergence of drug-resistance and hypervirulence in *K. pneumoniae* has severely limited therapeutic options. Hypermucoviscosity (HMV) is an important *K. pneumoniae* virulence factor that manifests as a 'tacky' bacterial colony due to changes in capsule chain length. Two genetically encoded mechanisms regulating HMV are the regulator of mucoidy phenotype (*rmpD*) and *wzc* activity. We have previously shown that difference in growth medium also alters HMV, but specific nutrient signals and mechanisms involved are still unclear. To address this knowledge gap, we hypothesized that extracellular nutrients such as sugars distinctly induce changes in *K. pneumoniae* HMV without impacting capsule abundance.

Methods: To investigate, we cultured K. pneumoniae strain KPPR1 in M9 minimal medium supplemented with varying sugar concentrations, and measured HMV and capsule production using sedimentation resistance assay and uronic acid quantification, respectively.

Results: Our results demonstrated that all tested sugars, including metabolizable and non-metabolizable sugars, significantly suppressed the mucoidy, while capsule abundance was not impacted similarly. This finding indicates that sugar import in *K. pneumoniae* distinctly regulates HMV. Moreover, sugar supplementation led to significant downregulation of *rmpADC*. To further elucidate the mechanism tying sugar transport to *rmpD* transcription and mucoidy, we screened a transposon library covering ~70% of the KPPR1 genome to identify genes required for suppressing mucoidy in sugar-supplemented M9 medium. The transposon screen identified genes involved in carbohydrate and amino acid transport and metabolism, suggesting their role in sugar-mediated HMV suppression.

Conclusion: These findings collectively suggest that host-derived sugars could act as nutrient signals, selectively regulating *K. pneumoniae* hypermucoviscosity during infection. Further defining the mechanism by which sugars modulate hypermucoviscosity and how this observed phenotype manifests *in vivo* would contribute to better understanding of *K. pneumoniae* pathogenesis.

Exploring Hemispheric Lateralization: Implications for Parkinson's Disease and Cell Transplantation Therapies

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Keywords: Parkinson's Disease (PD), Movement Disorders, neurodevelopmental disorders

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Background: Hemispheric lateralization refers to the distinct information-processing properties exhibited by the cerebral hemispheres of the human brain (1). There is a well-established specialization of function in the two hemispheres, with the left hemisphere primarily specialized for language function in right-handed individuals. In contrast, the right hemisphere is mainly specialized for visuospatial function in right-handed individuals and ambidextrous individuals with no hand preference (2). Evidence also suggests that paw preference in rats is similar to human handedness (3). Despite the evolutionary development of hemispheric specializations in humans, their role in cell transplantation for Parkinson's disease (PD) remains poorly studied. Previous studies have indicated that cell transplantation in the striatum of the dominant hemisphere, as opposed to the non-dominant hemisphere in 6-hydroxydopamine lesioned rats, resulted in improved motor behavior (4). However, the potential underlying factors for the improvement in motor behavior have not been explored. This experiment aims to investigate whether lateralization exists in the case of the substantia nigra pars compacta (SNpc) and striatum between the dominant and non-dominant hemisphere animal groups.

Methods: We hypothesize that animals within the dominant hemisphere will exhibit a significantly higher population of dopaminergic neurons, as well as variation in volume in SNpc and striatum compared to the non-dominant hemisphere animal group. (N=15) Sprague Dawley rats will be assigned to a paw preference test to determine the degree of handedness (right, left, or ambidextrous) as a measure of hemispheric dominance. Subsequently, a rodent behavioral battery test (RBBT) will be performed, followed by euthanasia, Cresyl violet (CV) staining and Tyrosine hydroxylase (TH)-immunohistochemistry. Stereological quantification of TH expression will be done on SNpc and striatum in each hemisphere using Stereo Investigator (MBF Bio.) software. The potential finding of variation in intrinsic factors between dominant hemisphere and non-dominant hemisphere animals is crucial for understanding successful cell transplantation in PD patients.

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Synthetic Psychoactive Cathinones (SPCs): Predicting Toxicity using *In Vitro* and *In Vivo* Models

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Background: In 2021 a staggering 33,000 American lives were lost to psychostimulant overdoses, accounting for over 30% of all drug overdoses that year. Synthetic psychoactive cathinones (SPCs) are novel psychoactive substances with effects like cocaine, methamphetamine, and methylenedioxymethamphetamine (MDMA). SPCs are of great concern because their abuse liability and potential for adverse effects, including lethal overdose, are largely unknown. Cell culture can help streamline toxicity assessment of new drugs of abuse.

Methods: For zebrafish studies, 5-day post fertilization (dpf) wildtype larval fish were exposed to various concentrations of SPCs to determine lethal dose 50 (LD50). For In vitro studies cell viability was assessed using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT). HepG2 (hepatic), AC-16 (cardiac), and SH-SY5Y (neural) cells were exposed to each SPC to determine the half-maximal inhibitory concentration (IC50).

Results: The LC50 values in zebrafish exhibited a correlation with the IC50 values of both the HepG2 (R^2= 0.8653, F(1,6)= 38.53, p= 0.008) and SH-SY5Y (R^2= 0.5762, F(1,6)= 8.158, p= 0.0289) cells. However, there was no significant correlation between zebrafish lethality and AC-16 toxicity (R^2= 0.3182, F(1,6)= 2.801, p= 0.1452).

Conclusion: The toxicity evaluation of SPCs in HepG2 and SH-SH5Y cell lines predict lethal toxicity in zebrafish. The similarity in toxicity patterns between these cell lines and zebrafish strengthens the potential utility of predictive *in vitro* models of SPC-induced lethal toxicity. The correlation between the toxicity in both cell lines and zebrafish indicates that the adverse and lethal effects of SPCs involves cellular mechanisms rather than physiological factors. Therefore, cell culture can provide insights into the cause of *in vivo* lethality and reduce the number of vertebrate subjects necessary for the study of the toxicity of these drugs, in line with the 3R principle of animal research.

MultiomicMenu: Streamlining Multiomics Data Interpretation for Insights into Neuronal Responses to Glutamate Treatment

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Background: The exponential growth of omics data poses a significant challenge to biomedical researchers. The development of high-throughput multiomics technologies has opened new avenues for understanding complex biological phenomena, yet the sheer volume of data often overwhelms human cognitive capacity. This "data deluge" has hindered the efficient interpretation of omics results, limiting their applicability in fields like precision medicine. To address this bottleneck, we present the "MultiomicMenu," an interactive web application designed for the interpretation of multiomics data. Our objective is to introduce the MultiomicMenu and demonstrate its utility in a practical use case. We applied this software package to analyze RNAseq and kinome array data obtained from rat neurons subjected to glutamate treatment. Utilizing the PCSF algorithm for multiomic data integration implemented by the Kinograte R package, we sought to confirm glutamate's known association with cytotoxicity, apoptosis, and stress pathways.

Methods: We employed the Kinograte R package to create an integrated protein-protein interaction (PPI) network from the transcriptomic and kinomic datasets. Node prizes and edge costs were assigned based on absolute log fold change or z score, respectively, and STRING-DB interaction confidence. Gene-set enrichment analysis was performed using the enrichR R package to identify dysregulated pathways. The MultiomicMenu facilitated functional interpretation through interactive network and pathway visualizations.

Results: The MultiomicMenu allowed us to identify and visualize significantly altered pathways, revealing clusters associated with cytotoxicity, apoptosis, and stress. The PPI network highlighted key

"hub" genes involved in these pathways, providing valuable insights into the molecular mechanisms underlying glutamate-induced neuronal responses.

Conclusion: Our study demonstrates the effectiveness of the MultiomicMenu in streamlining the interpretation of complex multiomics data, enabling researchers to uncover biologically relevant insights with ease. This interactive web application holds promise for accelerating discoveries in the field of systems biology and advancing the application of omics data in precision medicine and beyond.

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Assessing the abilities of Factor H-Fc IgG fusion protein variants as a therapeutic against *Burkholderia pseudomallei*

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Introduction: *Burkholderia pseudomallei* (Bp) is a Gram-Negative bacterium and is emerging as a global health threat, including the America's, causing melioidosis and lethal sepsis. Because Bp has a $LD_{50} \geq$organisms, it is designated as a Tier 1 select agent due to its bioweapon potential. Bp is naturally resistant to most antibiotics and there is no vaccine, thus there is a great need for therapeutics.

Methods: One of Bp's important virulence mechanisms is its ability to evade the host complement system. We have identified a surface protein expressed by Bp that can bind host Factor H, which is a negative regulator of the complement cascade and thus promotes immune evasion. Focusing on this mechanism, we are collaborating with Planet Biotech which has generated several chimeric molecules which contain the host binding site for Factor H and the other portion consists of the Fc region of human immunoglobulin G. Thus, this chimera should competitively bind to the bacterial surface, eliminating their ability to bind functional Factor H, and the IgG Fc region should activate the complement cascade to mediate direct and/or opsonophagocytic killing by immune cells.

Results: Our preliminary studies indicate that a subset of the initial constructs were able to bind to Bp, initiate C3 deposition, and generate membrane attack complexes (MAC) on their surface using ELISA. Based on these findings, we are now testing a second generation of constructs. Our current findings indicate that a subset of these new constructs are able to bind to, elicit C3 deposition, and generate MAC on Bp's surface better than the original construct. These chimera's are also able to promote direct killing of Bp strains.

Conclusion: Future studies will test their ability to promote opsonophagocytic killing by neutrophil/macrophages and protect mice from challenge with Bp.

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Characterization of the Role of Shikimic Acid in Vascular Smooth Muscle Cells

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Keywords: Vascular smooth muscle cells (VSMC), Shikimic acid (SA), platelet derived growth factor (PDGF)

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Background: Vascular smooth muscle cell (VSMC) phenotypic modulation from a contractile to a synthetic state is central to the etiologies of multiple vascular wall diseases such as atherosclerosis, hypertension, and post-angioplasty restenosis. Shikimic acid (SA) is a chemical derived from a variety of plants and microorganisms and has been found to exhibit diverse pharmacological activities in multiple cell/tissue systems, such as antioxidant, anti-inflammatory, and pro-proliferative effects. However, the role of shikimic acid in the vascular system is unknown. The purpose of this study is to examine the effect of SA in regulating VSMC proliferation and migration.

Methods: Using human coronary artery smooth muscle cells (hCASMC), we investigated the effects of SA (10 mM) in vitro on (i) serum-induced VSMC phenotypic switching by immunoblotting or qRT-PCR analyses, (ii) platelet-derived growth factor-BB (PDGF-BB, 30 ng/ml) activation of proliferative signaling, (iii) serum- or PDGF-BB-induced VSMC proliferation by performing WST-1 and CyQUANT assays, and (iv) serum-induced VSMC migration by performing scratch wound assay.

Results: SA enhanced the expression of CCND1, a proliferation marker, at both mRNA and protein levels. Consistently, SA enhanced the effects of PDGF-BB on proliferative signaling, including ERK1/2, Akt, and mTORC1 signaling. Conversely, SA activated antiproliferative signaling components including the AMPK/autophagy pathway. Proliferation and migration assays revealed no significant differences after SA treatment as compared to PDGF-BB or serum.

Conclusion: Our findings indicate that SA activates both pro- and anti-proliferative signaling components in VSMCs and suggest that the net outcome of activation of these diverse signaling pathways has little effect on VSMC proliferation or migration.

Hypertensive mice are more susceptible to experimental malaria

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Keywords: RBC, BPH, Plasmodium, Reticulocytes, Blood Pressure, Anemia

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Background: Global prevalence of hypertension is on the rise, especially in developing countries where infectious diseases, such as malaria, are also rampant. Whether hypertension could predispose or increase susceptibility to malaria, however, has not been extensively explored. Previously, we reported that hypertension is associated with abnormal erythrocyte physiology and anemia. Since erythrocytes are target host cells for malarial parasite, *Plasmodium*, we hypothesized that hypertensive patients with abnormal erythrocytes physiology are at greater risk or susceptibility to *Plasmodium* infection.

Method: To test this hypothesis, eight weeks old normotensive (BPN/3J) and hypertensive (BPH/2J) mice were characterized for their erythrocyte physiology and subsequently infected with green fluorescent protein-tagged *Plasmodium yoelii* (*P. yoelii*), a murine-specific non-lethal strain.

Results: When compared to BPN mice, BPH mice displayed microcytic anemia and their erythrocytes were highly resistant to osmotic hemolysis. Further, BPH erythrocytes exhibited an increase in membrane rigidity and an altered lipid composition, as evidenced by higher levels of phospholipids and saturated fatty acid, such as stearate (C18:0), along with lower levels of polyunsaturated fatty acid like arachidonate (C20:4). Moreover, BPH mice had significantly greater circulating $Ter119^{+}$ $CD71^{+}$ reticulocytes, or immature erythrocytes. Upon infection with *P. yoelii*, BPH mice experienced significant body weight loss accompanied by sustained parasitemia, indices of anemia, and substantial increase in systemic pro-inflammatory mediators, compared to BPN mice, indicating that BPH mice were incompetent in clearing *P. yoelii* infection.

Conclusions: Collectively, these data demonstrate that aberrant erythrocyte physiology observed in hypertensive BPH mice contributes to an increased susceptibility to *P. yoelii* infection and malaria-associated pathology.

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Effect of developmental pesticide exposure in Prairie voles as a model of neurodevelopmental disorders

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Keywords: Pyrethroids, Deltamethrin, Prairie Voles, Neurodevelopmental Disorders

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Background: Neurodevelopmental disorders (NDDs) are a class of lifelong incurable disorders with few treatments are biomarkers. They share common comorbidities including deficits in communication and learning, repetitive behavior, and hyperactivity such as attention deficit hyperactivity disorder (ADHD), Autism, and Developmental disability. The incidence of NDDs has been rapidly rising affecting almost 17% of children in the US. Heritability is still a crucial factor in the etiology of NDDs, however large meta-analysis data now consider environmental impact as a major contributor. Recent epidemiological studies have shown the effect of pyrethroid pesticides on pregnant women and risk factors associated with the proper brain development in the children. Pyrethroids are common household insecticides widely used in the US and considered relatively "safe" by the Environmental Protection Agency (EPA). Despite growing evidence of the complex gene-environment interaction in the etiology of NDDs, very few environmental factors have been studied.

Methods: Previously, our lab has studied the effects of deltamethrin (pyrethroid) in mice. Metabolomic analysis on whole male brain samples provided suggested a disruption in folate metabolism pathway as a result of developmental pesticide exposure. Our research aims to look at the effect of developmental pyrethroid exposure (DPE) in prairie voles as a model of neurodevelopmental disorders, and folic acid supplementation as a potential therapeutic strategy.

Results: We exposed pregnant vole dams to 3mg/kg of deltamethrin two weeks prior to pregnancy, during pregnancy and throughout lactation. A subset of pesticide exposed voles were supplemented with 5'MTHF (folate vitamer). Following weaning, the offspring grow to adulthood and are subjected to a battery of behavioral tests to look at deficits in five different domains namely communication, cognition, social interaction, repetitive behavior, and locomotion. After concluding behavioral tests their brains are harvested along with other tissues for further studies. In order to understand molecular disruptions in the folate metabolic enzymatic pathway, we conducted western blot analysis in five participating enzymes in the folate metabolic pathway, namely Dihydrofolate Reductase (DHFR), Methyltetrahydrofolate

Reductase (MTHFR), Serine hydroxymethyl transferase (SHMT), Methyl tetrahydrofolate dehydrogenase (MTHFD), Methionine synthase (MTRR), and Folate receptor alpha (FOLR1).

Conclusions: Our results show that developmentally exposed prairie voles had deficits in communication, cognition, repetitive behavior, and locomotion (hyperactivity). Previously harvested brains from the mice study were used to look at disruptions in molecular mechanisms because of DPE. Our data also suggest that folate supplementation alleviates some of the behavioral deficits. Disruptions in enzymatic expression was observed in FOLR1, MTHFR, and SHMT1. Such disruptions could be an effect of treatment or a compensatory mechanism for changes caused by developmental pesticide exposure.

Role of Insulin Signaling in Prostaglandin Synthesis

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Background: This study explores the impact of the insulin/FOXO pathway on prostaglandin E2 (PGE2) synthesis in hypothalamic astrocytes. Previous research established insulin's role in activating FOXOs, leading to the expression of PTGS (COX-1, 2) and PTGES genes, ultimately driving PGE2 synthesis in astrocytes and influencing fertility. While earlier findings indicated insulin's ability to regulate PGE2 pathways in distinct astrocyte cell lines, questions remained about its specific effects on hypothalamic astrocytes.

Methods: To address this, astrocyte cell lines and primary astrocytes were isolated and treated with insulin or a control. Quantitative PCR and western blotting confirmed that 250nM of insulin induced Cox-2 expression within 30 minutes. Concerns about nonspecific signaling led to a decision to treat at 100nM for 6 hours. Transcriptomic analysis of RNAseq data revealed insulin's down-regulation of sterol and cholesterol biosynthesis pathway genes in male hypothalamic astrocytes. Kinome array analysis identified differentially phosphorylated kinases in the presence of insulin, with some sex-specific patterns.

Results: Notably, insulin phosphorylated AKT1, AR, P53, mTOR, RAF1, CDK1, GYS2, and MAPK10 in both sexes. MAPK1 showed male-specific phosphorylation, while MAPK3 and ISR2 displayed female-specific phosphorylation. In the presence of insulin, increased activity was observed in genes related to autophagy (more pronounced in males), various cancers, insulin resistance, type II diabetes, insulin signaling, FOXO signaling, and GnRH signaling and secretion (more prominent in females).

Conclusions: In conclusion, this study sheds light on how the insulin/FOXO pathway influences PGE2 synthesis in hypothalamic astrocytes. Insulin induces COX-2 expression and modulates pathways linked to sterol and cholesterol biosynthesis. Sex-specific phosphorylation patterns revealed by kinome analysis further contribute to our understanding of insulin and FOXO regulation in astrocytes, impacting PGE2 synthesis and associated pathways.

