NIH

Diversity Supplement Professional Development Workshop

August 30th – August 31st 2022

Book of Abstracts

Agenda

Tuesday, August 30th

11:00 a.m.	**Welcome Remarks and Meeting Goals** (Zoom) *Albert Avila, Ph.D., Director, Office of Diversity and Health Disparities, Deputy Director, Office of Research Training, Diversity, and Disparities, National Institute on Drug Abuse, National Institutes of Health*
11:05 a.m.	**NIH Diversity, Equity, Inclusion, and Accessibility (DEIA) Priorities and Initiatives** (Zoom) *Marie A. Bernard, M.D.* *Chief Officer for Scientific Workforce Diversity (COSWD),* *National Institutes of Health*
11:35 a.m.	**General Introduction to the NIH Grant and Review Process** (Zoom) *Joan Greve, PhD, Program Director* *SBIR Development Center, National Cancer Institute* *Matthew Lockhart, MBA* *Director, Division of Loan Repayment* *National Institutes of Health*
11:55 a.m.	**Demystifying NIH Research Funding Opportunities and the Review Process: Breakout Sessions by Career Level** (Zoom Breakout Sessions) <u>Breakout: Undergrad/Post-Baccalaureate</u> *Marguerite Matthews, PhD, Program Director, Office of Programs to Enhance Neuroscience Diversity, Division of Extramural Activities, National Institute of Neurological Disorders and Stroke, National Institutes of Health* <u>Breakout: Predoctoral Student</u> *Ashlee Van't Veer, PhD, Director, Office of Research Training and Career Development, Division of Neuroscience and Basic Behavioral Science, National Institute of Mental Health, National Institutes of Health* *Desirée Salazar, PhD, Diversity, Equity, and Inclusion Coordinator for Extramural Programs National Heart, Lung, and Blood Institute, National*

Institutes of Health

Jamie L Lahvic, PhD, Program Officer
Office of Strategic Extramural Programs (OSEP),
National Institute on Aging, National institutes of Health

Lauren E. Ullrich, PhD, Program Director, Office of Programs to Enhance Neuroscience Workforce Diversity (OPEN), National Institute of Neurological Disorders and Stroke, National Institutes of Health

Leslie Frieden, PhD, Extramural Training Officer, Research Training and Career Development Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health

Melissa Smarr, Ph.D., Health Scientist Administrator
Population Health Branch, Division of Extramural Research and Training
National Institute of Environmental Health Sciences

Breakout: Postdoctoral Fellow

Maria G. Carranza, PhD, NIA Training Officer
Division of Extramural Activities (DEA), National Institute on Aging,
National Institutes of Health

Robert Clay Rivers, PhD
Office of Minority Health Research Coordination
National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

Kenneth Gibbs, PhD, Program Director, National Institute of General Medical Sciences, National Institute of Health
Institutes of Health

Breakout: Investigator/Faculty

Albert Avila, PhD, Director, Office of Diversity and Health Disparities, Deputy Director, Office of Research Training, Diversity, and Disparities, Director, Office of Diversity and Health Disparities, National Institute on Drug Abuse, National Institutes of Health

Frederick L. Tyson, Ph.D., Program Director
Genes, Environment, and Health Branch
Division of Extramural Research and Training
National Institute of Environmental Health Sciences

1:15 p.m. **Lunch Break** (Zoom or Gather Town networking)
Time allowed to continue discussing funding opportunities within breakout sessions

1:45 p.m. **Meet a PO: Small Group Breakout Session with POs from Institutes and Centers**(Gather Town)

2:15 p.m. **Diversity Supplement Scholar Poster Session 1** (Gather Town)

3:15 p.m. **Break**

3:30 p.m. **A Journey to Becoming an NIH Funded Investigator (Panel Discussion)** (Zoom)

Moderators:

Houmam Araj, PhD, Director of Lens/Cataract, Oculomotor/Neuro-Ophthalmology, Ocular Pain Programs, and Conference Grants
National Eye Institute, National Institutes of Health

Leslie Frieden, PhD, Extramural Training Officer, Research Training and Career Development Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health

Panelists:

Ana Ortiz
PhD Candidate
Department of Neuroscience
UT Southwestern Medical Center

Dionna W. Williams, PhD
Assistant Professor
Department of Molecular and Comparative Pathobiology
Johns Hopkins University School of Medicine

Jessica Scoffield, PhD
Assistant Professor
Department of Microbiology
University of Alabama Birmingham

Lillian J. Brady, PhD, MS
NIH/NIDA – MOSAIC K99/R00 Scholar
Academic Pathways Postdoctoral Research Fellow
Department of Pharmacology
Vanderbilt University

Thomas K.M. Cudjoe, MD, MPH
Robert and Jane Meyerhoff Endowed Assistant Professor
Division of Geriatric Medicine and Gerontology
Johns Hopkins University School of Medicine

4:55 p.m. **Day 1 Closing Remarks/Q&A/Adjourn**
Lanay M. Mudd, PhD, Program Director, Clinical Research in Complementary and Integrative Health Branch, Division of Extramural Research, National Center for Complementary and Integrative Health, National Institutes of Health

Wednesday, August 31st

11:00 a.m. **Day 2 Welcome Remarks**
Shakira Nelson, PhD, Program Director, Division of Training, Workforce Development and Diversity, National Institute of General Medical Sciences

11:05 a.m. **Cultivating Curiosity: On Becoming and Being a Scientist**
Alejandro Sánchez Alvarado, PhD
Executive Director and Chief Scientific Officer, Stowers Institute for Medical Research Howard Hughes Medical Institute, Principal Investigator Faculty, The Graduate School of the Stowers Institute for Medical Research

11:35 a.m. **Get to Know Your NIH Institute/Center** (Zoom Breakout Sessions)
Participants will spend an hour meeting with staff from the NIH Institute that funded their diversity supplement.

12:30 p.m. **Lunch Break and Optional Networking Session** (Gather Town)
Time to continue the conversation with IC leadership or meet with other scholars in a smaller setting

1:15 p.m. **Diversity Supplement Scholar Poster Session 2** (Gather Town)

2:15 p.m. **Transition to Zoom**

2:20 p.m. **Resubmissions, Resources and Resilience (R^3)** (Zoom)
Albert Avila, PhD, Director, Office of Diversity and Health Disparities, Deputy Director, Office of Research Training, Diversity, and Disparities, National Institute on Drug Abuse, National Institutes of Health

Jamie Mihoko Doyle, PhD
Clinical and Translational Science Awards (CTSA) Program, DCI, National Center for Advancing Translational Sciences (NCATS)

3:15 p.m. **How to Choose a Mentor and Navigate the Relationship** (Zoom)
Introduction by Eunyoung Kim, PhD, Scientific Research Analyst, Office of the BRAIN Director, National Institute of Neurological Disorders and Stroke, The BRAIN Initiative

Fátima Sancheznieto, PhD
Assistant Researcher, University of Wisconsin – Madison
President, Future of Research

3:45 p.m. **Science Careers Panel Discussion** (Zoom)

Moderators:

Desirée Salazar, PhD, Diversity, Equity, and Inclusion Coordinator for Extramural Programs National Heart, Lung, and Blood Institute, National Institutes of Health

Reiko Toyama, PhD, Program Director,
Developmental Biology and Structural Variation Branch
Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health

Panelists:

Caleph B. Wilson, PhD
Business Development Manager, Axion Biosystems

Gloriana Trujillo, PhD
Director, Academic Teaching Programs, Center for Teaching and Learning, Stanford University

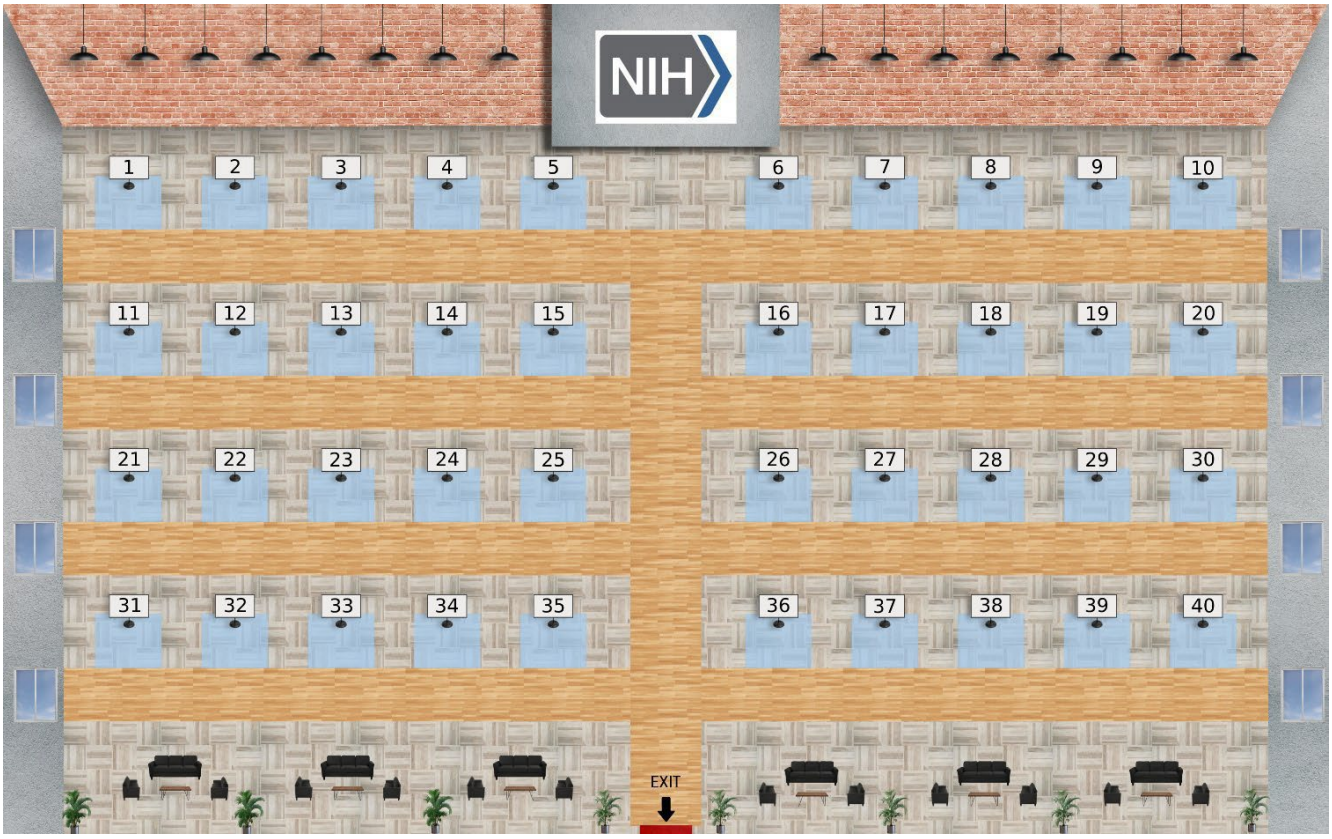
Janine Low-Marchelli PhD
Manager, Technical Information Services, The Jackson Laboratory

Rashada Alexander, PhD
Director, Science and Technology Policy Fellow Program at AAAS

4:55 p.m. **Closing Remarks** (Zoom)
Marguerite Matthews, PhD, Program Director, Office of Programs to Enhance Neuroscience Diversity, Division of Extramural Activities, National Institute of Neurological Disorders and Stroke, National Institutes of Health

Poster Sessions – Tuesday, August 30th

Session A



1 **An Adaptive Excitation Source for In-Vivo Cell Tracking with Multiphoton Microscopy**
Alejandro Simon, The BRAIN Initiative

2 **Caregiver Adverse Childhood Experiences and Child Externalizing Problems: The Role of Caregiver Resilience**
Tre Gissandaner, ECHO/OD

3 **Children's Screen Time and Associations with Academic Skills**
Linsah Coulanges, NICHD

4 **The Effect of an Unconditional Cash Gift on Intimate Partner Violence for Low-Income Families with Infants and Toddlers: Evidence from the Baby's First Years Study**
Maya Escueta, NICHD

5 **The Temple Tour: Relating episodic memory and spatial navigation in children and adults**
Kim Nguyen, NICHD

6 **UV Exposure, Diagnostic Scrutiny and Melanoma Incidence in US counties**
Adewole Adamson, NCATS

7 **Biocompatibility of Geopolymers for Bone Tissue Regenerative Engineering**
Ange-Therese Akono, NCATS

8 **The Effect of Thyroid-Stimulating Hormone (TSH) on Brown Adipose Tissue (BAT) in Humans**
Alina Gavrila, NCATS

9 **Investigation of keratoconus tear extracellular vesicles phenotype**
Brenna Hefley, NEI

10 **Partial deletion of Lecithin:retinol acyltransferase (Lrat) inhibits prodromal characteristics of diabetic retinopathy**
Emma Lessieur-Contreras, NEI

11 **Evaluating the Role of Extradenticle in the Ventral margin of the Drosophila eye**

Jasmine Warren, NEI

12 **The Role of RUNX1 in CM Cell Cycle Activity and its Impact on Cardiac Regeneration**
Kaelin Akins, NHLBI

13 **Gene Knockdown of Transporters and Receptors in Drosophila Blood Brain Barrier Alters Sleep**
Ashley Avila, NHLBI

14 **Expression and characterization of the TREM-Like Transcript-1 protein in the brain**
Yancy Ferrer-Acosta, NHLBI

15 **Bioengineered 3D in vitro strategies to investigate phenotypic and genotypic differences in lymphatic network sprouting**
Yarelis Gonzalez-Vargas, NHLBI

16 **Cardiovascular Health Classification using Arterial Dispersion Ultrasound Vibrometry**
Hadiya Harrigan, NHLBI

17 **Prefrontal-medullary circuit attenuates stress reactivity**
Sebastian Pace, NHLBI

18 **Associations between Neighborhood Built Environment and Cardiovascular Risk in Adults Residing in Caribbean Small Island Developing State**
Kern Rocke, NHLBI

19 **Inhibition of purinergic receptor signaling and its role in the protection of endothelial integrity during Plasmodium falciparum infection**
Marilyn Vasquez, NHLBI

20 **Identification of a novel candidate molecular mediator of QT interval prolongation and arrhythmia risk**
Lamario Williams, NHLBI

21 **Unexpected Heterogeneity In Reporting Bioethical Issues Arising From Advances in Chimera Research From Online New**
Briana Lopez-Patino, NHGRI

22 **Properties of the Reovirus Attachment Protein affect Particle Stability**
Max Garcia, NIAID

23 **Selenoprotein I deficiency in T cells promotes differentiation into tolerant phenotypes while decreasing Th17 pathology**
Lance Gergory Nunes, NIAID

24 **Paradoxical Effect of Frizzled2-Fc on Bone Mass for the Treatment of Osteogenesis Imperfecta**

Mary Adeyeye, NIAMS

25 **Determining the functional role of Osteocytic genes that have potential clinical relevance to human bone fragility**
Clarissa Aguirre, NIAMS

26 **The epigenetic regulation of epidermal stem cells during adult skin wound healing**
Meagan Branch, NIAMS

27 **Low Vitamin D status associates with quadriceps atrophy following ACL reconstruction**
Jean Fry, NIAMS

28 **Trimethylamine N-Oxide Mitigates Cell Death Due to Heat Injury in Monolayer Culture and Osteochondral Explants**
Andy Garcia, NIAMS

29 **The Role of Interface Geometry and Appendages on the Microscale Mechanics of the Skin**
Omar Moreno Flores, NIAMS

30 **Synovial Tissue Metabolomic Profiling Reveal Biomarkers of Synovial Inflammation in Patients with Osteoarthritis**
Jessica Murillo Saich, NIAMS

31 **Synthetic Hydrogels for Muscle Satellite Cell Transplantation in Dystrophic Diaphragms**
Nia Myrie, NIAMS

32 **Cell Adhesive an Growth Factor Peptide Mimetics Cooperatively Influence Osteogenic Activity of Mesenchymal Stem Cells in 3D Culture**
Sydney Neal, NIAMS

33 **Design and Evaluation of Biphasic Dual Drug Delivery System for the Treatment of Osteoarthritic Pain**
Erick Orozco Morato, NIAMS

34 **Regulation of Myoblast Differentiation And Skeletal Muscle Formation By The Extracellular Protease ADAMTS10**
Keron Rose, NIAMS

35 **Determining the Role of Collagen XI on Collagen Fibril Deformation and Sliding in Developing Mouse Patellar Tendon**
Jaime Santillan, NIAMS

36 **A New Switching Technique for Multinuclear Study of Duchenne Muscular Dystrophy**
Edith Valle, NIBIB

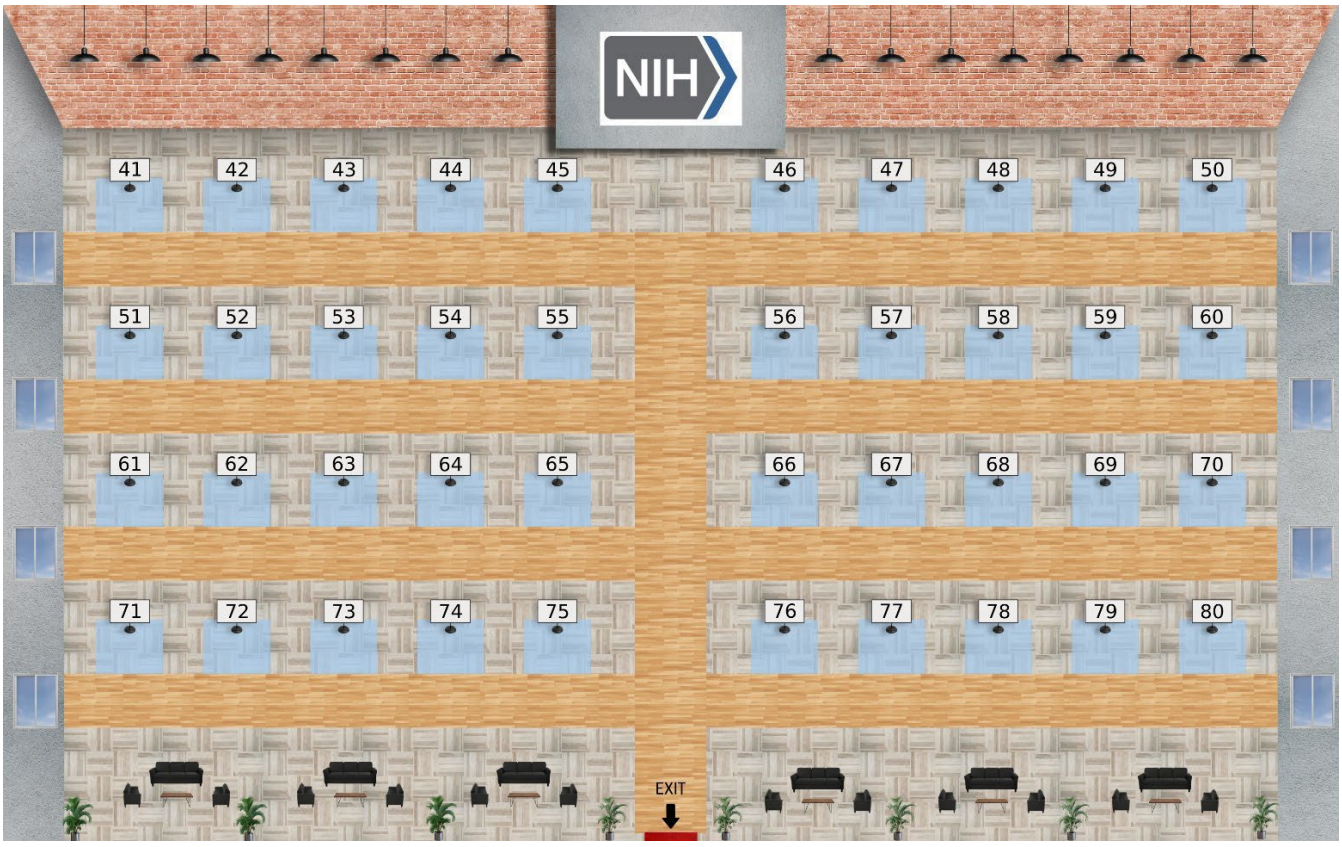
37 **Characterization of PDGFRalpha/beta heterodimer-specific dynamics**
Maria Campana, NIDCR

38 **X-ray Crystal Structure of Treponema denticola TDE0362 Protease Domain**
Nicholas Clark, NIDCR

39 **In vitro 3D human gingival tissue model to study oral microbiome: computational model to estimate long-tern culture conditions**
Anyelo Diaz, NIDCR

40 **Using the Burrowing Test to Detect Affective State Behaviors in Rat Neuropathic Pain Models**
Brianna Doan, NIDCR

Session B



41 **Collagen architecture and stiffness affect vascular homeostasis**
Cristiane Franca, NIDCR

42 **Examination of magnesium hydroxide nanoparticle antibacterial activity and identification of bacteriostatic or bactericidal effects of magnesium hydroxide and magnesium oxide nanoparticles in pathogenic bacteria**
Patricia Holt-Torres, NIDCR

43 **Jaw Bone Length is Altered by Pharmacological Inhibition of Matrix Metalloproteinase-9**
Claire Houchen, NIDCR

44 **From Microbiologist to Medical Device Manufacturing- Transition Through NIDCR Diversity Funding and Mentoring by NuShores Biosciences LLC**
Amairani Paredes Lester, NIDCR

45 **Polymeric Nanoparticles for Endosomal Delivery of Pain Therapeutics**
Parker Lewis, NIDCR

46 **Neuronal subclass specificity of mechano-gated current responses in trigeminal ganglion neurons innervating masseter muscle**
Karen Lindquist, NIDCR

47 **Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) quantitative parameters, Ktrans and Ve, for monitoring of mandibular injury/osteoradionecrosis**

Jillian Rigert, NIDCR

48 **Susceptibility of enamel to acid attack in Krt75 tm1Der knock-in mouse model**
Brent Vasquez, NIDCR

49 **Spatial analysis of Wnt and Fzd receptor expression in adult mouse liver**
Jenesis Gayden, NIDDK

50 **The Role of Mitochondria in Skin Aging and Healing**
Keisha Hardeman, NIDDK

51 **Biological Underpinnings of Emotional Eating in Middle Childhood**
Erika Hernandez, NIDDK

52 **Investigating the Role of Mitochondria in Eosinophilic Esophagitis**
Jazmyne Jackson, NIDDK

53 **GLS1 dependent regulation of the IL-17 axis shapes NAFLD pathogenesis**
Jarren Oates, NIDDK

54 **Understanding the signals that promote beta cell growth in the setting of exocrine insufficiency**
Danielle Overton, NIDDK

55 **Colorimetric β-galactosidase Assay Facilitates the Indirect Measurement of cAMP Production via the Activation of Gs-Pathways in the Melanocortin Receptors Subtypes**
Ricardo Rosas, NIDDK

56 **Impact of COVID-19 on Individual Behavior and Household Exposure Related to Smoking, Vaping and Marijuana Use Among Adults with Asthma**
Luz Huntington-Moskos, NIEHS

57 **Investigating the fetal hepatic proteomic response to in utero dimethylbenz[a]anthracene**
Imaobong Inyang, NIEHS

58 **Depleted uranium exposure disrupts mitochondrial morphology, metabolism, and genomic stability**
Phillip Kalaniopio, NIEHS

59 **Multi-'omics analysis identifies PCB-regulated pathways in Nonalcoholic Fatty Liver Disease**
Belinda Petri, NIEHS

60 **Oxidative metabolism effects on the activity of the Sonic Hedgehog pathway inhibitor piperonyl butoxide**

Kenneth Rivera Gonzalez, NIEHS

61 **Time Series Clustering for the Integration of p53 Protein Dynamics and Transcriptomics in Single Cells**
Emily Ackerman, NIGMS

62 **Super-resolution imaging of unusual metal-responsive transcriptional regulation mechanisms in bacteria**
Felix Alfonso, NIGMS

63 **History-dependent collagen remodeling enables cell invasion across environments of distinct stiffness and dimensionality**
Jose Almeida, NIGMS

64 **Cyclin-Dependent Kinase Motif Enrichment for Isobaric Trigger Channel-Based Substrate Analysis**
Dominique Baldwin, NIGMS

65 **Understanding the Mechanism of Host Glutathione Depletion by Helicobacter pylori**
Maia Baskerville, NIGMS

66 **Spermidine Enhances the Contractile Force of Single-Cell Human Induced Stem Cell-Derived Cardiomyocytes Post Doxorubicin Treatment**
Cheavar Blair, NIGMS

67 **Structure and Candidate Biosynthetic Gene Cluster of a Manumycin-type Metabolite from Salinispora pacifica**
Gabriel Castro-Falcón, NIGMS

68 **Efficacy of the Rac/Cdc42 inhibitor MBQ-167 in combination therapy with Paclitaxel**
Ailed Marie Cruz Collazo, NIGMS

69 **Regulation of the maintenance of the [PIN+] prion by the evolutionarily conserved non-prion domain of the [PIN+]-forming protein, Rnq1**
Irina Derkatch, NIGMS

70 **Solid-Phase Photochemical Modification of Peptides via Charge-Transfer Complexes**
Mahmoud Elkhalifa, NIGMS

71 **Investigating the role of METTL16 in SAM homeostasis and U6 snRNA function**
Juliana Flaherty, NIGMS

72 **Nickel-Catalyzed Cascade Cyclization of Alkene-Tethered Alkylpyridinium Salts**
Bria Garcia, NIGMS

73 **Development of Novel Therapeutics for Depression**
Monica Gonzalez, NIGMS

74 **Identifying neuronal circuits that coordinate sugar and water ingestion**
Amanda Gonzalez Segarra, NIGMS

75 **Tissue-Specific Role of FXR in Non-Alcoholic Steatohepatitis Development in Female Mice**
Henry Zakiyah, NIGMS

76 **Modular Synthesis of Unnatural Peptides via Rh(III)-Catalyzed Diastereoselective Three-Component Cross-Coupling Reaction**
Christopher Lamartina, NIGMS

77 **Diversity and dispersal contribute to animal microbiome resistance and resilience to disturbance**
Rebecca Maher, NIGMS

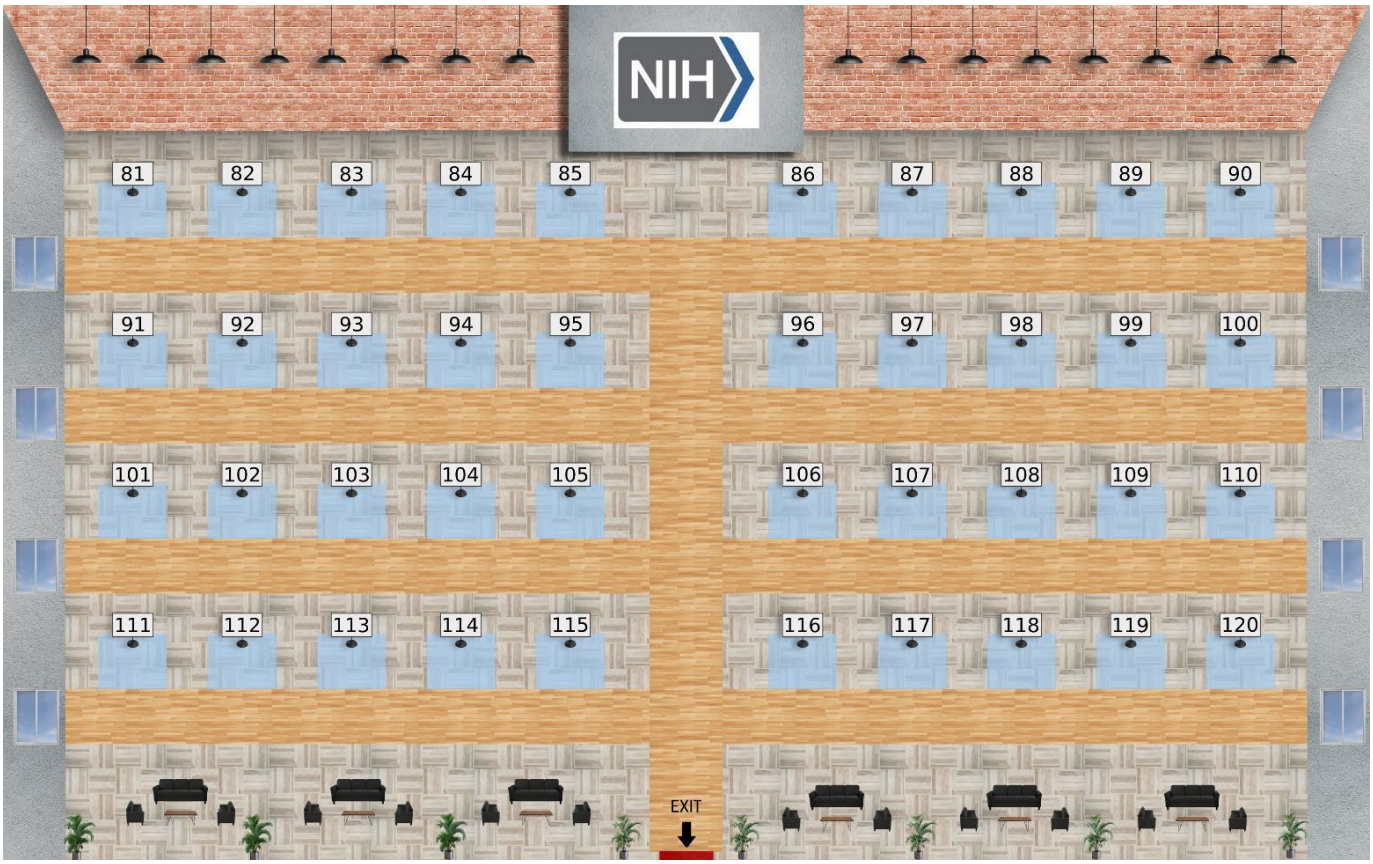
78 **The Unkempt RNA binding satellite protein promotes PLK4-induced centriole overduplication**
Abraham Martinez, NIGMS

79 **Determining the mechanism of Kinesin-1 dependent translocation of the meiotic spindle to the cortex in C. elegans**
Alma Martinez Peraza, NIGMS

80 **Investigating the Role of the p97 Adaptor UBXD8 in Peroxisome Function**
Iris Montes, NIGMS

Poster Sessions – Wednesday, August 31st

Session C



81 **The Sex-Specific Effects of Adolescent Intermittent Ethanol Exposure on Hippocampal Neurogenesis and Neurotrophic Response Following Abstinence**
Kala Nwachukwu, NIGMS

82 **Regulation of the budding yeast meiotic proteome**
Maia Reyes, NIGMS

83 **Aurora kinase B is required to maintain mitochondrial quality in aging oocytes**
Mayra Romero, NIGMS

84 **Characterizing ESCO1's Interaction with Chromatin**
Jeffrey Schoen, NIGMS

85 **Generation of SIX3 Mutants for Human Neural Differentiation Studies**
Carolina Torres Rojas, NIGMS

86 **Estimating the Kinetics of mRNA 3' End Cleavage**
Leslie Torres Ulloa, NIGMS

87 **DNA-binding and degradation of mitochondrial genome maintenance exonuclease (MGME1) is enhanced by a 5′-phosphate**
Kathleen Urrutia, NIGMS

88 **Engineering of LwaCas13a Protein with Enhanced Collateral Activity for Ultrasensitive Nucleic Acid Detection**
Jeffrey Vanegas, NIGMS

89 **Yeast PI31 Inhibits the Proteasome by a Direct Multisite Mechanism**
Benjamin Velez, NIGMS

90 **Fast conformational exchange and low populated states as cornerstones in protein allostery**
Darex Vera-Rodriguez, NIGMS

91 **Blockade of CCR3 during fluid resuscitation from hemorrhagic shock**
McWayne Weche, NIGMS

92 **Inhibitory and excitatory responses to feedforward auditory inputs in the mouse posterior parietal cortex**
Constanza Bassi, NIMH

93 **Advancing Measurement of Clinical Supervision to Support the Implementation of Evidence-Based Practice**
Mimi Choy-Brown, NIMH

94 **Examining Socio-cultural barriers to mHealth tools**
Jacqueline Duong, NIMH

95 **Investigating sex-differences in the epigenetic regulation of nuclear protein degradation in the amygdala**
Kayla Farrell, NIMH

96 **Patient derived SYNGAP1 mutations disrupt excitatory networks in human models of neurodevelopmental disease**
Ilse Flores, NIMH

97 **Using Race-Conscious Framework to Explore Pathways Structural Racism May Impact the Effects of Racially Diverse Mental Health Workforce: A Critical Review with Case Example**
Eric Kyere, NIMH

98 **Type I interferon signaling drives microglial phagocytosis of whole neurons during postnatal development**
Christian Lagares Linares, NIMH

99 **The role of sex and cell-type specific protein degradation in fear memory formation**
Taylor McFadden, NIMH

100 **The Effect of Cultural and Linguistic Concordance on Family Engagement with Child Behavioral Health Services**
Rocio Nunez Pepen, NIMH

101 **Sex differences in the selection of stress coping strategy as a predictor of post- stress avoidance behavior**
Kailyn Price, NIMH

102 **PrEParados: A Multi-Level Social Network Model to Increase PrEP Enrollment by Latino MSM Self-Identified as Gay, Bisexual or Straight in Miami**
Edda Rodriguez, NIMH

103 **Cortical Organoids as a Model for the Study of Neurodevelopmental Disorders**
Soraya Sandoval, NIMH

104 **Development and Usability Testing of a Chatbot to Promote Mental Health Services Use Among Individuals with Eating Disorders Following Screening**
Jillian Shah, NIMH

105 **Cognitive and motivational consequences of paternal snord116 knockout in dopaminergic cells**
Layla Vasquez, NIMH

106 **How do Gender Diverse Students Identify their Sexual Orientation?**
Melissa Vazquez, NIMH

107 **The association between interpersonal factors, barriers to mental health help-seeking behaviors, and dysthymia among Black and Latinx youth**
Carolina Vélez-Grau, NIMH

108 **Data-Driven Analysis of the Intergenerational Effects of Maternal ACEs on Offspring Subcortical Neurodevelopment**
Albert Wakhloo, NIMH

109 **The effect of low intensity pulsed ultrasound on peripheral nerve regeneration**
Jenica Acheta, NINDS

110 **Intraspinal Microstimulation Intended for Motor Rehabilitation Modulates Spinal Nociceptive Transmission**
Maria Bandres, NINDS

111 **Resolving the molecular basis for the translocation of a fungal pathogen from blood to brain**
Amelia Bennett, NINDS

112 **Increased nuclear DNA damage and activation of the DNA damage response in LRRK2 G2019S Parkinson's disease models**
Claudia Gonzalez-Hunt, NINDS

113 **The Effect of an Antioxidant Coating on the Acute Recording Performance of Planar Silicon Intracortical Microelectrode Arrays**
Ana Hernandez Reynoso, NINDS

114 **Mitochondrial Trafficking as a Target for GBM Therapy**
Javier Lepe, NINDS

115 **Sex-dependent cognitive dysfunction following repeated mild TBI in adolescent animals may be dependent on alterations in acetylcholine and corticotropin releasing factor expression**
Taylor McCorkle, NINDS

116 **Oncostatin M induces nociceptive signaling and satellite glial activation in human dorsal root ganglia**
Juliet Mwirigi, NINDS

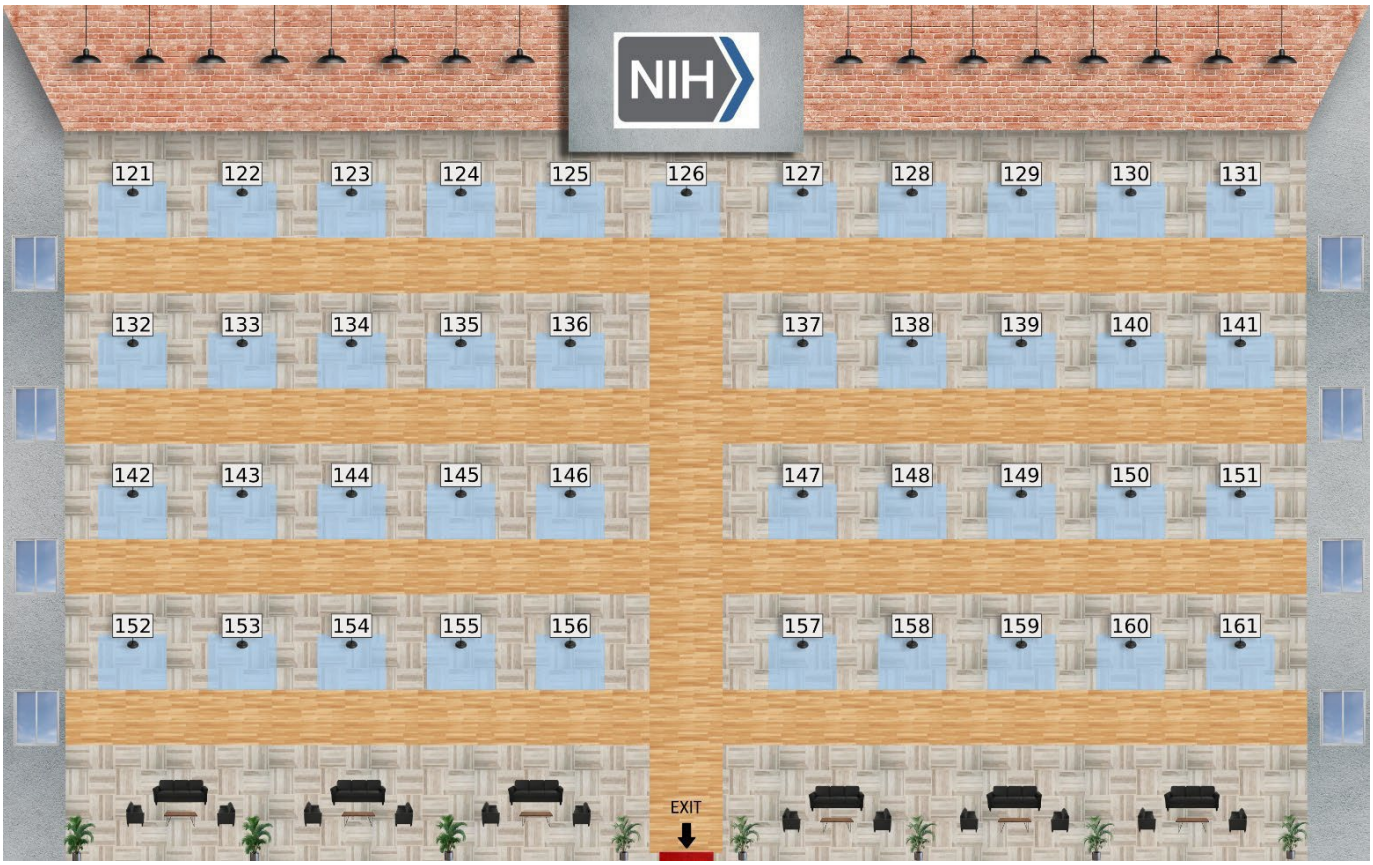
117 **Genipin rescues sensory neuron defects in Familial Dysautonomia**
Kenyi Saito-Diaz, NINDS

118 **Developing a Collaborative Community Intervention to Train Chronic Kidney Disease Family Caregivers in providing Decision Support**
Shena Gazaway, NINR

119 **Race/Ethnic Disparities in Insulin and Sulfonylurea Use in Older Patients with Type 2 Diabetes**
Dana Abdelgadir, NIA

120 **Stress Accumulation and Sleep among Black Adults Living in the Rural South**
Olutosin Adesogan, NIA

Session D



121 **Identifying Distinctions and Commonalities between Elder Mistreatment and Late-life Intimate Partner Violence: A Study of ADRD Caregiving Dyads**
Elizabeth Avent, NIA

122 **Cognitive Reserve Moderates the Association Between Cerebral Blood Flow and Language Performance in Older Adults with Mild Cognitive Impairment**
Einat Brenner, NIA

123 **An Epithelial Organoid Approach to Understanding Stemness in the Intestine**
Saxton Cruz, NIA

124 **Understanding Barriers to the Digital Collection of Mobile and Wearable Device Data to Monitor Health & Cognition in Older Adults: A Scoping Review**
Ibukun Fowe, NIA

125 **The Effect of APOE on Lipid Droplet Dynamics in Microglia**
Cassi Friday, NIA

126 **The Neighborhood Context and All-Cause Mortality among Older Adults in Puerto Rico**
Catherine Garcia, NIA

127 **Short-term high-fat diet consumption impairs long-term potentiation in the aged hippocampus**
Brigitte Gonzalez Olmo, NIA

128 **Optimizing Induced Neuron Cultures from Aged Human Fibroblasts for Electrophysiology Studies**
Melissa Hernandez Kelley, NIA

129 **The Moderating Effect of Race and Mediating Role of Systemic Inflammation on Racial Disparities in Incident Dementia: A Decomposition Analysis**
Cesar Higgins Tejera, NIA

130 **Fall Prevention Through Wearable Robotics**
Monroe Kennedy, NIA

131 **A machine learning-based brain age biomarker more sensitive to chronic pain**
Chavier Laffitte Nodarse, NIA

132 **Asymmetric Predominance of Neuronal Atrophy in Primary Progressive Aphasia due to Pick disease**
Vivienne Lubbat, NIA

133 **Chronic Psychosocial Stress Mediated Cerebrovascular Dysfunction In Novel Hepatic Xanthine Oxidase Knockout Mice Model**
Crystal Oudomvilay, NIA

134 **Modulation of TIMP2 mediates transcriptomic changes within hippocampus associated with plasticity**
Sarah Philippi, NIA

135 **Rural-Urban Disparities in Allostatic Load in the United States**
Alexis R. Santos, NIA

136 **Addressing Disparities in ADRD Through More Inclusive Research: A Community Engagement Pilot During COVID-19**
Charles Windon, NIA

137 **A Developmental Perspective on Bilingualism and Musical Expertise**
Hilda Parra, NIAAA

138 **Insula to BNST circuit adaptations following restraint stress and alcohol withdrawal associated negative affect**
Anne Taylor, NIAAA

139 **Cocaine induced neurophysiological alterations to reward predictive cues following outcome devaluation in the infralimbic and dorsolateral striatum**
Leighelle Adrian, NIDA

140 **The Association Between Perceived Discrimination and Suicidal Thoughts and Behaviors in ABCD: Does Race and Ethnicity Moderate Findings?**
Laika Aguinaldo, NIDA

141 **The Role of Family Functioning and Race/Ethnicity on the Efficacy of an Opioid Misuse Prevention Videogame Intervention for Adolescents**
Kammarauche Areni, NIDA

142 **Game-Based Digital Biomarkers of Cognitive Risk for Adolescent Substance Misuse**
Kammarauche Areni, NIDA

143 **Meth, Guilt, and Spiritual Awakening**
Rachel Berenshteyn, NIDA

144 **Mechanistic Insight into Microbial Regulation of Psychostimulant Abuse**
Angela Carter, NIDA

145 **All In? A Qualitative Exploration of Diversity, Organizational Stress, & Buy-In among MAT Staff in Justice Populations**
TaLisa Carter, NIDA

146 **Activated CD4+ T cells induce epithelial cell death in an IFN-γ dependent mechanism**
Michelle Cruz, NIDA

147 **Emotion Dysregulation and Nicotine Consumption: Assessing the Moderating Role of Physical Activity Among Female, Daily Smokers**
Isabela Cruz-Vespa, NIDA

148 **Positive Emotion as a Risk and Protective Factor for Substance Use Among Indigenous Youth**
Noah Emery, NIDA

149 **Opioid withdrawal as a catalyst for positive and negative outcomes**
David Frank, NIDA

150 **The role of municipal policies and practices in the sustainability of community-adopted interventions**
Greer Hamilton, NIDA

151 **Parameters influencing food intake in a conditioned overconsumption task**
Darielle Lewis-Sanders, NIDA

152 **Simultaneous & sequential cocaine+alcohol PSU alters neurocircuitry of cocaine seeking**
Javier Mesa, NIDA

153 **Increased probability of fentanyl adulteration in cocaine decreases cocaine demand**
Cecilia Nunez, NIDA

154 **Mitragynine Reverses Paclitaxel Chemotherapy-Induced Peripheral Neuropathy and is Mediated via Opioid Receptor Involvement**
Yuma Ortiz, NIDA

155 **Leveraging Qualitative and Administrative Data to Characterize Delivery of Substance Use Services for Youth Involved in the Juvenile Justice System in Rural Indiana**
Gabriela Rodríguez, NIDA

156 **Prenatal Tobacco Exposure on Brain Morphometry and Cognitive Measures in the Adolescent Brain Cognitive Development (ABCD) Cohort**
Pedro Rodriguez Rivera, NIDA

157 **Preventing Substance Misuse and Substance Use Disorder by Examining Service Provider Interactions and Ethnic Identity**
Steven Stone-Sabali, NIDA

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Diversity Supplement Professional Development Poster Sessions

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Session A
Tuesday, August 30th, 2022

An Adaptive Excitation Source for In-Vivo Cell Tracking with Multiphoton Microscopy

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Improvements made upon conventional multiphoton microscopes (MPMs) through the use of an adaptive femtosecond excitation source (AES) have enabled an increase in spatiotemporal resolution while reducing power requirements by more than 20 times. In this study, we leverage the increased temporal resolution offered by AES to enable the in vivo tracking of cell (e.g., microglia, lymphocyte, etc.) migration within transgenic mice. To achieve this we propose adaptive illumination for tracking (AIT). AIT seeks to perform tracking while maintaining the benefits provided by AES, including the reduction in power requirements. This salient feature of AES in particular requires significant preprocessing time, however, due to gain transient effects observed during the burst-mode amplification of the pulsed laser used for imaging. This effectively makes power savings during tracking impractical. No closed form solution currently exists to describe the gain transient for a non-periodic pulse train, and thus we propose the use of a deep neural network to predict the shape of the gain transient, allowing us to significantly decrease preprocessing time.

Caregiver Adverse Childhood Experiences and Child Externalizing Problems: The Role of Caregiver Resilience

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Introduction: Children with externalizing problems are at risk for short- and long-term difficulties. Caregiver adverse childhood experiences (ACEs) have been identified as risk factors for offspring externalizing problems. Research has investigated potential variables that account for the relation between caregiver factors and child externalizing problems or evince incremental validity in predicting externalizing issues above ACEs. However, research has typically focused on variables that positively correlate with both externalizing problems and ACEs. Thus, the extant literature is limited regarding constructs that may be associated with lower child externalizing problems. Particularly, resilience may be an adaptive caregiver characteristic that negatively relates to child externalizing problems, even when adjusting for caregiver ACEs. The primary aim of this study was to examine the extent to which various measures of caregiver resilience showed incremental validity in predicting lower child externalizing problems above the effect of caregiver ACEs (both cumulative ACEs and distinct ACEs classes).

Method: Participants were families recruited from the community and a local Head Start program. The sample size for this study was 125 caregiver-child dyads. Child ages ranged from 3 to 5 years old, and most caregivers were biological mothers (80%). Caregivers were asked demographic information and completed measures assessing caregiver ACEs, caregiver resilience (individual resilience, social-ecological resilience, and positive childhood experiences (PCE)), and child externalizing problems.

Results: Caregiver PCE was the only type of caregiver resilience that significantly related to decreased child externalizing problems ($r = -.25$). Latent class analysis was used to determine the presence of distinct ACEs classes, with model fit indices suggesting a 3-class solution: 1) a caregiver high emotional neglect class; 2) a caregiver high sexual abuse class; and 3) a caregiver high cross-domain ACEs class. Caregiver PCE was not significantly related to decreased child externalizing problems above caregiver ACEs class membership ($B = -.16$, $p = .11$) or cumulative ACEs ($B = -.12$, $p = .24$).

Conclusions: Overall, caregiver PCE was the only type of caregiver resilience to demonstrate a significant negative association with child externalizing problems, but this relation became statistically non-significant when adjusting for caregiver ACEs. This study highlights the importance of assessing a caregiver ACEs, specifically when working with caregivers seeking parent training for disruptive child behaviors, given the association between ACEs and child externalizing problems. From a prevention perspective, providing at-risk caregivers with parenting education may also be an important point of intervention in addressing both infant and parent concerns. Studies should continue to identify ecological and family strengths and differential impacts as they relate to child externalizing problems.

Children's Screen Time and Associations with Academic Skills

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Children's screen time has increased dramatically in the past few years, but the implications of the combination of content and context (e.g., parental screen monitoring, and device type) of screen use for children's early learning remains understudied. In this longitudinal study of 128 4 and 5-year-old children, using time diary data to measure children's screen time and direct child assessments of academic and cognitive skills, we examined whether the content and contextual factors related to screen time predicted children's academic skills. Children's overall screen time was about 158 minutes, of which 40% was classified as educational. Compared to use of mobile devices, children spent more time watching TV and parents monitored about 60% of children's screen time. Cluster analyses detected three unique groups of screen use: Cluster 1 was "low total, unmonitored, and non-educational TV," Cluster 2 was called "moderate total, educational mobile devices," and Cluster 3 was "highest total, educational TV and mobile devices." Children in Cluster 2 scored marginally higher in literacy skills at age 5 than peers in Cluster 1, and significantly higher in these skills than Cluster 3. No cluster differences were found for number or spatial skills. Findings suggest that contextual features of screen time combine in meaningfully different ways across families, and these combinations of factors appear to predict children's literacy skills.

The Effect of an Unconditional Cash Gift on Intimate Partner Violence for Low-Income Families with Infants and Toddlers: Evidence from the Baby's First Years Study

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Using data from the Baby's First Years study, this paper estimates the effect of an unconditional cash gift on a mother's exposure to intimate partner violence (IPV) before her child's second birthday. It also examines how these effects interact with the mother's relationship status, the residential status of her partner, and her relationship with the child's biological father. These results will inform decision-makers about how unconditional cash might impact IPV for a racially and ethnically diverse sample of low-income households with very young children in the U.S.

The Temple Tour: Relating episodic memory and spatial navigation in children and adults

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Navigation and episodic memory are two fundamental cognitive processes that guide mature decision-making. Conceptually, they are linked by reliance on accurate retrieval of spatial and temporal context and accurate integration of different types of information. However, the extent and nature of interdependence at behavioral and neural levels is unclear, with some recent evidence suggesting they are mechanistically distinct. In this study, we investigate how spatial navigation and episodic memory relate to each other behaviorally, both in young adults and in children. We developed a real-world tour task in which young adults take a guided walk through a novel environment and encode sixteen distinct events. We assess their knowledge of the environment and episodic recollection of the events. Linear modeling will shed light on how these two systems are related and how spatial abilities map onto episodic memory. Data collection is ongoing, but these results tease apart how these two systems relate overall and componentially.

UV Exposure, Diagnostic Scrutiny and Melanoma Incidence in US counties

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Importance: Although ultraviolet (UV) exposure is the conventionally reported risk factor for cutaneous melanoma, an alternative exposure is diagnostic scrutiny: the more physicians look for and biopsy moles, the more melanoma they find.

Objective: To assess the relative importance of proxies for UV exposure and diagnostic scrutiny in explaining geographical patterns of melanoma incidence.

Design: A cross-sectional ecological study combining environmental data relevant to UV exposure (from a variety of sources), Health Resources and Services Administration (HRSA) data relevant to diagnostic scrutiny, and Surveillance Epidemiology and End Results (SEER) data on melanoma incidence.

Setting: County-level analysis of the 727 continental U.S. counties reporting to SEER. (among a total of 3108 counties)

Participants: Non-Hispanic White population diagnosed with melanoma during 2012-2016.

Exposures: Three UV proxies (UV daily dose, cloud variability, and temperature variability) and three diagnostic scrutiny proxies (median household income, dermatologists and primary care physician supply).

Main Outcomes and Measures: Melanoma incidence (in situ and invasive cancers).

Results: In total 235,333 melanomas were diagnosed. Proxies for UV exposure changed gradually across geography, melanoma incidence and proxies for diagnostic scrutiny changed abruptly across contiguous counties. UV daily dose, a variable the NCI specifically developed for melanoma analyses, was uncorrelated with incidence ($r = .03$, $p>.20$). For context, smoking prevalence was highly correlated with lung cancer incidence in the same counties ($r = .81$, $p<.001$). Melanoma incidence was correlated with median household income ($r = .43$, $p<.001$). Counties with no dermatologists and shortages of primary care physicians had the lowest incidence, while counties amply supplied with both had the highest – despite having lower mean UV daily dose. There was little relationship between melanoma incidence and melanoma mortality ($r = .09$, $p=.05$), while the analogous relationship in lung cancer was strong ($r = .96$, $p<.001$).

Conclusion and Relevance: The current geographical pattern of melanoma incidence across US counties is less related to proxies for UV exposure, more to proxies for diagnostic scrutiny. Incidence – the fundamental epidemiologic measure of disease frequency – now has little relationship with the feared outcome of melanoma: death.

Biocompatibility of Geopolymers for Bone Tissue Regenerative Engineering

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Musculoskeletal diseases affect 1 in 2 adults in the United States and result in an annual loss of 5% of the Nation Gross Domestic Product in both direct and indirect costs. An important subset is musculoskeletal trauma that creates a major challenge for restoring full function and appearance. The problem is crucial for patients who have endured massive injuries as well as for elders following an osteoporosis-induced hip fracture. Due to a reliance on titanium-based, polymer-based, and ceramic-based orthopaedic implants, standard tissue engineering methods often result in complications such as infection or bone degeneration due to a mismatch in both geometry and physical properties between the implant and the surrounding natural bone structure. Therefore, there is a gap of knowledge in novel functionally-graded materials for tissue regenerative engineering that are patient-specific, can mitigate bone loss, and promote bone proliferation around the host bone structure. We focus on a new class of materials, geopolymers that are X-ray and alkali-bonded amorphous inorganic polymers, and investigate their biocompatibility. We show that geopolymers are bioinductive. Specifically, we observed the formation of calcium phosphate crystals on the surface of pure geopolymer after incubation in simulated body fluid. We show that geopolymers are biodegradable using accelerated biodegradation tests. We show that geopolymers are biocompatible using mouse fibroblast cells. Finally, the mechanical properties of geopolymers are shown to be close to that of cortical bone. These results suggest that geopolymer materials can be used to replicate bone tissue behavior and induce bone regeneration. This work was supported by the National Institutes of Health under Grant NCATS 3UL1TR001422-06S2.

The Effect of Thyroid-Stimulating Hormone (TSH) on Brown Adipose Tissue (BAT) in Humans

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This project is designed to evaluate the effect of thyroid-stimulating hormone (TSH) on brown adipose tissue (BAT) in adult humans, to better understand the interaction between the thyroid axis and BAT. Studies using positron emission tomography /computed tomography (PET/CT) scanning have shown metabolically active BAT in adult humans, whose activity correlated inversely with body fat. We know that thyroid hormone (TH) plays an important role in brown adipocyte differentiation and controls adaptive thermogenesis in BAT by uncoupling of oxidative phosphorylation. Based on data from animal studies and findings from our prior study in adult humans, we hypothesize that circulating TSH has a direct local effect in increasing BAT mass and activity by stimulating local TH production.

Study 1. We aimed to evaluate whether recombinant TSH administration has a direct effect in increasing BAT mass and activity in adult humans. We studied 7 patients with thyroid cancer who underwent thyroid surgery and required recombinant TSH (rhTSH) as part of their standard treatment and follow-up. Subjects were evaluated at the time of the rhTSH administration (high TSH state) and 1-3 months later, on suppressive TH treatment (low TSH state). PET-CT scans were performed after wearing a cooling vest for 2 h (patient #1) or after resting for 2 hours at room temperature of 73-74F (patients #2-7). Indirect calorimetry, serum thyroid function tests, and metabolic parameters were measured in the two TSH states. Results. The difference between the BAT volume and BAT SUVmax measured in the high TSH and low TSH states was not statistically significant. Muscle (erector spinae) SUV max in both low TSH and high TSH states were also not statistically significant. Evaluation of BAT volume and activity change from a low TSH to a high TSH state with covariate adjustment is ongoing.

Study 2. We aimed to investigate the TSH and TH receptor expression and further characterize the local effects and interaction between TSH and TH in BAT. We collected peri-thyroidal BAT and subcutaneous white adipose tissue (WAT) (control) from 13 patients who underwent thyroid surgery. Serum thyroid function tests were measured at the time of the thyroid surgery. Results. Two patients had a high TSH (both taking methimazole and KI), 4 patients had a high normal TSH, 5 patients had a mid-normal TSH, 1 patient had a low normal TSH and 1 patient had a low TSH at the time of surgery. We are in the process of measuring mRNA expression for TSH receptors, TH receptors, β-adrenergic receptors, deiodinase 2 (DIO2) and uncoupling protein 1 (UCP1) to evaluate their relationship with serum TSH and TH levels.

It is expected that this project will provide enough preliminary data to apply for a more comprehensive grant to study the connection between TH, TSH, BAT, and anti-obesity/diabetes therapies. This is particularly relevant for public health, since we are in the midst of worldwide epidemics of obesity and diabetes.

Investigation of keratoconus tear extracellular vesicles phenotype

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Background: Extracellular vesicles (EV) are biological nanoparticles microvesicles, exosomes, and apoptotic vesicles based on their cellular compartment of origin. EVs are made of phospholipid bilayers and have the important role of horizontal transfer of protein and nucleic acids between non contacting cells and have been identified in virtually all bodily fluids. Keratoconus (KC) can be characterized by the thinning and bulging of the cornea. KC is a progressive disease that affects approximately 1:500 patients worldwide. In this study, we sought to investigate the phenotypic signatures of tear EVs in a small cohort of KC patients using the canonical EV markers of CD81, CD9, and CD63.

Methods: Tears were collected from 10 healthy (5 males and 5 females) and 9 KC (4 males and 5 females) subjects. Tear samples were collected passively from the lateral meniscus of the eye using a glass capillary tube, and processed for analysis using the ExoViewTM R100 platform.

Results: Analysis demonstrated that KC tear EVs had several significant phenotypic differences when compared to healthy controls. The subpopulations of EVs which colocalize CD9+ and CD63+ on single EVs was increased in KC compared to healthy tear EVs. KC EVs had lower CD81+ than EVs isolated from healthy subjects. KC EVs also showed higher CD81/CD9, lower CD63/CD9, and lower CD63/81 colocalization, when compared to their healthy counterparts. No differences were found in tear EVs between Healthy and KC samples on triple colocalization CD63/CD81/CD9. Sex stratification revealed both KC male/female EVs having higher CD9+ and CD63+, compared to corresponding healthy male/female EVs. Notably, KC female EVs showed lower CD81+ than healthy female EVs, and KC male EVs showed a higher CD81+ population compared to healthy male EVs. Additionally, KC male/female EVs had higher colocalizations of CD81/CD9 and lower colocalizations of CD63/CD9 when compared to healthy male/female EVs. KC male EVs showed higher colocalization of CD63/CD81 and lower CD63/CD81/CD9 than healthy male EVs. KC female EVs also showed lower colocalization of CD63/CD81 and higher CD63/CD81/CD9 than healthy female EVs. Both Healthy and KC EVs had a diameter range of 50 to 200nm, with the majority being between 50 and 80 nm suggesting a majority of exosome population and a minor population of apoptotic vesicles.

Conclusion: For the first time, we were able to isolate EVs from KC tears and determine their phenotype as determined by disease status and sexes. It is clear that notable phenotypic differences exist in tear EVs derived for KC patients, compared to healthy subjects. Diagnostic and treatment implications of these tear EV differences remain unknown, however based on these preliminary clinical studies further investigations are warranted.

Partial deletion of Lecithin: Retinol acyltransferase (Lrat) inhibits prodromal characteristics of diabetic retinopathy

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Purpose: Evidence suggests that the cells from the outer retina play an important role in the pathogenesis of diabetic retinopathy (DR), but whether or not the visual cycle activity is suboptimal in diabetes, and whether decreasing or not that activity is a therapeutic approach to inhibit DR is controversial. Here we used the Lecithin:retinol acyltransferase (Lrat) knockout mice to investigate the effects of visual cycle inhibition on the development of early DR.

Methods: Diabetes was induced in male C57Bl/6J (WT) and in Lrat heterozygous (Lrat+/-) and homozygous (Lrat-/-) mice using streptozotocin. Retinoid metabolism, and biochemical and physiological abnormalities in the retina were evaluated at 2 months of diabetes using published methods. Vascular histopathology, permeability and retinal thickness were evaluated at 8 months of diabetes. Non-retinal effects of inhibition of the visual cycle were examined in leukocytes ex vivo at 2- and 8-months of diabetes.

Results: At 2-months of diabetes visual function assessed by OKT and ERG was not significantly affected by diabetes, whereas in Lrat+/- and Lrat-/- was partially and severely attenuated respectively. Rhodopsin levels in WT and Lrat+/- diabetics were similar to corresponding values of 11-cis-retinal as determined from retinoid analyses when compared to WT nondiabetics. Lrat+/- mice differed in their level of all-trans-retinyl esters by 50% less when compared to WT and diabetes had no influence in the total levels. No all-trans-retinyl esters were detected in the Lrat-/- groups. The diabetes-increase in retinal superoxide was significantly inhibited in Lrat+/- and Lrat-/- diabetic mice when compared to WT diabetics. At 8-months of diabetes, thickness of the ONL by OCT was similar between WT and Lrat+/-, but Lrat-/- showed total loss of photoreceptor cells. Degeneration of retinal capillaries was significantly inhibited in Lrat+/-. Similar trends were apparent in the diabetic Lrat-/- mice, but mortality in this group precluded further evaluation. The diabetes-induced increase in retinal vascular permeability was inhibited in the inner plexiform and inner nuclear layers of Lrat+/- mice, but not in the outer plexiform layer. The leukocyte-mediated cytotoxicity against retinal endothelial cells at 2- and 8-months of diabetes was inhibited in Lrat+/- and Lrat-/- mice when compared to WT diabetic mice.

Conclusions/interpretation: Lrat+/- diabetic mice are protected from the development of retinal vascular pathology in early DR.

Evaluating the Role of Extradenticle in the Ventral margin of the Drosophila eye

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Many kinds of eye diseases, including those that result in blindness originate from genetic mutations of key genes involved in eye morphogenesis. Extradenticle (exd), is a TALE homeobox family transcription factor that is used in a variety of molecular processes such as embryogenesis and development of the central nervous system. However, it's role in patterning the Drosophila eye has not been completely understood. The compound eye of Drosophila is composed of 800-unit eyes, called ommatidia, which are arranged in a stereotyped hexagonal array. This organization, down to the directional angle of the ommatidia, is key to proper vison in adult flies. This specific cell organization is achieved by a wave of cell differentiation called a morphogenic furrow. This furrow arises from a single point on the most posterior end of the eye-antennal imaginal disc and migrates anteriorly in a single wave of differentiation. This patterning event involves several signaling pathways to properly pattern the undifferentiated cells that composed the eye field. My preliminary findings have shown a role for exd in retina patterning, which revolves around the regulation of cell differentiation in the ventral side of the Drosophila eye. During a genetic screen I found that reductions in levels of Exd leads to the inappropriate release of a second wave of differentiation from the ventral margin of the eye field. This was accomplished by utilizing RNAi and a unique driver, c311-GAL4, that drives expression in a specific tissue layer of the imaginal disc known as the peripodial epithelium. This ectopic patterning results in a completely disorganized retina. A possible cause for the disorganized retina is that wingless (wg) expression is lost on the ventral margin when exd is knockdown. It has been well documented that wg signaling in the eye acts as a repressor to other key patterning genes. I hypothesize that the loss of exd leads to the loss of the ventral repression pathway of the eye field. Additionally, I conducted a genetic screen to identify potential binding partners of exd that functional in patterning the Drosophila eye. The results of my screen showed that the knockdown of hth in the peripodial epithelium phenocopies the loss of exd. This data shows that hth and exd maybe functioning as co-factors to properly spatially restrict the initiation and progression of cell differentiation in the Drosophila eye disc.

The Role of RUNX1 in CM Cell Cycle Activity and its Impact on Cardiac Regeneration

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Factors responsible for cardiomyocyte proliferation could serve as a potential therapeutic to stimulate endogenous myocardial regeneration following insult, such as ischemic injury. A previously published forward genetics approach assessing the frequency of a rare and presumed proliferation-competent subpopulation of cardiomyocytes, mononuclear diploid cardiomyocytes (MNDCMs), led us to the transcription factor RUNX1. It has been established that RUNX1 induction in cardiomyocytes is increased after injury. Here, we examine the effect of RUNX1 on cardiomyocyte cell cycle and establishment of the MNDCM population during postnatal development and cardiac regeneration using both cardiomyocyte-specific gain-and loss of function mouse models. We hypothesize that RUNX1 overexpression (OE, gain-of-function) increases cardiomyocyte cell cycle activity with expansion of the MNDCM population, thereby extending the neonatal regenerative window and positively impacting adult cardiac remodeling post injury.

During postnatal development, RUNX1 KO decreased postnatal cardiomyocyte cell cycle activity, while RUNX1 OE extended the period of cell cycle activation. This extension of cell cycle activation observed in RUNX1 OE mice is complete with cytokinesis resulting in an expansion of the MNDCM population and total cardiomyocyte endowment. To determine whether RUNX1 could similarly regulate cardiomyocyte cell cycle in a regenerative and non-regenerative model, we induce P6 and 8-week MIs to measure cell cycle activity and cardiac function post injury. RUNX1 OE neonatal mice with a P6 MI displayed no difference in cardiomyocyte cell cycle activity 7 post injury compared to control littermates. However, RUNX1 OE in adult mice with an 8-week MI showed increased cardiomyocyte cell cycle activity with completion of cytokinesis 2 weeks post injury and limited improvement in cardiac function 28 days post injury. At first glance, RUNX1 appears to influence cardiomyocyte cell cycle activation in the context of normal postnatal development, a phenomenon which may not translate to the injury context for RUNX1 KO neonates. Yet, we are examining this possible discrepancy with additional experiments to fully understand the role of RUNX1 in cardiomyocyte cell cycle activity and its impact on cardiac regeneration.

Gene Knockdown of Transporters and Receptors in Drosophila Blood Brain Barrier Alters Sleep

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Sleep is a requirement for biological organisms such as humans to survive, but the molecules that regulate the homeostatic drive to sleep are still largely unknown. It has been shown that during sleep the CNS clears out waste. To investigate if endogenous molecules could be involved in driving sleep we conducted a RNAi screen in the Drosophila BBB. Our research show that knockdown of transporters and receptors at the blood brain barrier can affect sleep quantity and quality. Knockdown of the genes CG13743 and CG6126 in the drosophila BBB resulted in decreased sleep in both male and female flies. The amino acid transporter CG13743 resulted in decreased total sleep when knocked down. Knockdown of a carnitine transporter also resulted in decreased sleep, which was rescued with dietary carnitine. These results point to other biological process that could be involved in homeostatic sleep which can have important implications in the treatment of insomnia or other sleep disorders.

Expression and characterization of the TREM-Like Transcript-1 protein in the brain

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Cerebral blood vessel dysfunction can trigger stroke and add to the vascular component of other forms of neurological dysfunction. Even though these diseases lead to mortality, basic knowledge in general Vascular Biology and its interactions with blood components are lagging. Several neurological disorders such as stroke and Alzheimer's Disease (AD) have the characteristic feature of vascular injury affecting the blood-brain-barrier (BBB) and brain homeostasis. One identified platelet-abundant protein, TREM Like transcript (TLT-1), binds fibrinogen and mediates the inflammatory aspects of platelet/fibrinogen interactions. This protein can release its N-terminus (sTLT-1) and enter endothelial cells, affecting their cytoskeletal dynamics. We hypothesize that sTLT-1 interaction with the BBB endothelial cells (BECs) will induce molecular signaling that affects brain endothelial cell's cytoskeletal rearrangement and BBB permeability. To start answering this question, we first tested whether TLT-1 could be expressed in brain glial cells, which are cells with immune functions, and some interact with endothelial cells directly, possibly affecting BBB function. We studied TLT-1's presence in isolated primary astrocytes, microglia, neurons, whole-brain lysate, and negative/positive controls were used. Isolated murine astrocytes, a murine microglial cell line (SIMA9), neurons (N2a), and mouse brain lysate showed the presence of TLT-1 in brain. Interestingly, these results were also confirmed in non-human primate brain lysates. We further studied the expression of TLT-1 in human brain tissue by immunofluorescence and found expression of this protein in brains colocalizing with astrocytes, and also expressed in other cells. Ongoing RNA-Seq studies are being carried out to confirm its production within astrocytes. These preliminary data are the first to report the presence of TLT-1 in murine, non-human primate brain, and human tissue, introducing a molecule important in coagulation, leukocyte infiltration, and hemostasis that could have similar critical roles in the CNS.

Bioengineered 3D in vitro strategies to investigate phenotypic and genotypic differences in lymphatic network sprouting

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Millions of people are affected by debilitating conditions related to the lymphatic system, which is responsible for maintaining homeostasis and immune cell transport, but the mechanisms that govern the formation of lymphatic vessels (LVs) are still understudied. Lymphatic dysfunction can be characterized by the hyperactivation or hypoactivation of lymphangiogenesis, the process by which new lymphatics are formed. Post-developmentally, LVs are thought to arise from pre-existing vasculature such as collecting LVs (CLVs). These vessels differ from lymphatic capillaries in that they have a surrounding layer of lymphatic muscle cells (LMCs) generating the contraction that transports lymph, around the luminal layer of lymphatic endothelial cells (LECs). LMCs are considered to have a role in promoting lymphangiogenesis. Although, studies have been able to identify mediators of lymphatic capillary lymphangiogenesis, there are no in vitro platforms, apart from CLV explant studies, to investigate the role of muscle cells and sprouting of the lymphatic network from the CLV. Further, there is an unmet clinical need to define the molecular mechanisms of post-natal lymphangiogenesis and drivers of dysregulated growth and to utilize biomaterials strategies that aid in the regeneration of lymphatics. Lymphatic malformations (LMs) are a form of rare (1:4000 live births) vascular anomalies that result from somatic mutations, and which are usually apparent after birth. The heterogeneity of this disease has challenged our knowledge of the molecular underpinnings that drive LMs and has hindered the development of a standard care of treatment for these patients. To this end, I present a bioengineered platform that incorporates the major components of the CLV; LECs and LMCs and a basement membrane coating around LECs to support assembly of lymphatic networks. Next, I introduce a reverse engineering approach to model the dysregulated growth that occurs in LMs, using patient-derived biopsies. This research proposes to use an established poly (ethylene glycol) (PEG)-based hydrogel platform to 1) recapitulate CLV network sprouting using primary lymphatic cells in a 3D spheroid, 2) reverse engineer LMs from patient vascular anomaly biopsies, and 3) to elucidate the phenotypic and genotypic effects of matrix stiffness on sprouting lymphangiogenesis.

Cardiovascular Health Classification using Arterial Dispersion Ultrasound Vibrometry

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Background: Arterial stiffness plays a fundamental role in cardiovascular health. To assess arterial health noninvasively, we use arterial dispersion ultrasound vibrometry (ADUV): an acoustic radiation force (ARF) causes propagating waves in the arterial wall that are measured with ultrasound. A long-standing problem in this field is developing a fast, reliable map from propagating waves to arterial health. Therefore, we propose an end-to-end classification framework: from ADUV data to arterial health. Our main framework contributions are treatment of high-dimensional signals, optimal selection of interpretable features, and training of Support Vector Machines (SVMs).

Statement of Contribution/Methods: The framework steps are feature extraction, feature selection, and classification. Our current framework evaluation considers 59 subjects: 9 healthy subjects (HS) and 50 unhealthy (US) subjects, including 28 with atherosclerotic cardiovascular disease (ASCVD) and 22 with cardiovascular risk factors (CVDRF). During ADUV, we applied ARF on the carotid artery at 10 points, cardiac stages (CS), throughout the cardiac cycle. Next, we extracted the most dominant mode from a 2D FFT and used the corresponding dispersion curve for classification. Averaging along the 200-800 Hz frequency range produced 10 windows of 50 Hz. From these 100 features (10 windows and 10 CS), we selected the most relevant using a greedy maximization of the following score, choosing one feature at a time: $S = (1/2)(F\_1\_t+(1-F\_1\_d))$. Here, F_1_t is the test F_1 score and F_1_d is the difference between the test and training F_1 score. Maximizing F_1_t and minimizing F_1_d will maximize S, so a high score demonstrates good classifier performance on the unbalanced dataset and consistency between training and test sets. We used a SVM with a Gaussian kernel as our classifier.

Results/Discussion: Greedy feature selection found a set of 3 features with a score of S = 90.0%. An exhaustive search revealed that this is the maximum score for 3 features, and 44 of the 161,700 combinations share this score. During training, using optimal hyperparameters, the best 3 features, and HS as the positive label, we obtained 94.9% accuracy, 66.7% sensitivity, and 100% specificity. For the test set, we obtained 95.0% accuracy, 66.7% sensitivity, and 100% specificity. These metrics show strong consistency across the training and test sets, indicating proper generalization of the SVM. Repeating the training and testing process 100 times, randomly selecting the training and validation sets (i.e., bootstrapping) resulted in a mean validation accuracy of 91.5% with a standard deviation of 7.3%. These results indicate repeatability and a mean accuracy better than classifying the unbalanced dataset as all US (86.4%).

Prefrontal-medullary circuit attenuates stress reactivity

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Organismal survival and adaptation to stress rely on brainstem catecholaminergic neurons. In particular, catecholaminergic neurons in the rostral ventrolateral medulla (RVLM) drive sympathetic activity and enable physiological adaptations, including vasoconstriction, corticosterone release, and glycemic mobilization. However, it is unclear how brain regions involved in the cognitive appraisal of stress regulate the activity of RVLM neurons. Our previous studies found that the rodent infralimbic prefrontal cortex (IL) integrates behavioral and physiological responses to stress. Thus, a potential IL-to-RVLM connection would represent a crucial link between stress appraisal and sympathetic reactivity. In the current study, we investigated a direct IL-to-RVLM circuit by targeting a genetically-encoded anterograde tracer under the control of the CaMKIIα promoter to the IL of adult male and female rats. Analysis revealed that IL terminals apposed RVLM neurons expressing the catecholamine-synthesizing enzyme dopamine beta hydroxylase. Further, IL input to catecholamine cells was widespread throughout the rostral to caudal extent of the VLM in both male and female rodents. Quantification of innervation density revealed that males had a larger proportion of VLM catecholamine neurons receiving IL inputs relative to female rats. Additionally, IL appositions were identified on GABAergic and glycinergic neurons in both sexes. Accordingly, we hypothesized that IL projections may activate local RVLM inhibitory cells to limit sympathetic output. To test this hypothesis, we injected a viral vector coding for channelrhodopsin-2 (ChR2) in the IL of males and females. Next, a fiber optic cannula was implanted dorsal to the RVLM to evoke IL synaptic glutamate release. Animals then received photostimulation during restraint stress with blood sampled to determine stress reactivity. Compared to controls, male rats expressing ChR2 on IL terminals had suppressed glycemic stress responses ($p < 0.05$). In contrast, stimulation of the IL-RVLM circuit in females did not affect glucose mobilization ($p < 0.05$). However, ChR2 decreased corticosterone responses to stress relative to control rats in both sexes (males, $p < 0.01$; females, $p < 0.05$). Thus, both male and female rats have a direct circuit from the IL portion of the mPFC to catecholamine-synthesizing cells of the RVLM that limits glucocorticoid stress responses, likely through the activation of local inhibitory neurons. However, the density of this circuit is greater in males, potentially accounting for reduced glycemic responses. Ultimately, excitatory/inhibitory balance at IL synapses in the RVLM may be critical for the health consequences of stress.

Associations between Neighborhood Built Environment and Cardiovascular Risk in Adults Residing in Caribbean Small Island Developing State

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Introduction: Over the past decade cardiovascular disease (CVD) has been the leading cause of mortality globally and in the Caribbean. Studies have highlighted the role of the built environment in increasing physical activity which is major protective factor to CVD burden. However, the geographic distribution of built environment features may be disproportionate and may be contributing factor to neighbourhood CVD burden.

Objective: To examine the assocation between neighborhood built environment attributes and 10-year CVD risk

Methods: A cross-sectional study was conducted among adults residing in Barbados. Participants were clustered within neighborhoods based on the survey design. Data on neighborhood level characteristics were estimated at the enumeration district level. Built environment features examined were: Residential Density; Street Connectivity; Land Use Mix; Greenspace Density; Walkability; Bus Stop Density. CVD risk was estimated using the ACC/AHA Pooled Cohort Equation for adults 40 years and older. Associations between BE features and 10-year CVD risk were examined using multi-level linear mixed effects regression models.

Results: Overall the average 10-CVD risk score (%) was 13.7 (95% CI: 12.5, 15.0). No significant differences in CVD risk were found between low and high walkability neighborhoods. We found that higher walkability, population density, residential density and street connectivity was significantly associated with lower 10-year CVD risk.

Conclusion: Our results showed that neighborhoods that are more walkable, higher population and residential densities, and street connectivity was associated with a lower predicted 10-year CVD risk. Promoting neighbourhood walkability and improvement of the built environment might help to improve population's cardiovascular health.

Inhibition of purinergic receptor signaling and its role in the protection of endothelial integrity during Plasmodium falciparum infection

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Malaria is a severe infectious disease that affects millions of people around the world, resulting in over 600,000 deaths in 2020 alone, with most casualties occurring in children. Fatal cases of malaria are caused by the parasite Plasmodium falciparum and two potentially lethal manifestations of this infection are cerebral malaria (CM) and acute lung injury, which can progress to the more severe acute respiratory distress syndrome (ALI/ARDS). P. falciparum – infected red blood cells (iRBCs) bind to endothelial cells in these organs and disrupt cell-cell junctions, ultimately compromising the integrity of the endothelial barrier and leading to edema and hemorrhaging. Given that endothelial disruption is a pathological mechanism that is linked to the development of CM and ALI/ARDS, we aimed to identify signaling pathways that can strengthen both the pulmonary and cerebral endothelium. To this end, we used an in vitro assay where lysates of P. falciparum iRBCs induce disruption of intercellular junctions of human pulmonary (HPMECs) and brain (HBMECs) microvascular endothelial cell monolayers in order to screen various inhibitors of receptors and signaling molecules potentially linked to inter-endothelial cell junction integrity. We found that cangrelor, an inhibitor of purinergic G-protein coupled receptors (GPCRs) P2Y12 and P2Y13 protects brain and pulmonary endothelial cells against the disruption induced by iRBC lysates. P2Y12 and P2Y13 are Gαi – coupled GPCRs that signal through the α subunit to inhibit adenylate cyclase, the enzyme that synthesizes cAMP. Preliminary flow cytometry studies showed that both P2Y12 and P2Y13 are expressed on brain endothelial cells, suggesting that the protective effect of cangrelor is mediated by blocking one or both of these receptors. Moreover, an antagonist of Gαi signaling, but not an antagonist of Gβγ signaling, mimicked the protective effect we observed with cangrelor on brain endothelial cells.

Given these results, we propose that blocking Gαi signaling through P2Y12 and/or P2Y13 leads to downstream changes in the signaling landscape that ultimately protect endothelial cells from parasite-mediated disruption. The inhibition of P2Y12 and P2Y13 signaling may lead to an increase in cAMP, which can activate two effectors: Protein Kinase A (PKA) and Exchange Protein Directly Activated by cAMP 1 (Epac1), both of which can negatively regulate enzymes involved in the phosphorylation and activation of the myosin light chain (MLC). MLC activation is a key step in actin-myosin contraction which can physically pull endothelial cells apart, resulting in increased barrier permeability. Therefore, inhibiting P2Y12 and/or P2Y13 may block their inhibitory effect on adenylate cyclase, resulting in an increase in cAMP and ultimately, a decrease in MLC phosphorylation and barrier disruption. In future experiments we will determine the involvement of cAMP and its effectors in protecting endothelial cells from parasite – mediated disruption. Overall, this study may help elucidate cellular signaling pathways that

strengthen the endothelium not only in the context of Plasmodium infection, but also in other diseases where the endothelium is compromised.

Identification of a novel candidate molecular mediator of QT interval prolongation and arrhythmia risk

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Unexpected Heterogeneity In Reporting Bioethical Issues Arising From Advances in Chimera Research From Online New

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Advances in organoid technology are enabling new insights into the function and organization of the human brain. Empirical ethics research suggests that the derivation and use of human brain organoids (HBOs) is generally well supported by patients (Boers et al., 2018, Bollinger et al., 2021), laymen (Haselager et al., 2020) and the US public (Evans, 2022). However, use of HBOs to create chimeras–animals composed of human and non-human biological parts–elicits significant public opposition compared to other in vitro applications of organoids. While conceptual studies among moral philosophers and bioethicists have speculated on a range of ethical issues raised by human-animal chimeras, including concerns about consciousness (Hyun ,2015), foundational distinctions between humans and non-humans (Robert, 2003), and other topics (Karpowicz et al., 2005), there has been significant attention to whether the moral status of experimental animals could be increased due to newly acquired human cognitive capacities. Work by Evan (2022) suggests, however, that public concern is primarily based on the perceived violation of categorical distinctions between humans and animals. Moreover, public concerns are similar regardless of whether the chimera was generated through genetic engineering, xenotransplants, and embryological hybrids. We sought to investigate whether news media coverage of scientific studies using chimeras reflects academic or public sources of concern.

Properties of the Reovirus Attachment Protein affect Particle Stability

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Structural metastability of viral capsids is pivotal for viruses to survive in harsh environments and to undergo timely conformational changes required for cell entry. Mammalian orthoreovirus (reovirus) is a model to study capsid metastability. Following initial disassembly of the virion mediated by proteases, a metastable intermediate called the infectious subvirion particle (ISVP) is generated. Previous work points to a role for outer capsid protein □1 in affecting the metastability of ISVPs. Using a monoreassortant virus, T3DF/T3DCS1, which contains a □1 encoding S1 gene from strain T3DC in a T3DF background, we recently showed that that □1 impacts ISVP metastability. T3DF/T3DCS1 encapsidates a lower amount of □1 than the parental T3DF virus. How the metastability is impacted by □1 and whether the lower encapsidation level of □1 is connected to this property is unknown. To better understand the link between □1 and ISVP metastability, we used forward genetics to isolate a T3DF/T3DCS1 mutant with a hyperstable capsid. We found a mutation in σ1, T132A. This mutation, in the trimerization domain of σ1, increased the stability of T3DF/T3DCS1 ISVPs, but did not increase σ1 encapsidation. To further corroborate the role of σ1 in ISVP stability, strain T1L viruses with chimeric σ1 were utilized. These viruses express σ1 protein that are engineered such that portions of □1 are derived from a T3 strain. These viruses display decreased ISVP stability and have lower σ1 encapsidation. Preparations of wild-type reovirus particles contain particles that encapsidate a range of 0-12 □1 trimers. By isolating particles based on the level of encapsidated □1, we found that lower σ1 encapsidation leads to lower ISVP stability. Together our work reveals that the levels of □1 encapsidated on the particle impacts the stability of ISVPs.

Selenoprotein I deficiency in T cells promotes differentiation into tolerant phenotypes while decreasing Th17 pathology

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Selenoprotein I (SELENOI) is an ethanolamine phospholipid transferase contributing to cellular metabolism and the synthesis of glycosylphosphatidylinositol (GPI) anchors. SELENOI knockout (KO) in T cells has been shown to impair metabolic reprogramming during T cell activation and reduce GPI-anchored Thy-1 levels, which are both crucial for Th17 differentiation. This suggests SELENOI may be important for Th17 differentiation, and we found that SELENOI was indeed upregulated early during the activation of naïve CD4+ T cells in Th17 conditions. SELENOI KO reduced RORgammat mRNA levels by decreasing SOX5 and STAT3 binding to promoter and enhancer regions in the RORC gene encoding this master regulator of Th17 cell differentiation. Differentiation of naïve CD4+ T cells into inflammatory versus tolerogenic T helper cell subsets was analyzed and results showed that SELENOI deficiency skewed differentiation away from pathogenic Th17 cells (RORgammat+ and IL-17A+) while promoting tolerogenic phenotypes (Foxp3+ and IL-10+). Wild-type (WT) and T cell-specific SELENOI KO mice were subjected to experimental autoimmune encephalitis (EAE), with KO mice exhibiting diminished clinical symptoms, reduced central nervous system (CNS) pathology and decreased T cell infiltration. Flow cytometry showed that SELENOI T cell KO mice exhibited lower CD4+RORgammat+ and CD4+IL-17A+ T cells and higher CD4+CD25+FoxP3+ T cells in CNS tissues of mice subjected to EAE. Thus, the metabolic enzyme SELENOI is upregulated to promote RORgammat transcription that drives Th17 differentiation, and SELENOI deficiency shifts differentiation toward tolerogenic phenotypes while protecting against pathogenic Th17 responses.

Paradoxical Effect of Frizzled2-Fc on Bone Mass for the Treatment of Osteogenesis Imperfecta

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Osteogenesis imperfecta (OI) is a group of genetically and phenotypically heterogeneous connective tissue disorders that results in low bone mass, bone deformity, and bone fractures. OI has an estimated prevalence of 1 in 15,000 births. Disruptions in multiple processes such as collagen synthesis, collagen posttranslational modification, signaling defects and intracellular trafficking lead to OI. The primary focus of medical therapy has been to increase bone mass and reduce fracture risk through medical and surgical treatment. The mainstay of treatment in this population is bisphosphonates, which reduces bone loss by suppressing bone turnover. However, no FDA-approved therapies are yet available for OI. Wnt signaling is an anabolic pathway in bone and activating the Wnt signaling pathways have been shown to increase bone formation. Surprisingly, very little is known about frizzled receptors, which are one of the main co-receptors that mediate the anabolic effects by the Wnt signaling pathway. In these studies, we show that Frizzled2-Fc (Fz2-Fc), a fusion protein of the Fz2 receptor and the Ig Fc, paradoxically increases bone mass in wild-type mice after a single dose of treatment even at one week post-treatment. We tested whether this reagent can be used therapeutically in the context of OI. We studied the potential treatment of both collagen and Wnt1-related forms of OI and investigated the mechanism by which Fz2-Fc increase in bone mass. We treated OI mouse models (Col1a2G610C+/-, Crtap, and Dmp1-Cre;Wnt1fl/fl) at 3 months old with Fz2-Fc weekly injections for 8 weeks. Bone tissues were collected for uCT analysis, bone histomorphometry, biomechanical testing, histology, and RNA and protein analysis. Our studies showed that repeated treatment with Fz2-Fc increases bone mass in not only wild type (WT) but also dominant (Col1a2G610C+/-) and recessive models of OI (Crtap-/- and Dmp1-Cre;Wnt1fl/fl). Mice treated long term with Fz2-Fc had significant increases in bone mass, bone surface and bone volume. This paradoxical effect could be translated into therapy and raises the questions about potential components of the Wnt signaling pathway that could be augmenting bone formation. While designed as a potential “sink” for Wnt ligand, this fusion protein in fact exerts strong anabolic effects on bone.

Determining the functional role of Osteocytic genes that have potential clinical relevance to human bone fragility

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The epigenetic regulation of epidermal stem cells during adult skin wound healing

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The epidermis is a vital tissue that protects our bodies against infection and environmental insults. Upon wounding, epidermal stem cells (EpSCs) undergo a transient transcriptional switch to increase proliferation and to migrate into the wounded epidermis. This switch, described as a "wound state," is transient occurring only during wound closure and transcriptional landscape of EpSCs returns to normal once the de novo epidermis is established. Regulation of this wound activated transcriptional switch is completely unknown. Recent research has suggested that chromatin remodelers may be responsible for this switch within the EpSCs during wound healing. Additionally, a key chromatin repressor, Polycomb Repressive complex (PRC), has been shown to be dynamic in a healing epidermis. Thus, I hypothesize that the loss of Polycomb-mediated repression in EpSCs will lead to defects in the speed of re-epithelialization. To analyze the phenotype resulting from the loss of Polycomb function in EpSCs during wound healing, we crossed a Keratin 14-Cre allele with conditional Eed (PRC2) or Ring1a/b (PRC1) alleles and induced a 1cm2 full thickness wound. We tracked speed of wound closure by measuring the area of the wound every day with ImageJ Software. We observed that there is a delay in wound healing in both of the PRC1 and PRC2 knockout murine models. This data suggests that Polycomb function plays a role in the ability for EpSCs to contribute to a wounded epidermis.

Low Vitamin D status associates with quadriceps atrophy following ACL reconstruction

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Protracted quadriceps atrophy and weakness after Anterior Cruciate Ligament (ACL) injury results in poor knee mechanics and contributes significantly to the development of posttraumatic osteoarthritis. The cellular and molecular underpinnings of poor functional recovery are unknown, limiting the development of evidence-based therapies. Vitamin D is a fat-soluble vitamin with critical roles for bone and skeletal muscle health.

PURPOSE: Our goal is to determine how circulating and skeletal muscle vitamin D indicators are affected by ACL reconstruction surgery (ACLR). We also sought to determine how vitamin D status associates with changes in skeletal muscle size, strength and bone mineral density following several months of recovery from ACLR.

METHODS: Twenty-one participants (Median [IQR]; age, 17 [16-18]; BMI, 25.4 [23.1-28.4], 47% female) who sustained an ACL tear received muscle biopsies from the injured and noninjured vastus lateralis at baseline and from the injured limb 1 week and 4 months after ACLR. Muscle biopsies were processed for western blotting and immunohistochemistry. Skeletal muscle protein content of vitamin D receptor (VDR) and vitamin D binding protein (VDBP) were assayed via western blot. Skeletal muscle fiber cross-sectional area (CSA) was assessed with immunohistochemistry using automated MyoVision software. Transcript abundance of vitamin D activating pathway regulators (VDR, and Cytochrome P450 2R1 [CYP2R1] and Family 27 Subfamily B Member 1 [CYP27B1]) were determined with RNA-seq, and differential gene expression was calculated comparing ACL-injured and Healthy, and 1 week post-ACLR and Healthy. Raw p-values were adjusted for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) step-up method. Serum was collected prior to ACLR, and at 1 week, 4 months and 6 month follow ups. Vitamin D status was determined by averaging 25-hydroxy vitamin D (25(OH)D) over the 4 time points with mean study 25(OH)D < 30ng/mL designated as “low status” and 25(OH)D ≥ 30ng/mL as “sufficient status.” Total 25(OH)D was assessed with LC-MS/MS (Mayo Clinic Laboratories). ELISA was used to measure 1,25-dihydroxy vitamin D (1,25(OH)D; Biovendor R&D RIS024R and RIS021R), vitamin D binding protein (VDBP; R&D Systems DY3778B-05 and DY008), and free 25(OH)D (Biovendor R&D KAPF1991). Bone density of the distal femoral epiphysis was determined with dual-energy x-ray absorptiometry (GE Healthcare) at study baseline and at 6 month follow up. An isokinetic dynamometer (Biodex) was used to determine peak isometric torque at study baseline and 4 and 6 month follow ups. GraphPad Prism was used to complete one way ANOVA to assess differences in vitamin D-related indicators. Two-way ANOVA/mixed effect analyses and independent samples t-tests were used to compare muscle and bone outcomes vitamin D status groups.

RESULTS: Circulating 1,25(OH) was substantially decreased ($p<0.0001$) 1 week after ACLR when compared with baseline. VDBP was significantly lower at 4 and 6 month follow up when compared with baseline ($p<0.001$). Free 25(OH)D and total 25(OH)D did not show significant changes. VDR and CYP2R1 mRNA were elevated after ACLR (adjusted $p<0.05$) but did not differ between the injured and non-injured limbs at study baseline. VDR and VDBP protein increased in the injured leg following ACLR ($p<0.05$ and $p<0.001$, respectively) and VDR protein tended to associate with VDR transcript number before and after ACLR ($p<0.1$ for both time points). Vitamin D status was associated with greater loss of skeletal muscle 4 months after ACLR in vastus lateralis as indicated by changes in mean skeletal much fiber CSA. Changes in bone mineral density and strength were not different between vitamin D status groups.

CONCLUSION: ACLR increases VDR expression in quadriceps, and preliminary results suggest alterations in vitamin D activity and turnover. Low vitamin D status associated with skeletal muscle atrophy but not strength or BMD 6 months after ACLR. Our results support a role for vitamin D in skeletal muscle recovery from ACLR and highlight the need for further research into micronutrient metabolism to aid in the recovery from injury.

Trimethylamine N-Oxide Mitigates Cell Death Due to Heat Injury in Monolayer Culture and Osteochondral Explants

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Osteochondral auto- and allografts are commonly utilized for the repair of large articular cartilage defects, whereby articular cartilage is harvested from non-weight bearing portions of a joint or a donor joint and is transplanted to the injury site. Tissue trephines and coring reamers are used to harvest these grafts; however, they often cause cell injury at the cut surfaces of donor and recipient sites. During drilling, cartilage is subject to temperatures reaching up to 80°C, causing significant iatrogenic injury. Cellular necrosis and/or apoptosis propagates radially inwards from the periphery of the graft resulting in significant damage to the entire graft and surrounding tissue during storage, compromising the duration of clinical benefit. In this work, we utilize trimethylamine N-oxide (TMAO), a naturally occurring osmolyte that has been implicated in protein stabilization and protein folding, to mitigate chondrocyte death due to heat injury. Cell response to heat injury at 43°C and 55°C in the presence or absence of 25mM TMAO was evaluated for 2D monolayer culture of articular chondrocytes and for osteochondral graft tissue. Biochemical analyses and live/dead imaging suggest that TMAO offers a chondroprotective effect against heat injury during short term storage. This work will inform strategies to improve osteochondral graft harvesting techniques, including storage media and irrigation solution formulation.

The Role of Interface Geometry and Appendages on the Microscale Mechanics of the Skin

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Understanding and correctly simulating the mechanics of skin is crucial for many medical applications, e.g. wound healing. Skin biomechanics have been primarily considered skin as one tissue. However, skin consists of multiple layers with different constituents and microstructure. Additionally, there are skin appendages such as hair follicles. Recently, multi-layer models of skin have been developed. However, these models have considered skin as a stack of the multiple layers but have ignored the interface geometry between layers and the presence of skin appendages. Here we fill this gap using a computational model of a representative volume element (RVE) of the skin microscale.

Our analysis shows that the homogenized stress does not depend on interface geometry, yet, interface geometry leads to heterogeneous stress at the microscale. It remains to determine if this heterogeneous stress distribution correlates with mechanical function or markers of disease. Hair follicles also cause heterogeneous mechanical response, opening research avenues for how the mechanics of the microscale can affect skin physiology, e.g. mechanosensation.

Synovial Tissue Metabolomic Profiling Reveal Biomarkers of Synovial Inflammation in Patients with Osteoarthritis

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Objective: Inflammatory responses are associated with changes in tissue metabolism. Prior studies find altered metabolomic profiles in both the synovial fluid (SF) and serum of osteoarthritis subjects. Our study determined the metabolomic profile of synovial tissue (ST) and SF of individuals with OA and its association with synovial inflammation.

Design: 37 OA ST samples were collected during joint replacement, 21 also had SF. ST samples were fixed in formalin for histological analysis, cultured (explants) for cytokine analysis by ELISA, or snap-frozen for metabolomic analysis. ST samples were categorized by Krenn synovitis score and picrosirius red. CD68 and vimentin expression was assessed by immunohistochemistry and semi-quantified using Image J. 1H-NMR was used to acquire a spectrum from ST and SF samples. Chenomx NMR suite 8.5 was used for metabolite identification and quantification. Metaboanalyst 5.0, SPSS v26, and R (v4.1.2) were used for statistical analysis.

Results: 42 and 29 metabolites were detected in the ST and SF respectively by 1H-NMR. Only 3 metabolites, lactate, dimethylamine, and creatine positively correlated between SF and ST. ST concentrations of several metabolites (lactate, alanine, fumarate, glutamine, glycine, leucine, lysine, methionine, trimethylamine N-oxide, tryptophan and valine) were associated with synovitis score, mostly to the lining score. IL-6, acetoacetate, and tyrosine in SF predicted high Krenn synovitis scores in ST.

Conclusion: Metabolomic profiling of ST identified metabolic changes associated with inflammation. Further studies are needed to determine whether metabolomic profiling of synovial tissue can identify new therapeutic targets in osteoarthritis.

Synthetic Hydrogels for Muscle Satellite Cell Transplantation in Dystrophic Diaphragms

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Background: Duchenne muscular dystrophy (DMD) is a genetic disorder caused by the absence of a functional form of the dystrophin protein in skeletal muscle fibers. Without the structural support of dystrophin, DMD patients experience muscle wasting and weakness. As a result, patients suffer from ambulatory disability and cardiorespiratory failure, the latter of which is a leading cause of premature death. This makes respiratory muscles, such as the diaphragm, an important therapeutic target. There is currently no cure for DMD; however, cell therapy is a promising treatment strategy. In the current work, our objective is to leverage synthetic hydrogels for localized delivery of healthy muscle stem cells, or satellite cells (MuSCs), to dystrophic diaphragm muscle. The central hypothesis is that these engineered hydrogels can encourage cell engraftment and fusion with host fibers to restore dystrophin and improve muscle function.

Methods: Hydrogels are synthesized using 4-arm polyethylene glycol macromers with end-functionalized maleimides (i.e., PEG-4MAL), and are functionalized with synthetic peptides that mimic proteins in the extracellular matrix (ECM). MuSCs expressing green fluorescent protein (GFP) are isolated from healthy mouse donors, encapsulated in hydrogels following the addition of a crosslinking peptide (VPM), and delivered to the hemidiaphragm of dystrophic mice. After 4 weeks, cell engraftment and dystrophin expression and localization are assessed using immunofluorescent staining. In a related study, ex vivo isometric force testing and in vivo shear wave elasticity imaging (SWEI) were used to measure muscle function in naïve mice with varying levels of disease severity at 12 months or 6 – 12 months of age, respectively. H&E and Gomori's Trichrome staining were performed to assess fibrosis and collagen deposition.

Results: We show that PEG-4MAL hydrogels adhere to and stay localized to the diaphragm for at least 7 days, MuSCs engraft to the diaphragm, and dystrophin is reintroduced to dystrophic fibers. In the related study, the percentage of fibrosis increases with disease severity. Isometric force produced by muscles following twitch stimulus tends to decrease with increasing disease severity, and a significant difference is observed between healthy and the most severe DMD model.

Discussion: These results demonstrate that PEG-4MAL hydrogels can be used for the localized delivery of MuSCs to the diaphragm and can reintroduce dystrophin to fibers. Our data also suggests that our in vivo and ex vivo methods can be used in conjunction to assess diaphragm function and predict muscle morphology/composition over time. On-going work involves increasing the area of cell engraftment and assessing various time points to determine if typical dystrophin localization is achieved. Isometric force testing and histology is being used to validate the SWEI system for evaluating diaphragm functionality and morphology. With success, both

measurement techniques will be used to determine whether there is functional improvement following MuSC transplant. This work can provide support for an alternative strategy that improves upon muscle stem cell therapy, particularly to the diaphragm muscle.

Cell Adhesive and Growth Factor Peptide Mimetics Cooperatively Influence Osteogenic Activity of Mesenchymal Stem Cells in 3D Culture

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Regenerative medicine applications of growth factors typically require supraphysiological doses to achieve clinically meaningful benefits. This leads to dangerous off target effects, such as ectopic bone growth and nerve damage caused by recombinant human BMP-2 (rhBMP2). Matrix immobilized growth factor mimicking peptides have been studied as potential substitutes that would prevent off-target effects of growth factors. However, these short peptides often lack the potency of full-length growth factors. During normal development, growth factors work together with ECM to cause tissue formation at much lower growth factor levels. We hypothesize that combining immobilized cell adhesive peptides with growth factor mimetic peptides will enhance the potency of short peptide mimetics of rhBMP2. We grafted cell adhesive cyclo-RGD (cRGD) and growth factor mimetic BMP-2 knuckle epitope (KE) via two orthogonal click chemistries: strain promoted azide-alkyne cycloaddition (SPAAC; for cRGD) and maleimide-thiol for the knuckle epitope (KE) of BMP2 onto alginate polymers. FRET assays on fluorophore surrogates suggest that when KE and cRGD were coupled to the same polymer, their separation was 6.3± 0.7 nm. Clonally derived mouse mesenchymal stem cells (MSCs) were exposed either to rhBMP2 or KE. ALP assays and preliminary RUNX2 staining demonstrated that immobilized KE peptide was comparable to rhBMP2 in osteogenic potency. We also observed a trend towards higher osteogenic potency when cRGD and KE were presented in close proximity to one another by being conjugated to the same polymer molecules.

Design and Evaluation of Biphasic Dual Drug Delivery System for the Treatment of Osteoarthritic Pain

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Osteoarthritis is one of the most prevalent musculoskeletal pain conditions leading to significant disability. Over 30% of patients suffering from OA conditions report significant joint pain. There is a significant need to address this challenging clinical condition. Current clinical interventions involve anti-inflammatories, local anesthetics, and opioid based drugs. There is increased interest in developing intra-articular therapies for the localized delivery of bioactive molecules. The major goal of our studies is to evaluate novel small molecules as potential pain modulators for intra-articular therapies. Here we will discuss the efficacy of curcumin, a natural small molecule derived from turmeric (Curcumina longa) in reducing inflammation, feasibility of developing a sustained delivery system for intra-articular injection, and in vivo pain modulation using an MIA induced osteoarthritic rat model.

Regulation of Myoblast Differentiation And Skeletal Muscle Formation By The Extracellular Protease ADAMTS10

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Acromelic dysplasias, including Weill-Marchesani syndrome (WMS), are rare connective tissue disorders that share musculoskeletal symptoms, including short stature, brachydactyly, thick skin, and a pseudomuscular build. In addition to the musculoskeletal presentations, individuals with WMS are characterized by lens dislocation, cataract formation, and cardiac anomalies. WMS can be caused by pathogenic variants in FBN1, ADAMTS10, ADAMTS17, or LTBP2. ADAMTS10 encodes a secreted metalloprotease that cleaves fibrillin-1 and fibrillin-2, if activated by furin processing, and promotes the deposition of fibrillin-1 into the ECM. Skeletal muscle in Adamts10 knockout mice has not been previously investigated. In contrast, a knock-in of an Adamts10 WMS mutation resulted in short stature and skeletal muscle alterations. The mechanistic role of ADAMTS10 in skeletal muscle formation and/or homeostasis, however, remains unknown. By querying the Tabula Muris and Myoatlas single cell RNAseq databases, we found that Adamts10 is expressed by satellite cells, which represent myogenic muscle stem cells that can differentiate and ultimately form myofibers in adult skeletal muscle, specifically after injury.

We utilized differentiation of murine C2C12 myoblasts as a model system for muscle satellite cells to determine the role of Adamts10 during myogenesis. We show that Adamts10 knockdown with shRNA compromised myotube formation and thus assigned Adamts10 a key role in myogenesis. Changes in the gene expression levels of markers for myoblasts (MyoD, Myf5), myocytes (Myog, Mrf4), or myotubes (myosin heavy chain, desmin), indicated that several stages of C2C12 differentiation are altered in ADAMTS10-deficient C2C12 cells. Collectively, we identified Adamts10 expression in two key cell types of skeletal muscle tissue and demonstrated a mechanistic relationship, where Adamts10 is required to promote myoblast differentiation during myogenesis.

Determining the Role of Collagen XI on Collagen Fibril Deformation and Sliding in Developing Mouse Patellar Tendon

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Stickler syndrome is a connective tissue disorder affecting about 1 of every 9000 newborns. This genetic disorder is caused by a mutation in the Collagen XI gene and manifests eye abnormalities, hearing loss, and joint problems. Collagen XI is most highly expressed during development related to its co-assembly with collagen I and II during heterotypic fibril formation. The goal of this study is to elucidate the role of collagen XI in developing patellar tendon through the analysis of D-band elongation and fibril sliding. To this end a tendon-targeted Collagen XI Knockout mice model was created. We hypothesize that collagen XI deficiency in developing tendon will reduce patellar tendon mechanical properties and increase collagen fibril deformation and sliding. Previous work showed that deficiency of collagen XI disrupts tendon structure, causing nuclear disorganization and the degradation of mechanical properties of extracellular matrix. Initially, we mechanically tested three different genotypes : Cre- control (WT), Scx-Cre;Col11a1flox/wt(HET), and Scx-Cre;Col11a1flox/flo (KO) to characterize their mechanical properties and determine the suitable % of low and high strain to be used for each group. There was no significant difference between the control group and the other genotypes. However, Knockout tendons were significant longer compared to HET and WT. This study provides evidence about the regulatory role of collagen XI in developing tendon; but also, further investigation is necessary to elucidate the effect of collagen deficiency at nanoscale and how it may affect the deformation mechanism of collagen fibrils.

A New Switching Technique for Multinuclear Study of Duchenne Muscular Dystrophy

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Background:

Magnetic resonance imaging and spectroscopy (MRI/MRS) have become increasingly useful in the study of Duchenne Muscular Dystrophy (DMD). Although most clinical studies focus on proton (1H) NMR, non-proton nuclei (or X-nuclei) also have potential to provide information relevant to disease diagnosis, severity, and response to treatment [1-4]. To enable multinuclear studies, radiofrequency (RF) coils that can be tuned to multiple frequencies are necessary. However, the most common methods for multi-tuning involve the use of lossy components, which impact the performance of the coil when compared to their single-tuned counterpart [5-7]. We developed a low-loss switching method that involves the use of liquid crystal elastomers (LCE). LCEs are stimuli-responsive materials that can be programmed to have reversible shape changes in response to stimuli such as temperature [8]. By incorporating light absorbing carbon black (CB) particles, shape change in the LCE can be triggered by light illumination. which enables their use as switches [9]. The LCE in our system is actuated by a remote infrared mechanism, which provides the potential to change the frequency of interest at any point with negligible impact to positioning and scan time.

Methods:

To test the switching method, two 4-cm loop coils, tuned to the 1H frequency at 3T (128 MHz), were constructed. One coil was used as a reference, and the other was used as the switching coil. An LCE based on two-step thiol-ene click reaction [10] with 0.4 wt% of CB was developed. To program the desired shape change, a thin 10 mm disk was 3D printed with an azimuthal print path. A small droplet of liquid metal (LM) was placed in the center of the LCE. Once illuminated with IR light, the thin LCE-CB disk morphed into a cone shape, allowing the LM to close the connection between the two open wires on the switching coil. Bench measurements were used to assess coil performance.

Results:

The LCE was successfully activated using the far-red chip on board LED, and the liquid metal successfully closed the current path on the switching coil. Bench measurements were acquired with the coil in the “on” state.

S11 measurements of both coils were better than -21 dB. The Q unloaded of both coils was the same, while the Q loaded only presented a difference of 5.2%, considered to be minimal.

Discussion:

Our lab group at Texas A&M University focuses on the development of multinuclear coils for diverse applications. We are currently investigating 1H (susceptibility-weighted imaging) and X-nuclei biomarkers for Duchenne Muscular Dystrophy. Multi-tuning has presented some challenges because of the addition of lossy components. A low-loss switching method has the potential to address these shortcomings and enhance sensitivity for imaging and spectroscopy of multiple nuclei.

Characterization of PDGFRalpha/beta heterodimer-specific dynamics

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The platelet-derived growth factor receptor (PDGFR) family consists of two receptors, PDGFRalpha and PDGFRbeta, that dimerize to form PDGFRalpha homodimers, PDGFRa/b heterodimers and PDGFRbeta homodimers. It has previously been impossible to study dimer-specific dynamics for PDGFRs because commonly-used antibody-based approaches do not allow for the visualization and purification of individual PDGFR dimers. Here, we highlight ongoing experiments that overcome these previous limitations in studying PDGFRa/b heterodimers using bimolecular fluorescence complementation (BiFC). We generated a cell line stably expressing C-terminal fusions of PDGFRa and PDGFRb with BiFC fragments corresponding to the N-terminal (V1) and C-terminal (V2) regions of the Venus fluorescent protein, respectively. We confirmed heterodimerization of the receptors and bimolecular fluorescence complementation upon PDGF-BB ligand stimulation of these cells via fluorescence microscopy and immunoprecipitation with a nanobody (GFP-Trap) that recognizes an epitope spanning the V1/V2 interface. We found that these receptors heterodimerize quickly in response to PDGF-BB ligand, with increased levels of receptor autophosphorylation at early time points of ligand treatment. Moreover, we provide evidence that PDGFRa/b heterodimers are rapidly trafficked into early endosomes, where they dwell for extended lengths of time. We further discuss current studies to identify PDGFR dimer-specific interacting proteins using mass spectrometry-based proteomics. These studies will impart valuable insight into the molecular mechanisms by which biological specificity is introduced downstream of PDGFR activation.

X-ray Crystal Structure of Treponema denticola TDE0362 Protease Domain

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Treponema denticola is a key pathogen in chronic periodontitis. During infection, Treponema denticola thrives within subgingival plaques formed in the periodontal pocket, despite direct interactions with the host immune system. To facilitate host immune evasion, Treponema denticola produces a number of virulence factors, including TDE0362, which cleave host immune proteins. TDE0362 encodes a two-domain protein with two conserved bacterial Ig-like domains at the N-terminus and a C-terminal cysteine protease domain (C362). C362 shows sequence and structural homology to the papain superfamily of cysteine proteases, with highest homology to IdeS, an IgG-specific protease from Streptococcus pyogenes. The survival of Streptococcus pyogenes is dependent on the ability to avoid the innate and adaptive immune responses. IdeS specifically cleaves the hinge region of IgG, dissecting the Fab and Fc domains, providing a defense against Fc-mediated phagocytic killing. Treponema denticola is also resistant to phagocytosis. However, despite the similarity to IdeS, C362 does not cleave IgG, suggesting a different mechanism for this resistance. We utilized Se-methionine incorporated protein in conjunction with SAD phasing methods and synchrotron radiation to elucidate the crystal structure of C362 to 2.19 Å. The structure contains 4 monomers in the asymmetric unit in space group P21. The observed structural architecture is conserved in each monomer and is similar to that seen in the IdeS crystal structure. Each monomer consists of 10 □-strands and 10 □-helices, which are organized into two distinct lobes, with the putative active site located between these two lobes. In its unliganded state, the putative active site residues are highly mobile, suggesting that substrate binding may be required for stabilization.

In vitro 3D human gingival tissue model to study oral microbiome: computational model to estimate long-tern culture conditions

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Introduction: In the field of oral tissue engineering, few advances have been achieved in terms of understanding the synergistic effects of host-pathogen interaction in oral flora. Current approaches lack the three-dimensional traits and bio-mechanical behaviors of human gingiva, thus resulting in the need to develop a physiologically and human relevant in vitro oral model to successfully comprehend host-pathogen imbalances and providing better insight into patient health, resulting in improved prevention strategies and clinical treatment for better ways for oral dysbiosis. Towards this goal, we have designed a 3D gingival tissue model composed of i) silk-based sponge to mirror the architecture of gingiva, ii) human primary cells to mimic the cytology of the gingiva, iii) an Arduino-controlled closed-loop bioreactor system to represent the oral cavity environment while circulating artificial saliva. Taken together, this model serves as a human-based in vitro model of gingiva which can be further implemented with microbiome and serve as a platform to study gingivitis and periodontitis. However, salivary flow (0.3-0.4 mL/min) is essential to ensure continuous lubrication of the oral mucosa, mechanical clearance that helps balance the oral microbiome, and ensures physiological host-pathogen interactions. Altered salivary flow can lead to dysbiosis by increasing the risk of dental caries, gingivitis, or periodontal disease (2). Thus, we focused on the development of the computational model to support and predict the bioreactor design for the long-term culture of the tissue model.

Methods: Shear stress assessment was carried out by Digital Particle Image Velocimetry (DPIV), fluorescent red microspheres were dispersed in artificial saliva solution within the customized bioreactor chamber while a camera captured the motion of the particles. A silk-based anatomical replica of an adult lower gingiva was placed in the chamber for an accurate estimate of the shear stress profile, while the chamber was connected to a peristaltic pump and different flow velocities were tested. Additionally, three inlet positions in the bioreactor chamber were screened, as the inlet position affects the velocity vectors at the gum-tooth level. The DPIV analysis was then conducted using a script/code written in MATLAB (MathWorks)- PIVlab2. To assess the shear stress, a shear rate was first computed as $S = v/\Delta h$, where

Using the Burrowing Test to Detect Affective State Behaviors in Rat Neuropathic Pain Models

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Chronic constriction injury of the infraorbital (CCI-ION) or supraorbital (CCI-SON) branches of the trigeminal nerve has been used as models for orofacial neuropathic pain and lumbar spinal nerve ligation injury (SNL) has been used as a model for peripheral neuropathic pain. For CCI-ION, behavioral hypersensitivity peaks about 3 weeks after injury and lasts for 7-8 weeks. For SNL, behavioral hypersensitivity peaks about 1-week post-injury and lasts for at least 10 weeks. So, these pain models are suitable for chronic neuropathic pain studies. Emergent data suggests that pain and affective states are interrelated and critical in mediating neuropathic pain states. However, the interaction of affective and neuropathic pain states in these chronic pain models has not been sufficiently studied. Burrowing is an ethologically relevant rodent behavior usually effective in hiding from predators. We hypothesize that neuropathic pain development may influence the affective states in these models. To test this hypothesis, we examined if changes in spontaneous burrowing behaviors could be detected in these models. Our preliminary results thus far showed that the optimal testing duration is 1 hour, and CCI-ION, SNL, but not CCI-SON, rats showed significant reductions in spontaneous burrowing behavior between 2-4 days post-surgery. These findings demonstrate that the burrowing test is effective in detecting pain-depressed behaviors in acute but not chronic pain states in a model specific manner.

Session B
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Collagen architecture and stiffness affect vascular homeostasis

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Microvasculature homeostasis is established through a dynamic interaction of endothelial cells (EC), pericytes (PC) and the extracellular matrix (ECM). Type I collagen is the most abundant ECM component. Collagen changes in stiffness, density and crosslinking can be seen in several physiologic and pathologic conditions such as aging, wound healing and cancer. However, whether collagen 3D architecture can affect pericyte differentiation or microvasculature homeostasis is still unclear.

To test this hypothesis, we used an organ-on-a-chip device to engineer pericyte-supported blood vessels. Briefly, 160-um channels were engineered using type I collagen that underwent fibrillogenesis under different temperatures (4, 16, 21 and 37°C). Thus, the microarchitecture and stiffness would vary from a soft reticular network (37oC) to a stiff fibrillar mesh (4oC). We then seeded human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) in the collagen channels. After 48h, samples were fixed and stained for actin (cytoskeleton), NG2 (pericyte differentiation) and CD31 (endothelial cell junction), imaged with a confocal microscope, and analyzed with Imaris.

Microvessels engineered in contact with a softer reticular collagen showed more pericyte differentiation ($p< 0.05$), inter-endothelial cell junctions ($p<0.05$) and effective barrier function. Conversely, stiffer fibrillar collagen was associated with less pericyte coverage, cell migration outward the vessel, and leakiness. When vessels were engineered without pericytes, those differences were drastically minimized.

Our data suggests that collagen architecture and stiffness affect pericyte differentiation, vascular morphology, and function. Moreover, pericytes may have an major effect in sensing the differences in collagen architecture and guiding vessel integrity.

Examination of magnesium hydroxide nanoparticle antibacterial activity and identification of bacteriostatic or bactericidal effects of magnesium hydroxide and magnesium oxide nanoparticles in pathogenic bacteria

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Magnesium oxide and magnesium hydroxide nanoparticle (nMgO and nMg(OH)2, respectively) antibacterial activity has been previously examined, but a lack of consistency in methods and materials used has culminated in conflicting results that limits their use when antibacterial activity is desired, but antibiotic use must be limited or avoided. Previously, Nguyen et al. (2018) developed a methodology that produced consistent results for nMgO MIC and minimum lethal concentrations (MLC 99.99) in bacteria and yeast species. We applied these methods to nMg(OH)2 using equivalent concentrations to nMgO (0-50 mM) and identified the nanoparticle MIC and MBC 99.9 for the identical bacteria species tested previously. Using the MBC values from Method 1, we examined nMg(OH)2 and nMgO antibacterial activities in real-time using spectrophotometry to determine if nMgO and nMg(OH)2 are bacteriostatic or bactericidal in logarithmic growth phase bacteria.

Jaw Bone Length is Altered by Pharmacological Inhibition of Matrix Metalloproteinase-9

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Defects in craniofacial bone are one of the most common birth defects; among these are defects in jaw length (micro- and macrognathia). Micro- and macrognathia negatively affect quality of life by interfering with mastication and breathing, but the only available treatment option is multiple invasive surgeries, making ameliorative pharmacological interventions highly desirable. Lower jaw bone modeling and remodeling during development is complex and not fully understood, but previous data from our lab demonstrated a role for bone-resorbing osteoclasts in establishing lower jaw length. Matrix metalloproteinase-9 (MMP9) is a proteolytic enzyme secreted by osteoclasts during bone resorption. Aligning with known increases in osteoclast activity over the course of jaw bone development, qPCR analysis of MMP9 expression in embryonic Japanese quail (Coturnix japonica) lower jaws indicates a 34-fold increase from the developmental stage just prior to onset of craniofacial bone resorption to the developmental stage when the facial skeleton is largely calcified ($n=7$/group, $p<0.0005$). We tested the effect of inhibiting MMP9 by delivering a single dose of a pharmacological inhibitor of MMP9 (iMMP9; 5mg/kg) to quail embryos in ovo over this same window of development. Morphologically normal quail have an upper jaw that extends beyond the distal tip of the lower jaw, and 90% of embryos given control saline had the normal lower jaw to upper jaw alignment ($n=16$). In contrast, 20% of iMMP9-treated quail had a lower jaw that was equal in alignment to the upper jaw and an additional 25% of iMMP9-treated quail had a lower jaw that protruded past the upper jaw ($n=20$). Control and iMMP9-treated quail skulls were scanned via microcomputed tomography and analyzed using Drishti software. iMMP9-treated quail had a significantly longer lower jaw bone than control quail, as well as a significantly higher lower to upper jaw ratio than control quail ($n=5$-6/group, $p<0.05$). Our data suggest manipulating bone resorption through pharmacological modulation of MMP9 activity is a potential option for altering lower jaw length developmentally.

From Microbiologist to Medical Device Manufacturing- Transition Through NIDCR Diversity Funding and Mentoring by NuShores Biosciences LLC

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Women and Minorities are under-represented in the science and engineering fields in general and in industry in particular. NuShores Biosciences LLC had the opportunity to participate in an administrative supplement for a NIDCR funding support for developing a dental bone graft. This supplement was to expose and early scientist from an under-represented population to the opportunities in a medical technology business.

Polymeric Nanoparticles for Endosomal Delivery of Pain Therapeutics

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Abstract: A reliable, non-habit-forming treatment for chronic pain has been elusive due to the rapid bodily clearance and short therapeutic effects of traditional opioids. To reduce opioid dependence, there is a need for a therapy which achieves robust pain inhibition for extended periods of time. Recent studies have shown that delivering drugs directly to spinal neuron endosomes yields more potent inhibition of key pain-signaling pathways. Additionally, nanomedicine has shown promise in promoting endosomal uptake for anticancer therapeutics. To translate this strategy to pain inhibition, it must be demonstrated that antinociceptive drugs can be efficiently encapsulated into nanoparticles, while controlling their release rate to prolong drug activity. Here, we develop a polymeric nanoparticle drug delivery platform for the sustained release of pain-signaling antagonists in spinal neuron endosomes.

Hydrophobic ion pairing (HIP), a method of solubility tuning, has been shown to promote the encapsulation of small molecule drugs within polymeric nanoparticles. We hypothesized that employing hydrophobic counterions during nanoparticle assembly would both increase the encapsulation efficiency of a calcitonin gene-like receptor (CLR) antagonist and slow the diffusion rate of this antinociceptive drug from the nanoparticle core.

The CLR antagonist and hydrophobic counterions were encapsulated by amphiphilic block copolymers via Flash Nanoprecipitation (FNP), a rapid and size-tunable nanoparticle assembly process. Synthesis parameters including counterion species, drug/counterion molar ratios, and solvent systems were varied to optimize nanoparticle drug loading (wt.%) and in vitro drug release rates.

Pairing of the CLR antagonist with pamoic acid resulted in nanoparticles (100 nm in diameter) with drug loadings of up to 6 wt.% and encapsulation efficiencies of up to 80%, with stability observed for over two weeks. These formulations demonstrated sustained drug release in sink condition release media for over nine days. Efficient co-encapsulation with a PAR2 antagonist was also demonstrated, for targeting of compensatory pain signaling pathways.

The reproducible encapsulation of an antinociceptive drug demonstrates the ability for endosomal uptake by spinal neurons, preventing rapid clearance. In achieving high drug loading compositions and sustained drug release on the order of weeks, such formulations are optimal for the potent and prolonged inhibition of the CLR pain-signaling pathway.

Neuronal subclass specificity of mechano-gated current responses in trigeminal ganglion neurons innervating masseter muscle

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The field of mechanotransduction remains the final frontier of somatosensory research. We recently demonstrated that the masseter muscle (MM) is primarily innervated by various types of myelinated neurons. Using reporter mice to label specific subsets of sensory neurons, determined by our single-cell RNA sequencing data, in combination with back labeling with WGA, we seek to characterize adaptation properties of mechano-gated currents using whole-cell patch clamp electrophysiology. The term "adapting" has been used to describe the decay of mechano-gated currents. Current adaptation can be divided into rapid-adapting (RA), intermediate-adapting (IA), and slow-adapting (SA). We hypothesize patterns of threshold activation and current characteristics unique to neuronal subtypes. Using the current signature method, action potentials and outward currents are additionally measured to categorize neuronal responses. We demonstrate neuronal subclass specificity of mechano-gated current responses.

Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) quantitative parameters, Ktrans and Ve, for monitoring of mandibular injury/osteoradionecrosis

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Abstract: Human papilloma virus (HPV)-associated head and neck cancers (HNCs) have been on the rise, impacting healthier and younger individuals in comparison to HPV (-) HNC. HPV-associated HNC cancer is generally radiosensitive with improved response and prognosis compared to HPV (-) cancers. The combination of younger age at diagnosis and favorable response to treatments leads to improved overall survival rates, however, patients are also living longer with radiation therapy (RT) attributable oro-dental sequelae. While less common than other RT oro-dental sequelae, osteoradionecrosis (ORN) is associated with high morbidity once developed. At present, diagnosis of ORN is reliant on subjective, non-standardized diagnostic criteria, anatomic imaging (ie dental panoramic), clinical examinations, and symptom development which may result in delayed diagnosis and late intervention when more advanced disease is present. An imaging biomarker may provide the opportunity for earlier detection of bone and vascular changes associated with development of ORN, leading to the ability to intervene earlier when treatment may be more conservative and treatment response is likely to be more favorable. Based on prior research from our group demonstrating that dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) can detect dose-dependent alterations in mandibular bone vascularity, providing biomarkers that are physiological correlates of acute mandibular vascular injury and recovery temporal kinetics, our group has acquired an Federal Drug Administration (FDA) letter of support (LOS) to investigate use of DCE-MRI parameters, Ktrans and Ve, as exploratory monitoring biomarkers of mandibular injury/osteoradionecrosis (ORN) in at risk patients with head and neck cancer (HNC) treated with radiation therapy.

Susceptibility of enamel to acid attack in Krt75 tm1Der knock-in mouse model

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Keratin 75 (K75), an epithelial hair keratin, was recently found in ameloblasts and enamel organic matrix. Carriers of A161T substitution in K75 present with the skin condition Pseudofollicullitis barbae. They also have changes in the structural and mechanical properties of enamel along with a higher susceptibility to dental caries. Krt75tm1Der knock-in mouse (KI) with deletion of Asn159 that is located two amino acids away from KRT75A161T in human can be a potential model for studying the role of K75 in enamel. To test the hypothesis that enamel in KI will be more susceptible to acid attack than in wild type (WT) mice, using in vitro demineralization experiments and μCT observations. Four hemimandibles from one and ten-month-old mice of each genotype were subjected to acid attack and contralateral hemimandibles were used as untreated controls. Hemimandibles were immersed in rhodamine solution, rinsed and microphotographed using fluorescence dissecting microscope. The mean fluorescence intensities of buccal surfaces of 1st molars were determined using Image J package. There was ~4-6 times increase in fluorescence intensity after acid attack in all experimental groups, indicating increased porosity of the treated enamel. In 1-month-olds fluorescence intensity of KI enamel was significantly higher than in WT ($p=0.037$), while no differences between WT and KI were observed in older animals. In addition, four hemimandibles from 1-month-old animals of each genotype were scanned with μCT scanner at 2 μm voxel size and subjected to the demineralization demineralization protocol. The same hemimandibles were scanned after demineralization and the scans of first molars from the same samples. In both genotype, some degree of surface demineralization was observed, however in KI subsurface lesions were also noticed. These results suggest that the Krt75tm1Der KI enamel is more susceptible to caries and it can be used as a model to study the role of K75 in enamel.

Spatial analysis of Wnt and Fzd receptor expression in adult mouse liver

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The liver performs critical metabolic functions through its organization into hexagonal units, often referred to as "metabolic units." Metabolic units are arranged along an axis that starts at the portal triad and ends at the central vein. Along this axis, hepatocytes exhibit expression differences that lead to 3 functionally distinct regions: Zone 1 is centered around the portal triad, while Zone 3 is located around the central vein, with Zone 2 intermediate to Zones 1 and 3. Such hepatic zonation confers zone-specific metabolic processes, e.g., gluconeogenesis in Zone 1 and drug detoxification in Zone 3 – a process known as "metabolic zonation." We previously showed the importance of Wnt pathway signaling in metabolic zonation as well as liver regeneration. Yet, the precise anatomic expression of Wnt signaling components including Wnt ligands (e.g., Wnt2, Wnt9b) or receptors including Frizzled (Fzd) receptors (e.g., Fzd1-10) and co-receptors (e.g., Lrp5/6), have not been characterized. Therefore, my aim was to create a spatial map of Wnt/Fzd signaling components in the adult mouse liver. Since Wnt signaling is highest in Zone 3 and least in Zone 1, I hypothesized that Wnt signaling components were expressed in a gradient, with preferential expression in Zone 3. To create a map of Wnt pathway expression I employed multiplex RNAscope, a quantitative approach for visualizing mRNA expression. Using this method, I defined each zone by quantifying mRNA expression of specific zonal markers. Zone 1 was defined by robust glutaminase2 (Gls2) expression around the portal triad with a progressive decrease in signal as I moved away from the portal triad. By contrast, glutamine synthetase (GS) expression was restricted to the region immediately surrounding the central vein, thus defining Zone 3. After establishing the location of each zone via RNAscope, I quantified the expression of Fzds 1-10, Wnt2, Wnt9b, and Lrp5/6 in each zone. Wnt2 and Wnt9b were preferentially expressed in Zone 3, as previously shown. Surprisingly most Fzd receptors were not zonated, but instead were expressed throughout the zones. The main exception is Fzd6, a negative regulator of canonical Wnt signaling, which exhibited zonated expression with highest expression in Zone 1. To define expression of the Wnt signaling components by hepatic cell type, we used a public resource database of mouse liver single cell RNA-sequencing data (Guiilliams et al., 2022). Surprisingly, Wnts and most Fzd receptors are not expressed in hepatocytes but rather in non-parenchymal cell types (e.g., endothelial cells). Overall, our results suggest that zonation is driven by preferential expression of Wnt ligands in Zone 3 rather than Fzd expression which are more uniformly distributed throughout the zones. Future directions include characterizing the mRNA expression of Wnt/Fzd signaling components in regenerating liver after partial hepatectomy.

The Role of Mitochondria in Skin Aging and Healing

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The skin is the body's largest organ, and has continual renewal and regeneration. It helps protect the body from pathogens, water loss, and wounding, and is susceptible to visible signs of aging, distress, and genetic skin disorders. Mitochondria play an important role in skin homeostasis. In this study we will use epidermal stem cell populations to understand processes of skin aging, wound healing and epidermal differentiation.

Biological Underpinnings of Emotional Eating in Middle Childhood

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Emotional eating, the use of food as a coping strategy to deal with negative emotions and stress, is linked to undesirable outcomes including high intake of energy-dense foods and obesity in childhood and adolescence. Up to 63% of children and adolescents engage in emotional eating; an alarming percentage given that this behavior may indicate a pathway toward obesity and is an antecedent of binge eating. Children's emotional eating tracks across the lifespan, increasing in prevalence from early childhood to preadolescence. As such, it is critical to understand individual factors that contribute to emotional eating.

Stress is linked to changes in eating behavior in adults but the relationship is understudied in children. Stress exposure activates two physiological pathways. The first of these is the sympathetic-adrenal-medullary (SAM) axis, which responds rapidly through neural and endocrine (i.e., epinephrine) routes to increased heart rate, skin conductance, and respiration. Pertinent for the current study, higher heart rate (HR) is associated with obesity in children, and higher skin conductance level (SCL), or electrical conductivity of the skin, is associated with disordered eating in adulthood. The second, and slower of these responses is mediated through the hypothalamic-pituitary-adrenal (HPA) axis, which results in the release of cortisol into the blood stream. This stage of the stress response cycle is considered to play a restorative role the resource-depleting effects of sympathetic activation, and is associated with a stimulation of appetite. This may be detrimental for optimal weight maintenance when children are exposed to chronic or sustained periods of stress. Chronic stress can be indexed by measuring concentrations of cortisol in the hair, reflecting cumulative exposure over the course of weeks. Higher hair cortisol is associated with greater BMI, waist circumference, and adiposity in children. Further, children's hair cortisol has been associated with poor dietary behaviors, including lower fruit intake and higher intake of sugary drinks and energy-dense foods. Furthermore, hair cortisol has been associated with children's emotional problems, which may predispose children to engage in emotional eating.

The current diversity supplement focuses on two novel additions to the parent project. First is a self-reported measure of eating behaviors (e.g., emotional eating) that children will complete during an interview. Second is an examination of how physiological markers of stress reactivity relate to children's emotional eating behavior. Stress reactivity will be examined at two timescales: (a) sustained chronic exposure to stress over the course of weeks and (b) acute autonomic reactivity associated with momentary increases in emotional eating. Children will complete the Dutch Eating Behavior scale, a 33-item measure that assesses restrained eating, emotional eating, and external eating, and is a validated measure for children at this age. Children will complete this scale in an interview with a trained researcher. Children's hair samples will also be collected, following an established protocol in the literature. We will collect HR and SCL using Empatica E4 watches. Children will receive the E4 devices during the in-

person visit along with a mobile device they will use to answer survey questions in real-time. Children will wear the E4 device for 10 consecutive days and will be asked to press a button on the E4 when they are eating, which will trigger surveys sent to the mobile device that will ask what children are eating and how intensely they are experiencing various emotions (e.g., sad, happy, angry, annoyed, overwhelmed, anxious). HR and SCL data surrounding children's eating will be extracted and processed using R version 3.6.2.

Investigating the Role of Mitochondria in Eosinophilic Esophagitis

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Background: Eosinophilic esophagitis (EoE) is a chronic, allergic inflammatory disease of the esophagus characterized by the infiltration of eosinophils, inflammatory immune cells and epithelial remodeling with an incompletely understood pathobiology. Recently, damaging variants were identified in the nuclear gene encoding the mitochondrial protein Dehydrogenase Transketolase Domain 1 (DHTKD1) in EoE patients indicating a potential role for mitochondria in EoE; however, their significance is currently unknown.

Methods: Mitochondrial number was evaluated in EoE or control esophageal biopsies by immunohistochemistry (IHC). An immortalized human esophageal keratinocyte cell line, EPC2-hTERT, was stimulated with the EoE-relevant cytokines tumor necrosis factor (TNF)-□, Interleukin (IL)-4, IL-5, IL-13 and IL-1□ for 7 days. Mitochondrial DNA (mtDNA) in cells and in culture media was evaluated by quantitative PCR (qPCR). Genetic depletion of mitochondria was achieved in organoids by Cre-mediated recombination in esophageal keratinocytes from TFAMloxp/loxp mice. Tamoxifen (TAM)-inducible depletion of Tfam in squamous epithelium was performed in vivo utilizing K5CreERT2;Tfamloxp/loxp mice. Esophageal biopsies were collected and Tfam expression was evaluated via qPCR and IHC.

Results: Preliminary data revealed an increased expression of the mitochondrial protein MTCO1 in esophageal epithelium of active EoE patients as compared to controls and inactive EoE patients. Given this we sought to delineate how the Eoe inflammation impacts mitochondria content. After exposing the immortalized human esophageal keratinocyte cell line, EPC2-hTERT, to a panel of Eoe-relevant cytokines, we observed an increase in mitochondrial DNA (mtDNA) within the esophageal keratinocytes in response to IL-13 specifically, while IL-4 and IL-13 induced the release of mtDNA into the media. Furthermore, knockdown of the mitochondrial transcription factor Tfam partially restored squamous cell differentiation in IL-13-treated esophageal organoids and a significant increase in Tfam expression was observed in EoE mice compared to controls.

Conclusions: Exposure to the EoE inflammatory milieu increases mitochondrial content in esophageal epithelial cells as well as release of mtDNA from esophageal keratinocytes. IL-13 specifically induces accumulation mitochondria that contributes to epithelial remodeling in EoE as demonstrated in vivo and ex vivo. Additionally, IL-4 and IL-13 promote release of mtDNA from esophageal epithelial cells. Future studies will utilize a DOX-inducible stable TFAM knockdown cell line to assess its depletion on squamous differentiation and barrier function in vitro.

GLS1 dependent regulation of the IL-17 axis shapes NAFLD pathogenesis

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The obesity pandemic has brought a dramatic increase in the incidence of non-alcoholic fatty liver disease (NAFLD). The immune system is a key causative link between obesity and NAFLD pathogenesis. Among various immune cells, hepatic CD4+ T cells have garnered attention as robust contributors to NAFLD pathogenesis. However, cellular processes that govern CD4+ T cells pathogenic functions in NAFLD remain poorly defined. Obesity, via modulation of cellular metabolism skews CD4+ T cells effector functions. Specifically, preferential utilization of glycolytic pathway in hepatic CD4+ T cells in obesity has recently been shown to promote CD4+ T cell IFN□ □and IL-17A production and to contribute to NAFLD pathogenesis. However, the contribution of other metabolic pathways to hepatic CD4+ T cells inflammatory potential in NAFLD remains underdefined. Amino acids and their transporters influence CD4+ T cell effector function. Moreover, glutamine (Gln), a non-essential amino acid, via glutaminase-1 (Gls1), instructs CD4+ T cell effector function. Whether and how glutaminolysis shapes CD4+ T cell effector function and NAFLD pathogenesis has not been studied. We show that, obese mice exhibit reduced hepatic Gln levels compared to lean controls. Further, Gln supplementation was sufficient to boost hepatic Gln levels and to restrict hepatic CD4+ T cell accrual, inflammatory vigor, and hepatocellular damage. Inhibition of Gls1, the rate limiting enzyme of Gln utilization via glutaminolysis, in CD4+ T cells increased their inflammatory vigor and exacerbated hepatocellular damage. Use of unbiased analyses of hepatic Gls1 deficient CD4+ T cells, compared to Gls1 sufficient controls, revealed robust differences in the regulation of IL-17-axis. Notably, hepatocellular expression of IL-17RA, the IL-17A cognate receptor, was found to be central to amplified hepatocellular damage and NAFLD pathogenesis in obesity. Together, these data suggest that targeted regulation of GLS1 signaling in CD4+ T cell may allow for the exploitation of the IL-17A axis driven alteration of hepatocyte function towards limiting obesity-driven inflammation and NAFLD pathogenesis.

Understanding the signals that promote beta cell growth in the setting of exocrine insufficiency

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The pancreas has commonly been considered two distinct organ systems wherein the endocrine compartment is responsible for hormone secretion and the exocrine compartment is responsible for the secretion of digestive enzymes. Although distinct, the endocrine and exocrine pancreas are functionally and structurally connected, working together to maintain metabolic homeostasis. Diseases of the exocrine can impact the endocrine, eventually leading to beta cell dysfunction as observed in Type 3c diabetes. Currently, there remains an incomplete understanding of the molecular pathways and communications that exist between cells of the endocrine and exocrine pancreas. Published studies and preliminary data from our lab reveal that hypusine biosynthesis plays a role in exocrine growth and function, which in turn may influence beta cell growth. Hypusine biosynthesis involves the post-translational modification of eukaryotic translation initiation factor 5A (eIF5A) by the enzyme deoxyhypusine synthase (DHPS) to form hypusinated eIF5A (eIF5AHyp), which functions in mRNA translation. Our studies demonstrate that loss of DHPS or eIF5A in the pancreas leads to reduced synthesis of proteins involved in exocrine growth and function, and resultantly loss of exocrine mass. Moreover, preliminary data suggests that the reduction in exocrine mass is accompanied by an increase in insulin area. We have subsequently generated mouse models of either exocrine ablation or eIF5A exocrine-specific deletion to determine if it is the loss of exocrine or the loss of hypusine biosynthesis that influences beta cell expansion.

Colorimetric β-galactosidase Assay Facilitates the Indirect Measurement of cAMP Production via the Activation of Gs-Pathways in the Melanocortin Receptors Subtypes

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The Melanocortin Receptors are GPCRs that primarily activate the adenylate cyclase via Gs-signaling pathways. The melanocortin 3 and 4 receptor (MC3R and MC4R) subtypes are expressed in the central nervous system and are of interested due to their roles in maintaining energy homeostasis.1 The Haskell-Luevano lab investigates and develop ligands targeting MC3R and MC4R,2 and suitable tools are required for our investigations. The development of the β-Galactosidase assay, by Chen and colleagues3, allows for the in-direct measurement of cAMP concentrations via the activation of Gs-signaling pathways. The colorimetric assay works by transfecting HEK293 cells with β-Galactosidase (lacZ) gene which fuses to five copies of the phosphorylated cAMP-response element (CREB) upon the increase production of cAMP. The lacZ gene can then produce β-Galactosidase, which can be used to indirectly measure cAMP production. This is measured by absorbance (405 nm) by introducing o-nitrophenyl-β-D-galactopyranoside (ONPG) substrate which is converted to ortho-nitrophenol via the cleavage of the sugar by β-Galactosidase. Herein, the 5-day β-galactosidase assay utilized in the Haskell-Luevano lab will be described.

Impact of COVID-19 on Individual Behavior and Household Exposure Related to Smoking, Vaping and Marijuana Use Among Adults with Asthma

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Rationale:

Across the United States, household, employment, and social routines have been substantially altered due to the emergence the COVID-19 pandemic. These altered routines may drive changes in smoking behaviors. The purpose of this study was to examine self-reported behavior changes and household exposure related to tobacco, vaping and marijuana use during the COVID-19 pandemic in a sample of adults with asthma.

Method:

An online, cross-sectional survey was conducted with those ≥18 years old, currently diagnosed with asthma, and able to read and write in English. The survey invitation was shared via email, social media, and ResearchMatch. Participants completed the Asthma Control Test (ACT) and items regarding their smoking behavior before and since the pandemic. Items to assess cigarette, marijuana, and vaping use included: "Do you smoke cigarettes?", "During the past 4 weeks, did you use marijuana/cannabis (e.g., joint, blunt, pipe, bong)?", "During the past 4 weeks did you use vaping products?". Questions also assessed presence of secondhand smoking behaviors in the home and if these behaviors had changed since the COVID-19 pandemic. ACT scores were dichotomized: ≤19 (uncontrolled asthma) vs. ≥20 (controlled asthma). Statistical analyses included descriptive statistics, chi squared statistics, and logistic regression.

Results:

Respondents (N=795) had a mean age of 43 years and were primarily female (81%), white (83%), and college educated (71%). Approximately 56% reported having an asthma exacerbation since COVID-19. Of participants, 4%, 5%, and 14% reported smoking cigarettes, vaping, and marijuana use, respectively. Household behaviors for cigarettes, vaping, and marijuana were 12%, 8%, and 15% respectively. For associations between smoking behavior and reduced asthma control, the adjusted odds ratios [CI] for cigarettes, vaping and marijuana were 5.79 (95% CI=2.28-14.66), 2.15 (95% CI=1.06-2.50), and 1.54 (95% CI=0.99-2.41), respectively. The adjusted odds ratio for current household smoking and reduced asthma control was 2.07 (95% CI=1.27-3.39).

Conclusions:

The onset of the COVID-19 pandemic in the US and the resulting routine changes have brought about substantial increases in smoking behaviors (tobacco, vaping, and marijuana). In addition to supporting the well-known impact of cigarette use on asthma, individual use of marijuana and

vaping were associated with uncontrolled asthma. Healthcare providers should remain diligent in asking about smoking behavior changes in the household during the COVID-19 pandemic.

Investigating the fetal hepatic proteomic response to in utero dimethylbenz[a]anthracene

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Exposure to environmental toxicants can negatively impact in utero fetal development. The impact of dimethylbenz[a]anthracene (DMBA), a polycyclic aromatic hydrocarbon present in wildfires and cigarette smoke, on the developing fetal liver remains unelucidated. This study investigated the hypothesis that in utero DMBA exposure alters the fetal hepatic proteome in a sexually dimorphic manner. Lean and hyperphagia-induced obese female mice were housed with males and the appearance of vaginal plugs was monitored and designated as gestation day (GD) 0.5. Females were exposed to corn oil (CT) or DMBA (1 mg/kg) for 7d via intraperitoneal injection (n = 10/treatment) from GD 7-14. On postnatal day 2, livers were collected from male and female offspring with biological sex confirmation by the presence of ovaries or testes and with litter considered as an "n = 1". Total protein (n = 3) was isolated and analyzed by LC-MS/MS. There were 34 and 21 differentially abundant proteins ($P < 0.05$) in the liver of females and males exposed in utero to DMBA, respectively. In females, the top five increased proteins were WAP, RPL6, LSM4, LSM6, NDUFB8 and the five proteins decreased to the greatest extent were S100A8, DDRGK1, ACAT1, FIP1L1, LAMTOR2 by DMBA exposure. In males, the abundance of DDX21, KRT13, CD81, KRT78, TTC30B were the top five increased and RPL8, SUB1, IVD, PSAT1, YWHAG were the five most decreased proteins due to DMBA exposure. In addition, the abundance of CD81, KRT78 and YWHAG proteins ($P < 0.05$) were altered in a sexually dimorphic manner due to in utero DMBA exposure. In females, four metabolic KEGG pathways were enriched ($P < 0.05$) and the abundance of three metabolic proteins (AGXT, ACAT1, and GCAT) were reduced. However, immune-related proteins, including CD81, were increased in males. Collectively, these findings support that the fetal liver respond to DMBA exposure in utero and that a sexually dimorphic response exists. Supported by R01ES030341-01 and R01ES030341-02S1 from NIEHS.

Depleted uranium exposure disrupts mitochondrial morphology, metabolism, and genomic stability

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A consequence of modern combat for people living or working near war zones is exposure to depleted uranium (DU). DU is released from armaments such as armor-piercing projectiles, which release shrapnel and produce uranium oxide particles (e.g., U3O8, UO2, UO3) upon striking a hard target. Humans near the impact are inevitably exposed to uranium oxides through inhalation of airborne particles, shrapnel injuries, or wound contamination. Case studies examining veterans of the first Gulf War have shown persistent body burdens of uranium, detectable in their urine decades after the initial exposure. Research in uranium chemical toxicology has yielded mixed results, raising uncertainty as to the risks of DU exposure and mechanisms of action. The central question is whether DU internalized through shrapnel or wound contamination causes persistent cellular and genetic damage. We address this question using a novel microimplantation method in zebrafish larvae to emulate DU shrapnel injuries and waterborne exposures to model inhalation and ingestion. Preliminary data show that mitochondria near internalized DU particles are more likely to contain disorganized cristae than mitochondria on the other side of the animal or in animals treated with a sham implant, suggesting proximity-dependent toxicity. At the physiological level, waterborne DU exposures at concentrations below federally mandated safe limits result in impaired cellular metabolism, possibly due to disrupted mitochondria like those observed in the implant study. Others have proposed that DU adduction to DNA's phosphate backbone activates DNA repair mechanisms and leaves a potential for unresolved DNA lesions or incorporation of mutations following the repair process. Here, we test the hypothesis that the ultrastructural and physiological mitopathies are results of uranium-induced mitochondrial genome damage. We are testing our hypothesis using two highly sensitive methods for quantifying DNA lesions, long-run real-time PCR (LORDQ) and semi-long run (SLR) real-time PCR. Results showing an increase in lesion frequency for uranium-exposed subjects would support our mechanistic hypothesis.

Multi-‘omics analysis identifies PCB-regulated pathways in Nonalcoholic Fatty Liver Disease

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Environmental exposure to polychlorinated biphenyls (PCBs) exacerbates high fat diet (HFD)-induced nonalcoholic fatty liver disease (NAFLD). However, the responsible mechanisms remain to be elucidated. Epigenetic regulation of hepatocellular toxicity includes altered miRNAs. An unbiased approach was to examine how PCB exposure alters the mRNA transcriptome, miRome, proteome, and changes in N(6)-methyladenosine (m6A) as an epitranscriptomic regulator in HFD-fed male mouse liver to regulate pathways leading to NAFLD. C57BL/6J male mice were fed a 42% fat diet (HFD) and exposed to Aroclor1260 (a non-dioxin-like PCB mixture, 20 mg/kg), PCB126 (a dioxin-like PCB, 20 µg/kg), combined Aroclor1260 and PCB126, or vehicle control for 12 wks. Liver mRNA, miRNA, and total RNA were isolated and sequenced (mRNA, miRNA, and m6A-RIP seq). Data were analyzed in comparison to our proteome-matched data for these samples. Compared to HFD control, fewer differences in mRNA expression were detected with Aroclor 1260 (124) or PCB126 (278) compared to the combined PCB exposure (5190). Similarly, more hepatic miRNAs were differentially expressed in HFD+PCB co-exposed (97) vs. HFD + Aroclor1260 (69) or PCB126 (50) exposed mice. Only 37 mRNAs and 4 miRNAs were commonly regulated in the three PCB exposures groups. Pathway enrichment analysis identified GO processes concordant with our previous reports of liver pathology including upregulation of NOTCH, Wnt/FZD/β-catenin, and PI3K/AKT/mTOR signaling. Inverse correlations were identified between dysregulated miRNAs and their putative mRNA targets for future analysis. Five liver disease-associated miRNAs that were previously reported as increased in serum from PCB-exposed human participants in the Anniston Community Health Survey cohort were examined in our mouse model. Upregulation of hepatic miR-99a-5p was identified with all PCB-exposures and miR-122-5p and miR-130a-5p were increased in HFD+PCB126-exposed mice. Together these findings suggest a regulatory role for PCB-exposure altered miRNAs on liver gene expression with some hepatic miRNAs that may be serum exposure markers. The results demonstrate that HFD+PCB exposure alters the hepatic miRome, mRNA transcriptome, m6A abundance, and proteome in a mouse model of NAFLD.

Oxidative metabolism effects on the activity of the Sonic Hedgehog pathway inhibitor piperonyl butoxide

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Disruption of the Sonic Hedgehog (Shh) pathway during critical periods of development can result in birth defects including holoprosencephaly, limb malformations and orofacial clefts, which are multifactorial and etiologically complex outcomes thought to result from gene-environment interactions. Identifying interacting factors that contribute to risk susceptibility is a key step towards designing birth defect prevention strategies. Genetic polymorphisms in metabolizing enzyme genes like the cytochrome P-450 (CYPs) enzyme family are primary drivers of inter-individual responses to xenobiotics. Here, we investigate how changes in the metabolism of the pesticide synergist piperonyl butoxide (PBO) influence its antagonistic activity against the Shh pathway. Traditional microsomal stability assays using human female microsomes are coupled with HPLC-MS and a Shh-responsive mouse embryonic fibroblast assay to detect the impact of metabolism on PBO activity. The results of this assay demonstrate that, as PBO undergoes metabolism, its antagonistic activity against the Shh pathway is reduced. Ongoing studies are incorporating recombinant human CYP enzymes to define specific isoforms that metabolize PBO and to determine how CYP-specific PBO metabolites impact Shh signaling. These studies focus on CYP isoforms with common human polymorphisms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) as a first but critical step in determining how common genetic predispositions may modulate the developmental impact of PBO exposure.

Time Series Clustering for the Integration of p53 Protein Dynamics and Transcriptomics in Single Cells

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Tumor suppressor p53 is a transcription factor that coordinates cellular repair and death processes after DNA damage. Changes in p53 levels over time (dynamics) regulate downstream processes and correlate with cellular outcomes. For example, oscillatory behavior leads to cell cycle arrest while non-oscillatory dynamics result in senescence. Notable heterogeneity in the p53 dynamics of genetically identical cell populations subjected to DNA damage necessitates the exploration of how distinct p53 dynamics impact the global transcriptional profile within individual cells. While it has traditionally been an experimental challenge to collect information on both p53 dynamics and transcriptomics in single cells, novel methods in the Lahav Lab enable the collection of p53 dynamics by live cell fluorescence imaging and scRNAseq measurements in the same individual cells, thus presenting the first opportunity to connect the data on a per cell basis.

To draw meaningful correlations between dynamical patterns and transcriptional outputs, it is first necessary to develop new methods of grouping the complex behaviors of p53 dynamic trajectories. Here, we evaluate the effectiveness of k-means and hierarchical clustering algorithms in clustering 72-hour time series measurements of p53 dynamics in MCF7 breast cancer cells after p53 activation with the DNA damaging agent cisplatin. Comparison of the two methods revealed that hierarchical clustering is better suited to confidently separate time series into a higher number of clusters. However, both methods were unable to group dynamics by shape, largely clustering time series by total magnitude. Future efforts will aim to stratify time series clustering to capture shape-based trends. Developing new clustering algorithms to classify protein dynamics based on shape will allow us to draw meaningful conclusions about how transcription factor dynamics govern transcriptional output and ultimately cellular outcomes.

Super-resolution imaging of unusual metal-responsive transcriptional regulation mechanisms in bacteria

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Biological processes in the gut microbiome heavily depend on the harmonious balance between microbial communities and a host. This balance is maintained by chemical and biophysical cues that are exchanged between organisms to coordinate behavior. Micronutrients such as transition metals have the potential to serve as chemical signals to coordinate behavior. Bacteria like higher organisms have developed highly sophisticated mechanisms to maintain cellular metal homeostasis. Metalloregulators are one among several classes of proteins that work to sense changes in metal concentrations inside the cells. For example, in E. coli the metalloregulator Zur senses a Zn2+ deficiency and derepresses znuABC transcription, a pump responsible for Zn2+ uptake, on the other hand, ZntR sense Zn2+ excess and activates zntA, responsible for cellular Zn2+ efflux. Previous studies on Zur and ZntR, have shown these proteins facilitate transcription switching of the genes responsible for Zn2+ import and efflux in cells, sensing the cytosolic Zn2+ concentration fluctuations. Zur in the metallated state binds tightly to the operator site of the znuABC promoter, the Zur Box, and represses Zn2+ uptake. Derepression of uptake under conditions of Zn2+ deficiency is either brought about by spontaneous unbinding of Zur from DNA, or via a Zur concentration dependent facilitated unbinding process, in which the incumbent Zur is dislodged by a freely diffusing cytosolic Zur.

History-dependent collagen remodeling enables cell invasion across environments of distinct stiffness and dimensionality

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Cells sense and migrate across mechanically dissimilar environments throughout development and disease progression. However, it remains unclear whether mechanical history of past environments alters future cell invasion. Here, we show that cells previously primed on stiff matrices, compared to soft, generate higher forces to remodel collagen fibers and promote invasion. This priming advantage persists in dense or stiffened collagen. We explain this history-dependent cross-environment cell invasion through a lattice-based model wherein stiff-primed cellular forces remodel collagen and minimize energy required for future cell invasion. Consistent with model predictions, depletion of yes-associated protein (YAP) destabilizes primed mechanoactivation before invasion. Releasing tension in collagen fibers via laser ablation or disabling fiber crosslinking by lysyl-oxidase inhibition disrupt the matrix remodeling necessary for cross-environment invasion. These results have implications for cancer, fibrosis, and aging, where a potential cell-to-matrix transfer of past mechanical history of cells may generate prolonged cellular response.

Cyclin-Dependent Kinase Motif Enrichment for Isobaric Trigger Channel-Based Substrate Analysis

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Cyclin dependent kinases (CDK) are key drug targets due to their central roles in the regulation of cell cycle progression and RNA Polymerase II elongation, which are key to cell growth in human diseases such as cancer. In this work, we have zoomed in on CDK targets of note using a CDK motif affinity reagent and an isobaric trigger channel-based analysis. CDK motif analysis enrichment requires a large amount of input material > 20 mg which is at least 4-8 times higher than input amounts required for titanium dioxide based phosphoproteomics. This isobaric trigger channel will allow for reproducible detection and quantitation of CDK substrates in a data dependent acquisition (DDA). Mammalian HEK293T cells are cultured to obtain 20 mg of protein for CDK motif analysis enrichment. The resulting lysate was then trypsin digested and underwent enrichment using the PTMScan® p(S/T)P kit (Cell Signaling). Enriched peptides are then TMT labeled and multiplexed with samples of titanium dioxide enriched phosphopeptides that were isolated from cells treated with varying concentrations of the CDK inhibitor flavopiridol. This approach will allow for increased phosphoproteome coverage with the CDK target trigger channel prioritizing acquisition of novel sets of phosphopeptides that were not observed in samples enriched with titanium dioxide alone. Recently published work has shown that an affinity-purified protein complex sample can be utilized at submolar concentrations as an isobaric trigger channel within a standard DDA shotgun proteomics workflow to quantitatively analyze 400 unique peptides. (Peck Justice, et. Al, Analytical Chemistry, 2021). Previous studies have also shown that PTM analysis is achievable via antibody-based enrichment to monitor phophopeptide changes. Specifically, phosphorylated Ser/Thr-Pro motifs were enriched in lysates the human gastric carcinoma cell line MKN-45 cells treated with c-Met inhibitor and PKC inhibitor. This enrichment step was integrated into standard LC-MS/MS workflows, allowing for the quantitation of 4,245 targets. These targets had an overlap ranging from 6.9% to 16.2% when compared to phospho-enriched lysate using immobilized metal affinity chromatography (IMAC) (Stokes, Rarnsworth, et. Al, Proteomes, 2015). As a result, CDK motif enriched peptides using the PTMScan® p(S/T)P kit will prioritize quantitation and analysis of an expanded set of phosphopeptide targets. This approach facilitates an expansion of phosphoproteome coverage through combination of phosphopeptide enrichment methods using a trigger-channel based DDA method.

Understanding the Mechanism of Host Glutathione Depletion by Helicobacter pylori

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Helicobacter pylori is the dominant bacterial species of the human gastric microbiota in approximately half of the global population. While most infected hosts remain asymptomatic, a subset develops chronic gastric inflammation or peptic ulcers. *H. pylori* infection is also the leading risk factor for the development of gastric cancer. One hallmark of *H. pylori* infection is the persistent generation of reactive oxygen species (ROS) in the gastric mucosa, which can cause oxidative DNA damage and potentiate cancer development. Healthy host cells rely on the antioxidant response to shield their DNA and other biomolecules from oxidative stress. Interestingly, *H. pylori* has been shown to subvert this response during infection by decreasing the levels of the antioxidant glutathione (GSH) in gastric cells through an unknown mechanism. Although ROS accumulation has been widely cited as the cause of GSH depletion, we have found that GSH oxidation does not account for the loss of reduced GSH in infected cells. Rather, our data indicate that the *H. pylori* enzyme and colonization factor γ-glutamyl transpeptidase (gGT) is the primary driver of GSH depletion. Our findings suggest that *H. pylori* also exploits host mechanisms to deplete GSH, dysregulating the expression of host GSH metabolic enzymes GSH synthase (GSS) and γ-glutamyl-cyclotransferase 1 (CHAC1). Together, these findings shed new light on how *H. pylori* influences the redox environment of the gastric mucosa and ultimately contributes to gastric disease.

Spermidine Enhances the Contractile Force of Single-Cell Human Induced Stem Cell-Derived Cardiomyocytes Post Doxorubicin Treatment

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Doxorubicin (DOX) is a chemotherapy used to treat childhood and adult malignancies, including pediatric leukemia and breast cancer. However, despite being a cornerstone for oncological care, the use of DOX is limited due to dose-dependent cardiotoxicity, which can lead to contractile dysfunction and heart failure. Yet, Dox is still widely used due to the lack of suitable replacements. The exact mechanism of Dox-induced cardiotoxicity is not fully understood. However, proposed mechanisms include sarcomere degradation and impairment of autophagy. To determine whether enhancing autophagy can restore contractile function, we treated μ-patterned single-cell human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), displaying sarcomere disarray and reduced contractile force post-Dox treatment with spermidine, an autophagy activator. The contractile force was quantified using traction force microscopy. There were five conditions (untreated control, Dox, maintenance medium post-Dox, spermidine post-Dox, and spermidine-only). We found that hiPSC-CMs treated with spermidine post-Dox produce 67% of the contractile force generated by control cells (.22 μN vs. .33 μN, not significant) and significantly (.22 μN vs. .11 μN, $p < .0001$) higher contractile force compared to cells in maintenance medium post-Dox. In the spermidine-only group, our results demonstrate that spermidine significantly (.57 μN vs. .33 μN, $p < .0001$) increases contractile force compared to control. Spermidine-only treated cells were also bigger (spread area = ~1716 μm2 vs. ~1353 μm2, $p < .00001$) than the control and had a more robust and organized sarcomere network. Preliminary qPCR results suggest that spermidine upregulates genes that control sarcomere function (e.g., MYH7) and organization (e.g., ANKD1). These data demonstrate that spermidine can enhance contractile force post-Dox treatment and increase hiPSC-CMs size and sarcomere network. The data further suggests that sarcomere homeostasis is one of the mechanisms spermidine regulates in hiPSC-CMs. This work opens the door for developing therapies that target autophagy and sarcomere proteostasis to treat cardiac dysfunction.

Structure and Candidate Biosynthetic Gene Cluster of a Manumycin-type Metabolite from Salinispora pacifica

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A new manumycin-type natural product named pacificamide (1) and its candidate biosynthetic gene cluster (pac) were discovered from the marine actinobacterium Salinispora pacifica CNT-855. The structure of the compound was determined using NMR, electronic circular dichroism, and bioinformatic predictions. The pac gene cluster is unique to S. pacifica and found in only two of the 119 Salinispora genomes analyzed across nine species. Comparative analyses of biosynthetic gene clusters encoding the production of related manumycin-type compounds revealed genetic differences in accordance with the unique pacificamide structure. Further queries of manumycin-type gene clusters from public databases revealed their limited distribution across the phylum Actinobacteria and orphan diversity that suggests additional products remain to be discovered in this compound class. Production of the known metabolite triacsin D is also reported for the first time from the genus Salinispora. This study adds two classes of compounds to the natural product collective isolated from the genus Salinispora, which has proven to be a useful model for natural product research.

Efficacy of the Rac/Cdc42 inhibitor MBQ-167 in combination therapy with Paclitaxel

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Triple negative breast cancer (TNBC) represents a challenge because of its aggressiveness and resistance to standard chemotherapy. Since TNBC lacks targeted therapy options, the frontline therapy is Paclitaxel, which targets actively dividing cells inducing subsequent apoptosis. Since dormant cells or metastatic cancer cells are not targeted by Paclitaxel, more targeted treatment options are needed. MBQ-167, a small molecule developed by us to target the metastasis drivers Rac and Cdc42, is highly effective in reducing tumor growth and metastasis in mouse models of TNBC. The purpose of this study is to test the hypothesis that MBQ-167 is a viable candidate for combined therapy with Paclitaxel in TNBC. Therefore, we tested the efficacy of MBQ-167 in combination with Paclitaxel in MDA-MB-231 human TNBC cells via MTT viability assays and caspase 3/7 apoptosis, using effective concentrations of MBQ-167 and/or Paclitaxel for 24, 48, 96 & 120 hrs. We found that individual MBQ-167 or the combination therapy was more effective at 48-120 hrs at reducing cell viability by ~80% compared to ~60% by Paclitaxel treatment alone and significatively increasing apoptosis. Next, we tested individual or combined MBQ-167 (5 mg/kg 5X a week) and Paclitaxel (10 mg/kg 1X a week) on SCID mice bearing MDA-MB-231 tumors, via intraperitoneal administration. We report a significant reduction in tumor growth and lung metastasis in response to combined MBQ-167 and Paclitaxel compared to either treatment alone. A more aggressive syngeneic model of 4T-1 mouse breast cancer cells in BALB/c immunocompetent model was used to determine the direct effect of MBQ-167 and Paclitaxel on metastasis. When 4T-1 tumors were surgically removed and MBQ-167, Paclitaxel, or the combination administered for 17 days, the combination treatment, but not individual compounds, significantly reduced lung metastases by ~80%. In conclusion, this study validates the clinical testing of MBQ-167 in combination with Paclitaxel as a potential therapeutic for TNBC.

Regulation of the maintenance of the [PIN+] prion by the evolutionarily conserved non-prion domain of the [PIN+]-forming protein, Rnq1

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Prions and prion-like amyloid aggregates are associated with devastating diseases, including Creutzfeldt-Jakob, Alzheimer's, Parkinson's, Huntington's, ALS, type 2 diabetes, and cancer. Accumulating evidence suggests that prion-like aggregates also play an important role in normal cellular physiology, e.g. by driving formation of mRNA processing bodies or localizing protein synthesis to activated synapses. [PIN+] is a prion form of the Rnq1 protein. [PIN+] promotes formation and elimination of other yeast prions. For example, the de novo formation of the [PSI+] prion essentially requires [PIN+]. [PIN+] also promotes aggregation of several proteins associated with human neurodegenerative diseases, including huntingtin and TDP43. Rnq1 can be divided into the N-terminal and C-terminal domains. The function of the N-terminal domain is still unknown, but it is not essential for [PIN+] formation or maintenance, i.e. "non-prion domain" (NPD). The C-terminal domain is prion-forming (PD): it contains several Q/N-rich determinants that can independently maintain [PIN+] and promote [PSI+] formation. Using a genetic screen, we identified the T27P mutation in the NPD of Rnq1 that leads to the loss of [PIN+] in a majority of cells after substituting T27P RNQ1 for WT Rnq1 in a [PIN+] strain. We hypothesize that the T27P mutation in the NPD modulates thepotential conformational flexibility of the PD within the amyloid aggregate thus creating a barrier for a transmission of the prion state from the WT RNQ1 to the mutant. Indeed, the mutation does not inhibit the de novo formation of [PIN+] in vivo. Also, after rare transmissions from WT Rnq1, the mutant [PIN+] prions can undergo gradual adaptation and eventually propagate stably. The distinct appearance of amyloid fibers formed by the mutant vs. WT Rnq1 in vitro further supports this conclusion. The transmission barrier can be rescued by deleting one of the QN-rich prionogenicity determinants in the Rnq1 PD. Interestingly, [PIN+] is the only prion found in natural yeast isolates, and its maintenance is strictly controlled by the interaction of the Sis1 chaperone with a site in the NPD. Analysis of RNQ1 orthologs from other fungal species reveals moderately conserved N-terminal domains (the sites of the above-mentioned mutation and Sis1 binding are conserved), and C-terminal domains that suggest evolutionary-driven amplification of Q/N-rich sequences with characteristic features of aggregation-prone prion domains. Based on this we propose that Rnq1's cellular function is to regulate aggregation of other amyloidogenic proteins.

Solid-Phase Photochemical Modification of Peptides via Charge-Transfer Complexes

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The compatibility of photochemistry with solid-phase peptide synthesis is demonstrated via the photochemical hydroalkylation to form C(sp3)−C(sp3) bonds between on-resin Giese acceptors and redox-active esters. Both iridium-based photocatalysts and Hantzsch ester led to high yields, with final reaction conditions producing full conversions within 30 minutes under ambient conditions. These conditions represent the first example of photochemical peptide modifications on resin. Furthermore, the conditions are utilized for two-carbon homologation and macrocyclization. We successfully demonstrated the use of Asp and Glu side-chains as N-hydroxyphthalimide radical precursors for two-carbon homologation. The strategy of tandem photochemical intermolecular homologation and cyclization is demonstrated on Atosiban. Finally, a photochemical charge-transfer mechanism which forms electron-donor-acceptor complexes directly between the two coupling partners – thiols and aryl halides – is being adapted to on-resin macrocyclization.

Investigating the role of METTL16 in SAM homeostasis and U6 snRNA function

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S-adenosyl methionine (SAM) is the methyl donor for nearly all cellular methylation events, so cells tightly regulate intracellular SAM levels. In most human cell types, SAM is synthesized by the SAM synthetase encoded by the methionine adenosyltransferase 2A (MAT2A) gene. To control SAM levels, cells regulate MAT2A mRNA accumulation by at least two mechanisms mediated by 6 regulatory hairpins (hp) in the MAT2A 3′ UTR. In the nucleus, the MAT2A transcript is regulated by SAM-dependent intron detention of the last intron. Upon SAM starvation, the dwell-time of the hp methyltransferase METTL16 on hp1 increases, resulting in splicing induction. In addition, MAT2A mRNA stability is subject to regulation mediated by hp2-6 and METTL16. Additionally, METTL16 is the U6 snRNA methyltransferase, and while the function of U6 snRNA methylation is unclear, 95% of U6 snRNA is methylated in humans. Furthermore, loss of U6 snRNA methylation in S. pombe causes global changes in splicing. The requirement of METTL16 for cell growth combined with its dual roles in MAT2A and U6 snRNA biogenesis have confounded functional studies in cells. To better define METTL16 function, we AID-tagged endogenous METTL16 to induce degradation of METTL16 within 2 hours. Using this system, we can genetically separate METTL16's distinct functions of MAT2A regulation and U6 snRNA methylation by complementing METTL16 degradation with different METTL16 variants. Broadly, this system will allow us to assess global changes in SAM levels, epigenetics, the epitranscriptome, and splicing following loss of METTL16, giving us new insight into the mechanisms of this multifunctional essential protein.

Nickel-Catalyzed Cascade Cyclization of Alkene-Tethered Alkylpyridinium Salts

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Heterocycles represent an important motif in pharmaceuticals and other bioactive molecules. To provide efficient access to these structures, we are developing a reductive cascade cyclization of alkylpyridinium salts. We hypothesize that this reaction proceeds via deamination to give an alkyl radical, which can then cyclize on the pendant alkene. Subsequent coupling with an aryl bromide then allows the formation of two new C–C bonds in one step. The model substrate is readily made from a 1,2-amino alcohol (74% overall yield over 3 steps from Boc-glycinol). Optimization studies show a promising 78% yield using two equivalents of alkylpyridinium salt. Scope studies are ongoing. The synthesis of other primary and secondary substrates is underway. We are particularly interested in the development of diastereo- and enantio-selective examples of this reaction.

Development of Novel Therapeutics for Depression

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Numerous studies indicate that depression and other neuropsychiatric disorders are caused by the atrophy of neurons in the prefrontal cortex (PFC).1-3 Classical psychedelics such as amphetamines, tryptamines and ergolines have been shown to promote plasticity, potentially counteracting the detrimental effects of these diseases.[4] These plasticity promoting compounds, termed psychoplastogens, can provide an important basis for the development of novel therapeutics. In the tryptamines class, the amine group in N,N-dimethyltryptamine (DMT) can adopt a variety of conformations. These conformations resemble structural motifs common in other classic psychedelics such as lysergic acid diethylamide (LSD) and ibogaine. To study the importance of amine placement, our lab has synthesized various tryptamine derivatives for structure activity relationship (SAR) studies. Currently, I am testing these derivatives using a variety of biological assays to determine the ability of these compounds to promote neural plasticity. By measuring changes in the size and morphology of the dendritic arbor of neurons, the number of synapses formed, and the number of spines, we can determine the effects of these compounds on structural plasticity in vitro. We can also assay hallucinogenic potential using the fluorescent sensor psychLight, recently developed by our lab in collaboration with the Tian lab at UC Davis.[5] These in vitro assays are an important step in screening compounds before advancing to in vivo studies.

References

1. Christoffel, D. J.; Golden, S. A.; Russo, S. J. Rev. Neurosci., 2011, 22, 535–549.

2. Duman, R. S.; Aghajanian, G. K. Science, 2012, 338, 68–72.

3. Qiao, H. et al., Neural Plast., 2016, article 8056370.

4. Ly, C. et al., Cell, 2018, 23, 3170-3182.

5. Dong, C. et al., Cell, 2021, 184, 2779-2792

Identifying neuronal circuits that coordinate sugar and water ingestion

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While the mechanisms animals use to eat are beautifully diverse, all organisms need to acquire nutrients from their environment to survive. Food ingestion is tightly regulated by internal signals of nutrient abundance to maintain homeostasis. Studies have identified four neurons called Interoceptive Subesophageal zone Neurons (ISNs) in Drosophila melanogaster that respond to endogenous hunger and thirst signals to oppositely regulate sugar and water ingestion. Upon activation, ISNs increase sugar ingestion but decrease water ingestion. While the molecular pathways that lead to ISN activation have been identified, it is still unknown how ISN activity translates to a behavioral response. The goal of this study is to examine how competing needs are coordinated by the nervous system by identifying and characterizing the neural circuit downstream of the ISNs. We used the full adult fly brain (FAFB) electron microscopy volume to trace the ISNs and the neurons with most synaptic connections from the ISNs. We identified a novel neuron which we named Bilateral T-shaped neuron (BiT), which is the neuron with the most synapses from the ISNs. We successfully created a split Gal4 fly line that genetically targets the ISN postsynaptic neuron BiT exclusively. Functional connectivity experiments revealed that the ISNs inhibit BiT. Consumption assays revealed that activation of BiT decreases sugar ingestion but increases water ingestion, demonstrating that sugar and water ingestion is still coupled downstream of the ISNs. Using a similar approach, we found that insulin producing cells (IPCs) were downstream of BiT. Functional connectivity experiments suggest that BiT inhibits IPCs. These data suggest that IPCs receive input on the hunger and thirst status of the fly from the ISNs via BiT to coordinate these competing needs. Future experiments involve characterizing a separate circuit we found that connects the ISNs to motor neurons controlling feeding.

Tissue-Specific Role of FXR in Non-Alcoholic Steatohepatitis Development in Female Mice

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Non-alcoholic fatty liver disease (NAFLD) is a spectrum of conditions characterized by lipid accumulation in the liver known as steatosis. Approximately 25% of the US population have NAFLD and approximately 30% of that population suffer from non-alcoholic steatohepatitis (NASH). NASH is the more severe and progressive form of NAFL that is characterized by liver steatosis, hepatocellular ballooning, inflammation, and fibrosis. Other than lifestyle changes, there is no current FDA-approved therapeutic for NASH; however, one emerging target is the Farnesoid X Receptor (FXR). FXR is a ligand activated transcription factor highly expressed in numerous tissues such as the liver and intestine and is a major regulator in bile acid (BA) homeostasis. BAs have been implicated in NASH development and progression and FXR deficiency in male mice leads to more severe NASH development. Because FXR negatively regulates BA production, synthetic ligands that are whole-body FXR agonists have been developed to treat NASH. Although beneficial, these whole-body modulators contribute to side effects such as cholesterol homeostasis imbalance, thereby explaining the importance of FXR tissue-specific activation in the development of novel therapeutics for NASH to negate any potential harmful consequences. Nothing is known for the role of FXR in females during NASH development, although gender difference in NASH development is reported. With this in mind, we are working to determine the tissue-specific role of FXR in NASH development in female mice. To better understand underlying tissue-specific mechanisms, 6-8 week old female mice in 4 genotypes: Wild-Type (WT, C57BL/6J, Fxr+/+), Liver FXR KO ((Fxrfloxed/floxed, Albumin Cre (+), FXR LKO), Intestinal FXR KO (Fxrfloxed/floxed, Villin Cre (+), FXR IKO), and Whole Body FXR KO (WB FXR KO, Fxr-/-), were fed either a low-fat control diet or a NASH "Fast Food" (FF) diet (Western diet with 21% milk fat, 1.25% cholesterol, and 34% sucrose) for 16 weeks. Serum biochemical analysis showed that Fxr-/- had decreased serum albumin compared to all other groups including the WT mice, indicating decreased liver function. Serum ALT and cholesterol values were elevated among all groups fed FF, with the largest increases seen in the FXR LKO/FF group. Serum triglycerides were decreased in all groups, except for the FXR LKO groups, further defining phenotypic characteristics in that model. Furthermore, serum ALP values were greatly increased in FXR LKO/FF mice compared to all other tissue-specific FXR KO groups, suggesting cholestasis. Total bilirubin was increased in Fxr-/-, FXR LKO, and FXR IKO mice in comparison to WT mice fed a control diet, which may indicate liver damage in the absence of FXR. FXR IKO displayed lower serum values for each parameter, except serum albumin and bilirubin, compared to all other groups including the WT. Female Fxr-/- and FXR LKO mice fed both a low fat and FF diet displayed minor increases in %BW change, while all other groups showed a more robust increasing trend. These data suggest that the FXR LKO model is more vulnerable and may exacerbate liver injury and in comparison, the FXR IKO is

less vulnerable and more protective against liver injury. These results suggest that hepatic FXR is more critical in suppressing liver injury during NASH development than intestinal FXR.

Modular Synthesis of Unnatural Peptides via Rh(III)-Catalyzed Diastereoselective Three-Component Cross-Coupling Reaction

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e present a novel Rh(III)-catalyzed technology that enables a 3-component carboamidation reaction for the construction of unnatural peptides. The reaction is diastereoselective and provides access to D-stereocenters, which are generally very difficult to synthesize by other means. The reaction encompasses a large scope of aryl boronic acids, dioxazolones, and acrylamides to provide rapid access to phenylalanine, tyrosine, and tryptophan derivatives. We apply our methodology to synthesis of various analogs of carfilzomib, a potent proteasome inhibitor.

Diversity and dispersal contribute to animal microbiome resistance and resilience to disturbance

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Microbiomes associated with invertebrate and vertebrate animal hosts can play a pivotal role in host health and fitness. However, disturbances ranging from medical intervention to environmental change can alter the diversity and composition of animal microbiomes. Given the host-microbe services that may be jeopardized by these alterations, we applied ecological theory as a framework with which to evaluate the degree of resistance or rate of recovery for animal microbiomes experiencing disturbance. Using longitudinal sampling and 16S community profiling, we demonstrate how a non-model invertebrate host (Acropora coral) uses rapid restructuring of the microbiome to cope with thermal stress. This sensitivity to disturbance, characterized by the proliferation of opportunistic taxa, is followed by a period of recovery to a low diversity stable state, thus demonstrating resilience. Further, metacommunity theory can be applied to animal microbiome systems in which hosts are considered habitat patches interconnected by microbial dispersal. We combined disturbance experiments and metacommunity theory to elucidate the effect of inter-host dispersal on microbiome resistance. In a model vertebrate zebrafish host (Danio rerio), we show that dispersal of microbes between hosts results in lower resistance to sublethal antibiotic disturbance compared to solitary fish with no dispersal. This suggests that inter-host dispersal may promote widespread colonization of disturbance-tolerant taxa that are otherwise absent in the local species pool of a solitary host. Fundamental processes of community ecology drive the assembly of animal microbiomes, including in the human gut. Insights from experimental systems such as the two animal microbiomes studied here, can inform the dispersal of pathogens and the recovery from antibiotics in human microbiomes.

The Unkempt RNA binding satellite protein promotes PLK4-induced centriole overduplication

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Centriole duplication drives assembly of the microtubule organizing center (MTOC) or centrosome, which occurs between G1 and S phase. PLK4 is the master regulator of centriole duplication that regulates early events in centriole duplication, centrosome assembly and satellite integrity. Overduplication of centrioles can lead to centrosome amplification (CA) multipolar mitosis, chromosomal instability (CIN), and aneuploidy. PLK4 OE promotes centriole overduplication by dysregulating assembly factors such as centriole structural proteins, satellites, and the PCM. How PLK4 dysregulates centrosome assembly factors to promote centriole overduplication remains unclear. We find that Unkempt (UNK), an RNA-binding zinc finger protein, is upregulated when PLK4 is overexpressed in RPE-1 cells, suggesting that UNK modulates PLK4 and/or centriole overduplication. We show that UNK localizes to the centrosome and to centriolar satellites. Using PLK4 overexpression in RPE-1 cells, UNK knockdown attenuates centriole overduplication, suggesting that UNK promotes PLK4 induced centriole overduplication. Moreover, upon UNK depletion in PLK4 induced cells, centriolar satellite proteins and early centriole assembly components, PLK4, CEP152, CEP192, and SAS6 are reduced. These data propose potential processes by which the UNK regulates centriolar satellites and early centriole assembly events in cells to promote PLK4 induced centriole overduplication.

Determining the mechanism of Kinesin-1 dependent translocation of the meiotic spindle to the cortex in C. elegans

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Cortical positioning of the meiotic spindle within an oocyte is required to expel chromosomes into polar bodies to generate a zygote with the correct number of chromosomes. In C. elegans the prophase nucleus migrates to the oocyte cortex and the metaphase spindle moves further toward the cortex, both in a kinesin-1 dependent manner. In contrast, yolk granules, mitochondria and kinesin-1 are transported inward, away from the cortex in a kinesin-dependent manner. The kinesin-dependent inward packing of yolk granules and mitochondria suggests the existence of microtubules with minus ends at the cortex and plus ends extending inward. Thus, the mechanism of outward translocation of the spindle has remained a mystery. Because the endoplasmic reticulum (ER) is organized by kinesin-1 in mammalian cells and the ER envelopes the C. elegans meiotic spindle, we first hypothesized that kinesin-1 moves the meiotic spindle through its action on the ER. Time-lapse imaging of GFP::signal peptidase-labeled ER in a kinesin loss of function mutant revealed no change in ER organization or dynamics during metaphase I, but an enrichment of ER at the cortex during the onset of anaphase I. Time-lapse imaging of EB1 or tubulin revealed microtubules with plus ends growing outward from the meiotic spindle, which suggested an alternative hypothesis. We hypothesized that transport of yolk granules and mitochondria on these microtubules extending from the spindle will generate a force in the opposite direction on the spindle. Because yolk granules and mitochondria are excluded from the cortex, there will be more pushing force toward the cortex, away from regions with high concentrations of yolk granules and mitochondria. One prediction of this hypothesis is that kinesin cargo will be closer to the meiotic spindle in a kinesin null mutant. As a preliminary test of this hypothesis, we have measured the empty space between the mitochondria and the meiotic spindle and surprisingly found an increased distance in a kinesin mutant (control: 2.2 +/- 5.7 um, unc-116: 4.9 +/- 7.2 um, P value < 0.0001 n=10). Because the direct cargo of kinesin is not known, we are analyzing the distance from the spindle to other organelles. As an alternative strategy to test this hypothesis, we are coupling tailless kinesin-1 directly to mitochondria and yolk granules to determine if this restores spindle translocation in kinesin mutant embryos.

Investigating the Role of the p97 Adaptor UBXD8 in Peroxisome Function

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[not submitted]

Diversity Supplement Professional Development Poster Sessions

Wednesday, August 31st, 2022

Session C & D: 1:15 p.m. – 2:20 p.m.

Session C
Wednesday, August 31st, 2022

The Sex-Specific Effects of Adolescent Intermittent Ethanol Exposure on Hippocampal Neurogenesis and Neurotrophic Response Following Abstinence

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According to the Centers for Disease Control and Prevention, alcohol is the most commonly abused drug among adolescents, and underage alcohol consumption accounts for more than 3,900 preventable deaths in the United States. Studies have shown that binge-like ethanol exposure during adolescence promotes dysregulation of inflammatory cytokine responses and a reduction of newly regenerated neurons in the dentate gyrus subregion of the hippocampus. These effects include changes in proliferation, regulation, differentiation, and maturation of neurons, and there is indication that such effects may be disproportionate between sexes. This study determined whether sex impacts the neurogenic makers Ki-67 and SOX2 as well as the proinflammatory cytokines TNF-α and IL-1β in adulthood after adolescent intermittent ethanol (AIE) exposure. To determine this, 16 adolescent male and 16 adolescent female rats underwent AIE with 10 doses of ethanol (5 g/kg) over a course of 16 days, on a 2-days on, 1-day off, 2-days on, 2-days off pattern, to mimic the sporadic drinking of adolescents. Half of each sex group received either AIE or adolescent intermittent water (AIW) as the control. In adulthood, animals were sacrificed and immunohistochemical techniques and ELISAs were used to distinguish AIE effects on sex-specific neurogenic markers and proinflammatory markers, respectively. A random number generator was used to assign experimental cohorts with cage numbers to blind experimenters to the treatment groups. Our results indicated that AIE exposure led to a significant decrease in neurogenesis in the dentate gyrus of the hippocampal formation indicated by reductions in the numbers of Ki-67+ and SOX2+ cells in male and female AIE-exposed rats. Additionally, AIE increased the protein expression of pro-inflammatory cytokines, TNF-α and IL-1β, in the hippocampus of male AIE-exposed rats only. Altogether, our findings indicate that AIE does reduce neurogenesis in the dentate gyrus subregion and pro-inflammatory cytokine expression in the hippocampus. The neurogenic impairment was not sex-specific as both male and female AIE groups had approximately a 10-20% decrease in the number of Ki-67+ and SOX2+ cells compared to their AIW control, though the pro-inflammatory cytokine increase was observed solely in male AIE-exposed rats. A persistent impairment in neurogenesis may alter hippocampally driven behaviors including memory consolidation and retrieval.

Regulation of the budding yeast meiotic proteome

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Our lab has generated rich datasets on the budding yeast meiotic transcriptome, translatome, and proteome to make global measurements of what is expressed as cells differentiate into gametes. These datasets can begin to elucidate the regulation of the meiotic proteome. Using start site profiling, we discovered that translation of alternative, in-frame truncations of important proteins, such as the MAPK protein Fus3, occurs during meiosis. From our analyses of the translatome and proteome, we discovered that most protein complexes are synthesized imprecisely then are post-translationally tuned during meiosis. Lastly, tracking gene expression trends can shed insights on new modes of regulation for well-known protein complexes during meiosis, such as the proteasome. Our data suggests that the proteasome undergoes a post-translational change in complex composition during meiosis, likely via targeted protein degradation and association with alternative activators.

Aurora kinase B is required to maintain mitochondrial quality in aging oocytes

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In humans, the female reproductive system ages more rapidly than other organ systems. A hallmark of reproductive aging is the decline of oocyte quality and quantity. Several factors including mitochondrial dysfunction contribute to accelerated oocyte aging. Mitochondria produce sufficient ATP to support the energy-demanding events in oocyte meiosis. However, dysfunctional mitochondria in aged oocytes are inefficient at producing adequate amounts of ATP and generate reactive oxygen species (ROS). ROS accumulation causes degradation of essential proteins such as components of the spindle assembly checkpoint (SAC), a critical signaling network designed to ensure cells remain euploid. We previously observed that oocytes from Aurora kinase B knockout (AURKB KO) females accumulate ROS, have SAC defects and have premature age-related infertility. Because AURKB KO females exhibit downstream mitochondrial dysfunction defects, we hypothesized that AURKB plays a role in maintaining proper mitochondrial function to protect oocyte quality during aging. To test our hypothesis, we evaluated mitochondrial function during oocyte maturation by comparing morphology and mitochondrial membrane potential in AURKB KO and wild-type controls. Wild-type oocytes have mitochondria spread throughout the cytoplasm with some localized around the meiotic spindle. In contrast, mitochondria in AURKB KO oocytes aggregates and forms larger clumps. The membrane potential, an essential component in ATP production, is decreased in AURKB KO oocytes compared to wild-type. These results suggest AURKB plays a role in maintaining mitochondrial quality but further experiments are needed to further evaluate the health of the mitochondria and specify the contribution of AURKB during oocyte aging.

Characterizing ESCO1's Interaction with Chromatin

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ESCO1 is an enzyme that regulates chromosome organization and gene expression. ESCO1's primary function appears to be acetylating Cohesin, a master regulator of genome architecture that organizes DNA into loops and is critical for normal nuclear structure and function. Acetylation of Cohesin by ESCO1 stabilizes Cohesin on DNA, promoting long residence time at functional sites. Factors that shape when, where, or how ESCO1 stabilizes Cohesin are not understood.

We have found that tethering ESCO1 to a specific location in the nucleus results in gross local rearrangement of chromatin. Strikingly, this local chromatin rearrangement occurs independently of ESCO1's acetyltransferase activity and of Cohesin. We mapped this activity to a small motif within ESCO1 and showed that this region of ESCO1 binds to DNA. Furthermore, we found that ESCO1 preferentially binds ssDNA, aligning with recent data showing Cohesin colocalizes with R-loops, sites of displaced ssDNA commonly produced during gene expression. Finally, the identified DNA binding domain (DBD) is needed for normal localization of ESCO1 onto chromatin.

We hypothesize that the DBD directs ESCO1 to specific chromatin structures to regulate Cohesin and gene expression. Experiments are ongoing to determine what DNA structures ESCO1 recognizes in vitro and in live cells. Using functional genomics techniques, we will determine the effect of DNA binding by ESCO1 on Cohesin localization and gene expression. These experiments will characterize the biological relevance of ESCO1's newly identified DNA binding activity.

Generation of SIX3 Mutants for Human Neural Differentiation Studies

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SIX3 is a transcription factor that controls gene expression in a spatiotemporal specific manner. SIX3 functions in Shh signaling, Wnt signaling, postnatal ependymal cell maturation, and in post-proliferative neurons of the hypothalamus and pituitary. However, we still do not know what regulates SIX3 expression during development in human brain. Also, it is unclear how SIX3 controls its targets in different developmental stages. More than 60 mutations in SIX3 have been causally associated with Holoprosencephaly (HPE), which occurs during the first few weeks of pregnancy in one in 5,000 live births. HPE is characterized by many signs including brain malformation, seizures, and developmental delay. Our lab previously obtained experimental findings suggesting that SIX3 gene is epigenetically regulated by the UTX-53BP1 axis during human neural differentiation. UTX is a lysine-27-specific demethylase of histone 3, and 53BP1 is a factor that binds to specific histones at double-strand break sites and contributes to the maintenance of heterochromatin and genome stability. We are generating SIX3 mutants in human embryonic stem cells to characterize mutant-SIX3 effects in neural differentiation and cortical organoids. Next, we will compare SIX3 mutants with UTX-KO lines. The successful completion of these experiments will illuminate the relationship between the UTX-53BP1 axis and SIX3.

Estimating the Kinetics of mRNA 3’ End Cleavage

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Intermediate mRNA processing events—splicing and 3’ end cleavage—often occur co-transcriptionally, with the interplay between transcriptional elongation rates and rates of each individual processing event often impacting choices that lead to alternative isoform production. Consequently, measuring the kinetics of these processes may shed light on how early gene regulatory decisions are made. Recent development of high-throughput sequencing techniques that capture nascent RNA over defined temporal intervals has made genome-wide kinetic profiling of RNA maturation possible. Though rates of mRNA splicing have been estimated globally, the rate at which an mRNA is cleaved and polyadenylated to complete the maturation process has never been investigated. Here, we present a novel computational method to estimate genome-wide kinetic parameters for mRNA cleavage rates. This method capitalizes on short-read sequencing data from nascent mRNAs isolated after a time-course of 4sU metabolic labeling to model the rate of mRNA maturation over time. To specifically measure cleavage rates, we first use patterns of read coverage from our sequencing data to approximate the position at which cleavage occurs and then estimate the fraction of reads derived from cleaved or uncleaved molecules at that site across time to model the rate of 3’ end cleavage. In-silico simulations of nascent 4sU-seq data show that our model can accurately and precisely estimate cleavage half-lives. We applied this method to nascent RNA-seq data from Drosophila melanogaster S2 cells to estimate polyadenylation-site (PAS) specific rates of mRNA cleavage at constitutively and alternatively used cleavage sites. We find that the rates of 3’ end cleavage are fast on average and are associated with the number of PASs in a gene and the density of PAS kmers near a site. Very short cleavage half-lives are associated with shorter transcriptional readthrough lengths and higher average intronic read coverage. Finally, to evaluate whether there is a kinetic relationship between splicing and 3' end cleavage decisions, we perform computational Gillespie simulations and nascent RNA-seq after treatment with a U1 antisense morpholino oligonucleotide (AMO). Our findings shed light on the timing of decisions involved in alternative PAS usage within genes and the variable efficiency of 3’ end cleavage and polyadenylation across genes.

DNA-binding and degradation of mitochondrial genome maintenance exonuclease (MGME1) is enhanced by a 5′-phosphate

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Mitochondria are main contributors of energy production in higher eukaryotes and play multiple roles in signaling and biosynthesis. Multiple copies of mitochondrial DNA (mtDNA) are present in a single mitochondrion and encode 37 genes essential for mitochondrial and cellular function. As mtDNA is susceptible to damage, it will undergo repair, degradation, and compensatory synthesis. A key factor involved in mtDNA degradation is human mitochondrial genome maintenance exonuclease 1 (MGME1). Despite previous studies, controversies exist regarding the polarity of MGME1-mediated DNA cleavage. It is also unknown how DNA sequence may affect the activity of MGME1. Such information is critical for understanding MGME1 and the overall mtDNA degradation mechanism. Herein, we use quantitative assays to examine the effects of substrate structure and sequence on the DNA-binding and enzymatic activity of MGME1. We demonstrate that MGME1 binds to and cleaves from the 5′-end of ssDNA, especially in the presence of a 5′-phosphate. MGME1 can tolerate certain modifications at the terminal end (i.e. base excision repair intermediate, 5′dRp). MGME1 processes different sequences with varying efficiency, with dT and dC-sequences being the most and least efficiently degraded. These results provide insights into the enzymatic properties of MGME1 and a rationale for the coordination of MGME1 with 3′ □ 5′ exonuclease activity of DNA polymerase γ in mtDNA degradation.

Engineering of LwaCas13a Protein with Enhanced Collateral Activity for Ultrasensitive Nucleic Acid Detection

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CRISPR-Cas13 has been rapidly developed for nucleic acid-based diagnostics by utilizing its characteristic collateral activity, easy programmability, and equipment-free nature. Despite recent progress in CRISPR based diagnostics, engineering the Cas13 enzyme is challenging due to its complex structural dynamics, but promising for point-of-care detection from raw patient samples. Here, we successfully employed a novel strategy to engineer the Leptotrichia wadei (Lwa)Cas13a protein by inserting different RNA binding domains (RBDs) to increase enzyme-substrate binding affinity. Two LwaCas13a variants achieved up to 58-fold enhanced collateral activity over the wild-type (WT) in a fluorescence-based assay. When using an electrochemical detection method, our variants achieved 50,000-fold sensitivity over the WT, showing a clear detection of ~1 attomolar (0.6 copy/μL) of the SARS-CoV-2 genome within 30 minutes. We are currently integrating our engineered LwaCas13a enzymes into a new diagnostic test for HIV with lateral flow strips. Most importantly, our engineered LwaCas13a enzymes can be further integrated into other fields including biological assessments and environmental surveillance.

Yeast PI31 Inhibits the Proteasome by a Direct Multisite Mechanism

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Cellular function requires the controlled degradation of proteins to prevent the accumulation of those that are aberrant or unnecessary. Proteasome inhibitors, usually targeting one of three unique active sites each of which is present twice, have been used to treat multiple myeloma and other cancers. The endogenous mammalian protein PI31, and S. cerevisiae counterpart Fub1, were found to inhibit all six active sites of the proteasome via an unknown mechanism. We found that Fub1 is enriched in mutant proteasomes with open gates that allow aberrant protein degradation. Through Cryo-EM and targeted mutations, we demonstrate that Fub1 inhibits proteasomes via a highly conserved C-terminus that forms a dimer and loops around the interior of the proteasome binding all six active sites. Fub1 demonstrates distinct strategies of avoiding cleavage at every active site allowing it to remain stably bound. This mechanistic data provides a novel framework for designing biology informed proteasome therapeutics.

Fast conformational exchange and low populated states as cornerstones in protein allostery

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Allostery is the ability of a macromolecule to intercommunicate between two or more distant sites. Due to its crucial role in signal transmission and regulation in cells, protein allostery has been studied as a baseline for the design and development of allosteric drugs to treat malfunctions resistant to other types of therapeutics. However, understanding the mechanisms that govern the allosteric model has been a challenge throughout the years and, as a result, only 0.5% of the total FDA-approved drugs are allosteric drugs. Here, we present our most recent discoveries on chorismate mutase (CM), a homodimeric yeast enzyme exhibiting classical allosteric transitions between a tense (T)-less active and a relaxed (R)-more active state. This T-to-R transition is achieved by the binding of small effectors and substrate in an allosteric fashion. Combining crystallographic data with nuclear magnetic resonance (NMR) spectroscopy, we show that the stabilization of CM's T- state in solution is achieved by key hydrophobic residues in-tandem with a structurally dynamic loop located at 28 Å from the active site and 20 Å from the effector binding site. Our NMR 1H-15N and 1H-13C data suggest that, while in cases where most of the protein's population remains in the T-state, these particular residues exhibit fast conformational exchange between the T and R states representative of a low populated R-state responsible of regulating CM's kinetics activity. These findings agree with recent data collected by our group consistent with flexible T-to-R transitions of methyl side chains at dynamic regions of CM. Our reports reveal a sophisticated allosteric model suggesting low populated states and local conformational T- to-R transitions as linchpins for protein allosteric communication. This detailed interpretation of protein allostery should eventually open opportunities to develop novel allosteric treatments and solutions to target multiple malignancies.

Blockade of CCR3 during fluid resuscitation from hemorrhagic shock

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Background:

Several lines of evidence suggest that chemokine (C-C motif) ligand 2 (CCL2), which activates chemokine (C-C motif) receptor 2 (CCR2), CCR3 and CCR5, contributes to hemodynamic instability during resuscitation from hemorrhagic shock (HS). We recently showed that blockade of the major CCL2 receptor CCR2 reduces fluid requirements and protects from hemodynamic decompensation during resuscitation from HS, whereas blockade of CCR5 was ineffective [1]. The effects of blockade of CCR3 after HS and fluid resuscitation (HS/R), however, are unknown. Thus, the aim of this study was to test whether the CCR3 antagonist SB328437 modulates fluid requirements and hemodynamic stability in Wiggers models of HS/R.

Methods:

Anesthetized male Sprague-Dawley rats were instrumented with arterial catheters. In series 1, rats were hemorrhaged to a mean arterial blood pressure (MAP) of 30 mmHg for 30 min. At t=30 min, animals were injected with 1 mL of vehicle (LR, n=4), 0.25 □mol/kg (n=3) or 1.1 □mol/kg (n=3) SB328437, followed by fluid resuscitation with Lactated Ringers (LR) solution to a MAP ≥60 mmHg or systolic blood pressure (SBP) ≥90 mmHg until t=90 min. In series 2, animals were hemorrhaged to a MAP of 30 mmHg for 60 min. At t=60 min, rats were injected with vehicle (n=6) or 1.1 □mol/kg SB328437 (n=6), followed by fluid resuscitation with LR to a MAP ≥60 mmHg or SBP ≥90 mmHg until t=300 min. Hemodynamics and fluid requirements were monitored continuously. Lactate, hematocrit and blood gases were measured in 30-60 min intervals. Data analyses: 2-way ANOVA/Bonferroni post-hoc. A 2-tailed $p<0.05$ was considered significant. Data are mean ± standard error.

Results:

Series 1: Groups were comparable at baseline; hemorrhage volumes to achieve target MAP were indistinguishable (Fig. 1A). All animals could be resuscitated to the target MAP (Fig. 1B). While treatment with 0.25 □mol/kg SB328437 did not affect resuscitation fluid requirements, treatment with 1.1 □mol/kg SB328437 reduced fluid requirements by 63%, as compared with vehicle treated animals (Fig. 1C). Lactate concentrations and blood gases were comparable among groups (not shown).

Series 2: Groups were comparable at baseline; hemorrhage volumes to achieve target MAP were indistinguishable (Fig. 2A). Subsequent MAP values during resuscitation were comparable (Fig. 2B). There were no significant differences in fluid requirements during the resuscitation period between the groups (Fig. 2C). All animals demonstrated a steep increase in fluid requirements after t=240 min, indicating hemodynamic decompensation. Lactate concentrations and blood gases were comparable among groups (not shown).

Conclusions:

CCR3 likely contributes to CCL2-mediated effects on blood pressure regulation after HS. Blockade of CCR3 reduces fluid requirements after 30 min of HS but does not reduce fluid requirements or prevent hemodynamic decompensation after prolonged periods of HS.

Inhibitory and excitatory responses to feedforward auditory inputs in the mouse posterior parietal cortex

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When sensory signals enter the brain, they are selectively processed by a highly organized hierarchy of regions in the cerebral cortex. In this cortical hierarchy, primary sensory areas serve as the entry point for sensory signals, transmitting information to higher-level association regions of the cortex via feedforward (FF) projections. Behavioral states flexibly modulate responses to incoming sensory inputs in primary sensory areas and in the posterior parietal cortex (PPC) (McGinley et. al. 2015; Pho et. al. 2018). Additionally, inhibitory interneurons have been proposed to be a key component in flexibly gating signals between brain areas due to their unique connectivity patterns and neuromodulator receptor expression (Tremblay et. Al. 2016). Here we characterize the light-evoked responses of PPC inhibitory and excitatory neurons to stimulation of incoming auditory FF projections in vivo. We use two-photon calcium imaging to measure fluorescent changes in activity of neurons in PPC and ChrimsonR, an excitatory optogenetic opsin, to stimulate incoming FF auditory axons in mice voluntarily running on a spherical treadmill. We calculate the light-evoked responses, the modulation of locomotion, and characterize the response dynamics of distinct cell-types. Preliminary results show variable light-evoked responses to stimulating the incoming FF signal across PPC neurons and across trials. These results highlight the dynamic processing of PPC neurons to incoming FF sensory signals.

Advancing Measurement of Clinical Supervision to Support the Implementation of Evidence-Based Practice

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Background: Clinical supervision is an attractive entry point for improving evidence-based practice (EBP) implementation in behavioral healthcare because it is nearly universally available in routine care settings and studies show that when supervisors use specific, evidence-based supervision strategies, therapists' EBP fidelity improves. Currently, very few measures assess clinical supervisors' use of evidence-based supervision strategies and those that are available have mixed psychometric strength and are not pragmatic. This study developed the Evidence-Based Clinical Supervision Strategies scale (EBCSS)—a pragmatic, theoretically grounded measure of two supervision strategies that are most strongly linked to improved fidelity across EBPs: supervisors' provision of data-informed feedback, and supervisors' use of active learning techniques (i.e., modeling and behavioral rehearsal).

Methods: The reliability and validity (factor, convergent, discriminant) of scores on the EBCSS were assessed at baseline in a randomized controlled trial of measurement-based care implementation. The sample included 155 therapists in 21 outpatient behavioral health clinics across 3 states. Confirmatory factor analyses tested the EBCSS's factor structure. Mixed effects analyses tested associations between scores on the EBCSS and supervisor availability, organizational climate for EBP implementation, and general dimensions of organizational climate. Item difficulty was assessed via item response theory analyses.

Findings: The hypothesized factor structure was confirmed ($\chi 2=7.70$, df=4, p=.103, CFI=.993, RMSEA=.077, average standardized factor loading = .80). Coefficient alpha was acceptable for both subscales (feedback, $\alpha=.73$; active teaching, $\alpha=.76$), which were correlated r=.60, p<.001. Robust associations between scores on the EBCSS and climate for EBP implementation (p<.05) provided evidence of convergent validity. Small associations with supervisors' general availability (p<.05) and smaller, non-significant associations with general dimensions of organizational climate (p > .05) provided evidence of discriminant validity. Scores on the EBCSS also varied in expected directions with therapist and supervision characteristics. IRT analyses indicated the most difficult feedback item was provision of feedback based on supervisor observation of sessions.

Implications: The EBCSS is a promising measure of behavioral health supervisors' adherence to two essential ingredients of effective clinical supervision for improved EBP fidelity. High quality measures of evidence-based clinical supervision have widespread utility for advancing the field.

Examining Socio-cultural barriers to mHealth tools

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Background/Purpose:

There is a lack of adequate health care, poor quality of services, and a high burden from unmet mental health needs among racial/ethnic minorities in the United States. Mobile health (mHealth) technology shows promise as a tool to reduce disparities among patients by overcoming barriers that reduce treatment accessibility, utilization, engagement, and effectiveness. Despite the tremendous opportunity to use digital strategies to address health disparities, progress has been slow. The purpose of this study is to examine:

RQ1: What are current socio-cultural barriers to traditional health care access?

RQ2: How would digital health tools reduce or amplify those barriers?

RQ3: What are people's attitudes/perceptions towards digital health tools?

Methods:

48 individuals from diverse, low-income families (annual income ≤ 33rd % for Miami- Dade County and adjusted for household size) were recruited from a more extensive study examining parent-child relationships using mobile tools. Consistent with the demographic of Miami-Dade, a U.S. "majority-minority" region, 52.1% of participants were racial or ethnic minorities, and 47.9% identified as Latinx for ethnicity. For race, 60.4% identified as White/European American, 35.4% as Black/African American, 2.1% as Asian/ Asian American, and 3% as other.

We utilize an exploratory sequential mixed methods approach to examine patient-centered outcomes in the field of digital health. Quantitative measures, which will address RQ1 and RQ3, assess general health management habits (44 items; Health Literacy Questionnaire), general technology use, (20 items; Technological Ease and Computer-based Habits Inventory), perceived skills at using technology for health (8 items; eHealth), and mobile device privacy and security concerns (3 items; Mobile Privacy & Security Concerns). Qualitative assessment, which includes a 45-minute structured interview, aims to address RQ 1-3 and will elicit attitudes and perspectives on participants' past experiences of mental health access and service in a traditional setting, where they get their resources on parenting and health, and how they currently feel towards digital tools in improving the health for themselves and their family. The structured interview questions are modified from the Cultural Formulation Interview, a tool intended to help health professionals gather and organize culturally relevant clinical information.

Planned Data Analyses:

Results from the questionnaire will be summarized to provide descriptive data of diverse families' attitudes toward mHealth. For the interview, we will utilize grounded theory, a coding,

consensus, and comparison approach to code and analyze cultural and clinical processes arising in the interview.

A subset of the transcripts will be reviewed to develop an initial coding taxonomy to capture these themes. Transcripts will be independently coded by a team of three trained research assistants and reviewed by master coders to ensure reliability using NVivo (Version 11). Qualitative thematic analysis via NVivo will be used to elaborate upon differences observed in code occurrence

Discussion:

Patients are the experts on their health; to prevent current health disparities from further turning into a digital health problem (or 'digital divide'), health care systems and professionals must find ways to engage patients more effectively, rather than the other way around. The rich descriptive and qualitative data from this mixed methods approach will provide critical preliminary information on how to optimize digital technologies to best benefit those needing it the most.

Investigating sex-differences in the epigenetic regulation of nuclear protein degradation in the amygdala

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Females are 3 times more likely than males to develop post-traumatic stress disorder (PTSD), despite females not reporting more traumatic experiences, implicating sex as a variable for PTSD development. The amygdala is the emotional control center of the brain and regulates fear memory formation. Within the amygdala, different molecular mechanisms, including protein degradation by the ubiquitin proteasome system (UPS) and altered DNA methylation, are necessary for fear memory formation. We previously found that K48 polyubiquitination, a major protein degradation mark of the UPS, is increased following learning and is necessary for fear memory formation in both sexes. Interestingly, 9-week-old, young adult female rats had higher levels of K48 polyubiquitination in the amygdala compared to males of the same age. The higher levels of K48 polyubiquitination in females were associated with increased DNA methylation of the promoter region of the major ubiquitin coding gene, Uba52. Together, these data indicate a possible mechanism for female predisposition to PTSD. In this study, we examined K48 polyubiquitination and DNA methylation of the Uba52 promoter in the amygdala of pre-pubescent (4-week-old) and post-pubescent (9-week-old) male and female rats to determine if the sex differences we previously observed are inherited or developmentally regulated. We observed increased K48 polyubiquitination levels and DNA methylation at the Uba52 promoter in females compared to males at 9 weeks, but not 4 weeks, of age. We also observed changes in DNA methylation at the promoter region of Uba52 between 4-week-old and 9-week-old animals of the same sex. Lastly, we found increased Uba52 expression levels in 9-week-old compared to 4-week-old females. Together, these data demonstrate sex differences in K48 polyubiquitination levels are developmentally regulated and may be controlled by changes in DNA methylation at Uba52.

Patient derived SYNGAP1 mutations disrupt excitatory networks in human models of neurodevelopmental disease

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The synaptic Ras/Rap GTPase-activating protein, SYNGAP1, is one of the most prevalent genes associated with developmental delay and intellectual disability. Consequently, mutations disrupting SYNGAP1 function are now defined as causative for SYNGAP1-related intellectual disability (SYNGAP1-ID). Like many other proteins associated with developmental delay, SYNGAP1 is highly enriched at the post-synaptic density (PSD) of mature glutamatergic synapses and has been established as a major regulator of synaptic function. Recent advances in stem cell biology and gene editing have allowed us to start investigating the role of SYNGAP1 mutations found in patients, and their role in synaptic dysfunction at early stages of synaptic and neuronal development. Here, we will characterize the role of SYNGAP1 mutations found in patients with ID, in excitatory synaptic signaling networks using human models of neurodevelopmental disease. For this reason we will use a combination of CRISPR/Cas9 gene editing technology, patients-derived iPSC neurons, multielectrode arrays (MEAs), Mass spectrometry and spine morphology analysis. We will show the impact of different mutations in SYNGAP1 on synaptic function and how these mutations disregulates synaptic signaling networks in immature synapses from excitatory neurons and their role in neurodevelopmental disease.

Using Race-Conscious Framework to Explore Pathways Structural Racism May Impact the Effects of Racially Diverse Mental Health Workforce: A Critical Review with Case Example

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Objective: To examine how structural racism my operate to undermine the efforts and effects of a diverse mental health workforce and provide a race-conscious framework to tackle the challenges.

Method: This study critically reviewed studies underscoring racialized structures within an organization that may systematically activate and uphold white racial worldview, over nonwhites. Theories of racialized organizations and a race-conscious framework were used for the literature reviews. Case analyses of qualitative interview data obtained from nine Black community mental health employees, who had stayed at their organization for at least 7 years, were also conducted to illustrate case examples.

Findings: Structural racism may sustain institutionalized white dominance through leadership and organizational processes and practices coded in race-neutral lenses to limit the effects of racially diverse mental health workforce.

Impact: This study sheds new insights into how structural racism may undermine workforce diversity and ways mental health organizations can retain and maximize the effects of diverse workforce to address racial disparities.

Type I interferon signaling drives microglial phagocytosis of whole neurons during postnatal development

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Microglia, the resident immune cells of the brain, monitor the central nervous system under physiologic conditions and respond to local changes in the neural environment. Microglia perform multiple roles in the developing brain, including neuronal engulfment and synapse pruning. However, whether distinct molecular profiles are associated with these different functions was unknown. We recently identified a type I interferon (IFN-I)-responsive microglial population in the developing cortex that was specifically associated with the engulfment of whole neurons. Type I interferon (IFN-I) signaling is canonically described as a viral response pathway but its role in physiologic brain function remains to be elucidated. Previous work in our lab showed that mice deficient for Ifnar1 accumulate neurons with double-stranded DNA (ds-DNA) breaks in their nucleus, a marker of neuronal stress, suggesting that this pathway is required to eliminate stressed neurons. I hypothesized that IFN-I signaling would be sufficient to induce an IFN-I responsive microglial state and restrict the accumulation of stressed (neurons. To test this, I performed injections of interferon-β into the brains of neonatal mice. I observed an increase in IFN-I responsive microglia (IFITM3+) and a decrease in cortical neurons with ds-DNA breaks. Taken together, my data suggests that interferon-β can promote a microglial state associated with the engulfment of neurons and restricts the accumulation of DNA-damaged neurons in the developing brain.

The role of sex and cell-type specific protein degradation in fear memory formation

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Post-traumatic stress disorder (PTSD) affects nearly 5% of the world's population, however, current treatments have limited efficacy in reversing the symptoms of this disorder. Females are more likely than males to develop PTSD, though the mechanisms controlling this sex-dependent predisposition remain equivocal. Ubiquitin-proteasome mediated protein degradation in the amygdala has been widely implicated in the formation of fear memories that underlie PTSD. However, previous studies on UPS-mediated protein degradation have focused exclusively on males, so little is known about whether similar UPS mechanisms control the formation of fear memories in females. Recently, we found that CRISPR-dCas9 mediated repression of the ubiquitin gene Uba52 and the proteasome gene Psmd14, which reduces the protein degradation process, in the amygdala impaired contextual fear memory in both sexes. These results suggest that both males and females need protein degradation in the amygdala for fear memory formation. Interestingly, in an unbiased proteomic analysis we found that males and females had increased protein degradation in neurons and astrocytes in the amygdala following fear conditioning. This data raises important questions about the cell-type specificity of the protein degradation process during fear memory formation and whether this varies between sexes. Here, using novel cell-type specific CRISPR-dCas9 plasmids, we found that inhibition of protein degradation in astrocytes in the amygdala impairs contextual fear memory in males, but not females. Conversely, inhibition of neuronal protein degradation impaired fear memory in both sexes. Together, these data provide the first evidence that males and females differ in the cell-type specific requirement for protein degradation during fear memory formation.

The Effect of Cultural and Linguistic Concordance on Family Engagement with Child Behavioral Health Services

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Abstract Title: The Effect of Cultural and Linguistic Concordance on Family Engagement with Child Behavioral Health Services

Background: Cultural stigma and limited English proficiency (LEP) are associated with decreased use of behavioral health services (BHS). Conversely, patient-provider ethnic and linguistic concordance has been associated with improved behavioral health outcomes. Community health workers (CHWs) are often employed to support patient engagement. Yet little is known about how cultural and linguistic concordance between CHWs and parents affects stigma and family engagement in BHS.

Objective: To describe how cultural and linguistic concordance between CHWs and families affects engagement in child BHS.

Design/Methods: As part of a larger implementation trial of CHWs serving 3-12 year-old children with behavioral health needs, families are paired with a Vietnamese or Hispanic/Spanish-speaking CHW who helps them connect to BHS. Purposeful sampling is being used to recruit parents in non-concordant or concordant pairs, defined as the parent and CHW speaking the same preferred language and sharing the same ethnicity. Semi-structured qualitative interviews were coded using thematic analysis. Following an explanatory mixed methods model, interview data will be triangulated with data from the child's medical record to evaluate associations with receipt and intensity of services and time to completion of service goals.

Results: We completed 22 interviews (Black/African-American n=9, white n=5, Asian n=6, mixed-race n=2, Hispanic n=9) resulting in 14 concordant and 8 non-concordant pairs. Analysis of transcripts identified four emergent themes: (1) Culturally concordant dyads were more likely to report feeling "understood" or "empathy" by their CHW. Language concordant families felt it was "easier" to speak to a native speaker compared to using an interpreter. Spanish language concordant families, specifically, described feeling more comfortable expressing personal opinions. (2) Non-concordant families reported being able to communicate their questions or concerns regardless of language differences. (3) All parents were open to their child receiving BHS. (4) Stigma, described as fear of judgement from family or community members, or generally preferring not to share information outside of their immediate family, was common regardless of concordant status.

Conclusions: Early findings suggest language and cultural concordance has some positive impacts not reported among non-concordant dyads. However, parents working with non-concordant CHWs did not report barriers related to language and cultural differences. Reports of

stigma were common and played a role in families' comfort disclosing their child's behavioral health diagnosis.

Sex differences in the selection of stress coping strategy as a predictor of post-stress avoidance behavior

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The global prevalence of generalized anxiety and major depression is higher in females than in males. While the experience of stress is ubiquitous, the development of anxiety and depression is associated with maladaptive stress responses. In order to probe the relationship between stress coping, sex, and stress- linked behavioral outcomes, we exposed male and female mice to subchronic variable stress and assessed the correlation between coping during tail suspension stress (TSS) and avoidance behavior. We found a negative correlation between total time mobile during TSS and total time spent in open arms in the elevated plus maze (EPM) for both sexes, $r(12) = -0.60$, $p=0.02$. This suggests that more time spent mobile during TSS is associated with more avoidance in the EPM. We also found significant differences between total immobility in males and females between the first and second day of TSS, suggesting changes in stress coping strategy selection over time. On the second day of TSS, females spent significantly more time mobile than males (Mann- Whitney $U=4$, $n1=5$, $n2=7$, $p=0.03$). While we observed trends in avoidance behavior with males spending more time in closed arms during the EPM, there was no significant difference in total distance traveled, total open arm time, or open arm entries between males and females. These data suggest that differences in coping styles during stress are predictive of avoidance behavior after stress. Analysis of sex- dependent coping behavior dynamics over time is ongoing. We will pursue the specific role of GABAergic cell types in the ventral tegmental area during transitions between mobility states, and their role in post- stress avoidance behavior.

PrEParados: A Multi-Level Social Network Model to Increase PrEP Enrollment by Latino MSM Self-Identified as Gay, Bisexual or Straight in Miami

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Latinx men who have sex with men (LMSM) are not sufficiently accessing PrEP services in Miami-Dade Florida, an epicenter of the US HIV epidemic. This insufficient uptake is alarming because, in 2018, 27% of new HIV cases were in Latinx individuals. Previous works have found that when LMSM hear about PrEP from a friend, there is greater initiation. However, changes due to the SARS-CoV-2 pandemic have impacted socialization. Additionally, these changes in socialization and the ongoing pandemic have impacted anxiety and depressive disorders in US adults. In response to these needs this Diversity Supplement will use innovate social network modeling to: (1) examine the intersection of mental health disorders exacerbated by negative network structures and dynamics (e.g., isolation, loneliness) in LMSM's PrEP initiation; (2) understand how social and health service networks can increase LMSM's enrollment in bundled PrEP and mental health treatment programs. To achieve these goals Ms. Rodriguez will receive training in the etiology of mental health and HIV disparities, knowledge of relevant perspectives, practices, and problems in bundled mental health and PrEP randomized clinical trials, and training in cutting-edge social network analytical skills to fully leverage her ability to understand mechanisms influencing LMSM's social networks and their service networks. This work and training are being completed under the parent grant, PrEParados which is a three-year developmental R01 study that will determine how social networks impact PrEP uptake and adherence among LMSM. PrEParados is a cross-sectional, network study of 520 LMSM friends, formed into networks. Eligibility requires individuals to identify as a cis-gender, Latinx man, over the age of 18, who meet the CDC qualifications for PrEP.

Cortical Organoids as a Model for the Study of Neurodevelopmental Disorders

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Human induced pluripotent stem cells (iPSCs) have been a useful model in the genetic studies of neurodevelopment and neuropsychiatric disease. Cortical organoids derived from iPSCs can be cultured for more than a year, making them a promising tool in the study of neurodevelopmental disorders. However, it remains unclear whether cell-specific gene expression profiles are conserved between human cortical organoids and developing human brains. To address this question, Dr. Daifeng Wang and his research group have developed a machine learning framework, Brain and Organoid Manifold Alignment (BOMA), that aligns publicly available scRNA-seq data to identify conserved and specific developmental trajectories and gene expression profiles between brains and organoids. BOMA has shown similar gene expression profiles among cortical organoids and brains making them a promising tool to understand neurodevelopment and disease mechanisms. In this study, we have used quantitative immunohistology to experimentally validate the expression patterns of top differentially expressed genes (DEGs) between excitatory neurons in the developing human cortex and those in dorsal forebrain organoids identified by BOMA. We have applied cortical organoids as a model to study Fragile X Syndrome (FXS), an X-linked neurodevelopmental disorder caused by epigenetic silencing of the fragile X messenger ribonucleoprotein 1 gene (FMR1) and have found increased cell proliferation in FXS patient derived organoids. Overall, further understanding of the developmental gene expression patterns present in organoids as identified through computational approaches such as BOMA, should enable better use of organoids as models for studying human brain disorders such as FXS.

Development and Usability Testing of a Chatbot to Promote Mental Health Services Use Among Individuals with Eating Disorders Following Screening

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Objective: A significant gap exists between those who need and those who receive care for eating disorders (EDs). Novel solutions are needed to encourage service use and address treatment barriers. This study developed and evaluated the usability of a chatbot designed for pairing with online ED screening. The tool aimed to promote mental health service utilization by improving motivation for treatment and self-efficacy among individuals with EDs.

Method: A chatbot prototype, Alex, was designed using decision trees and theoretically-informed components: psychoeducation, motivational interviewing, personalized recommendations, and repeated administration. Usability testing was conducted over four iterative cycles, with user feedback informing refinements to the next iteration. Post-testing, participants (N=21) completed the System Usability Scale (SUS), the Usefulness, Satisfaction, and Ease of Use Questionnaire (USE), and a semi-structured interview.

Results: Interview feedback detailed chatbot aspects participants enjoyed and aspects necessitating improvement. Feedback converged on four themes: user experience, chatbot qualities, chatbot content, and ease of use. Following refinements, users described Alex as humanlike, supportive, and encouraging. Content was perceived as novel and personally relevant. USE scores across domains were generally above average (~5 out of 7), and SUS scores indicated "good" to "excellent" usability across cycles, with the final iteration receiving the highest average score.

Discussion: Overall, participants generally reflected positively on interactions with Alex, including the initial version. Refinements between cycles further improved user experiences. This study provides preliminary evidence of the feasibility and acceptance of a chatbot designed to promote motivation for and use of services among individuals with EDs.

Cognitive and motivational consequences of paternal snord116 knockout in dopaminergic cells

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Background: Prader Willi Syndrome (PWS) is a rare genetic disorder that is primarily caused by a loss of genes within a critical region on the paternal allele of chromosome 15. Phenotypically, PWS presents with perturbations of growth, motivation, and cognition, at distinct stages of development. Of the paternal alleles deleted on chromosome 15, whole brain knockouts of the paternal snord116 gene cluster in mice results in PWS phenotypes similar to humans. Missing from such analyses are the effects of paternal snord116 deletion localized to specific neuronal populations. This gap prevents us from discovering and developing pharmacotherapeutics that could alleviate specific PWS-associated endo-phenotypes. With dopamine implicated in both cognitive and motivational behavior, we chose to examine paternal deletion of snord116 in dopaminergic cells throughout the brain.

Methods: A CRE-loxP approach was used to determine consequences of deleting the paternal allele of snord116 in dopaminergic cells on growth, cognition and motivation. Experimental animals (CRE) have the paternal allele of snord116 in dopaminergic cells deleted and control animals have it intact. Animals were weighed postnatal days P1-30, and at P46 to measure growth (1). Auditory fear conditioning (AFC) was performed to investigate learning and memory (2). Finally, a progressive ratio schedule of reinforcement (PR) was used to assay motivation (3).

Results: CRE animals did not exhibit growth impairments as measured by body weight. Cognitive changes were seen in CRE animals during recall of extinction training as measured by decreased % freezing to the tone after extinction training after AFC. Motivation was not impacted in CRE animals as measured by breakpoint ratio in the PR test.

Conclusions: Paternal deletion of snord116 in dopaminergic cells resulted in cognitive changes. To determine whether these changes can be interpreted as an improvement or an impairment in learning and memory, additional learning and memory tasks will be performed.

How do Gender Diverse Students Identify their Sexual Orientation?

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Introduction: College student populations are reporting more diversity across several domains including sexual and gender identity. It has been noted that gender diverse individuals (i.e., individuals whose gender identity may not correspond with their sex assigned at birth) disproportionately identify as sexually diverse and furthermore that identifying as both a sexual and gender minority may be associated with some exacerbated health disparities beyond those already documented among either population separately. However, this intersection remains poorly understood. The current study aimed to explore how gender diverse students identified their sexual orientation to inform future intersectionality research.

Methods: 213 gender diverse students (i.e., gender identity other than cisgender) participated in a survey about their gender and sexual orientation identities and related experiences. Participants responded to an open-text response question asking them to list their sexual orientation identities and to describe what each identifier means to them. Identifiers were grouped by sexual orientation identity (e.g., bisexual, queer). Thematic analysis was used to identify themes separately within each sexual orientation identity group.

Results: Almost all gender diverse students identified with at least 1 diverse sexual orientation (98%). Furthermore, gender diverse students identified with smaller/emerging and understudied subgroups of diverse sexual identities (e.g., queer, pansexual, asexual, demisexual). Thematic analysis revealed complex relationships between gender and sexual orientation identity regarding attraction to diverse genders, utilization of sexual orientation terms rooted in a gender binary or not, and navigating both in- and out-group interactions with use of different identifiers.

Conclusions: It is important to consider diverse sexual orientations among gender diverse students. Participants often described the meaning of sexual orientation identifiers in relationship to their own, or other, diverse gender identities; future research should consider these intersections and their implications for measurement of identities as well as outcomes.

The association between interpersonal factors, barriers to mental health help-seeking behaviors, and dysthymia among Black and Latinx youth

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Objective. Dysthymia is a milder yet chronic form of major depression, occurring in 4% of adolescents globally. Its onset in adolescence tends to continue into adulthood, with worse outcomes than depression, due to its chronicity and inconspicuousness. Although the last two years of COVID-19 had contributed to increased rates of depression among adolescents, particularly Black and Latinx youth (BLY), there is limited understanding of the factors associated with chronic depression among these youth. Additionally, barriers to help-seeking for mental health may further heighten the risk for chronic depression among BLY. For BLY, interpersonal factors may be a salient risk factor for chronic depression. Belongingness has been established as a protective factor for depression, and perceived burdensomeness has been associated with depression among adults. Still, there is limited understanding of the role of these factors in dysthymia among BLY, and even less is known about how these factors may impact barriers to help-seeking. This study aimed to examine the association between dysthymia, thwarted belongingness, perceived burdensomeness, and barriers to mental health help-seeking among BLY.

Methods. This was a cross-sectional and exploratory study (N=61 BLY, ages 13-17). It drew from structured baseline assessments conducted between 2021 and 2022 in New York City as part of an ongoing larger NIMH intervention for depressed Black youth and a sub-study with non-depressed BLY. Adolescents were recruited from schools and youth community centers and completed measures of depressive symptoms, interpersonal needs, and barriers to mental health help-seeking behaviors. Descriptive, correlational, and regression analyses were conducted.

Results. The majority of adolescents self-identified as male (72.1%), straight (85.2%), and Latinx (72.1%). The mean age was 15.1 (SD=1.3), and most were born in the U.S. (82%). Almost half of the youth (44.3%) reported symptoms consistent with dysthymia on the PHQ-9. Correlational data indicated that thwarted belongingness was positively associated with perceived burdensomeness ($r(59) = .32$, $p < .05$). Barriers to help-seeking were associated with identifying as male ($r(59) = -.26$, $p < .05$) and were positively and moderately associated with thwarted belongingness ($r(59) = .30$, $p < .05$). Perceptions of low usefulness of therapy were positively associated with high levels of thwarted belongingness ($r(59) = .50$, $p < .01$), greater perceived burdensomeness ($r(59) = .41$, $p < .01$), and barriers to help-seeking ($r(59) = .51$, $p < .01$). Regression analyses indicated that the presence of perceived burdensomeness increased the odds of having dysthymia among BLY (OR= 8.5, p=.013) after adjusting for thwarted belongingness, barriers to help-seeking, sex assigned at birth, and age. Compared to female-identified BLY, male-identified BLY had decreased odds of dysthymia (OR=.053, p=.002).

Conclusion. Interpersonal factors, particularly perceived burdensomeness, may play a role in the onset or maintenance of dysthymia and increased barriers to mental health help-seeking among BLY and should be considered in treating chronic depression. Decreasing perceived burdensomeness, particularly among Black and Latinx female adolescents, and encouraging Black and Latinx male adolescents to seek mental health services may be important strategies to reduce dysthymia.

Data-Driven Analysis of the Intergenerational Effects of Maternal ACEs on Offspring Subcortical Neurodevelopment

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Prenatal exposure to maternal ACEs is a risk factor for a variety of physical and psychological offspring health issues. However, the biological mechanisms underlying this relationship are not well understood. While previous research reports localized effects of maternal ACEs on offspring brain connectivity, studies examining the impact of maternal ACEs on offspring brain morphometry document an association between maternal ACEs and reduced grey matter volume across the whole brain. However, it is unclear whether these latter results are driven by distinct clusters of brain regions susceptible to maternal ACE effects or by genuine whole-brain volume reductions. Here, we use non-negative matrix factorization to produce sparse, interpretable, low-dimensional representations of infant subcortical grey matter volume data derived from the infant FreeSurfer pipeline. Using these low dimensional data representations, we carry out a data-driven analysis of the effects of maternal ACEs on clusters of covarying subcortical brain regions. In this way, we can test for ACE-driven changes in grey matter volume across the infant brain, without losing significant amounts of statistical power. While none of the resulting statistical tests are significant, we find some preliminary evidence that maternal ACEs are associated with volume reductions in a cluster of brain regions containing the putamen and palladium ($\beta = = -0.5354$, $p = .095$, 97.5% CI = [-1.166, 0.095])). Future work will repeat this analysis using whole-brain segmentations derived from the developmental human connectome pipeline.

The effect of low intensity pulsed ultrasound on peripheral nerve regeneration

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Peripheral nerve injuries are common conditions that can arise from trauma (e.g., compression, severance) and can lead to neuropathic pain as well as motor and sensory deficits. Although much knowledge exists on the mechanisms of injury and nerve regeneration, treatments that ensure functional recovery following peripheral nerve injury are limited. Schwann cells, the supporting glial cells in peripheral nerves, orchestrate the regenerative response following nerve injury, by converting to a "repair" phenotype. However, nerve regeneration is often suboptimal in humans as the repair Schwann cells do not sustain their repair phenotype long enough to support the prolonged regeneration times required for successful nerve regrowth. Thus, numerous strategies are currently focused on promoting and extending the Schwann cells repair phenotype.

Low intensity pulsed ultrasound (LIPUS) is a non-thermogenic and non-destructive continuous wave with medium frequency ultrasound, which has been shown to facilitate peripheral nerve regeneration. Yet it is still unknown how LIPUS affects the peripheral nerve. We applied LIPUS to the peripheral nerve for 20 days after injury and observe the effect on peripheral nerve regeneration. Here we show that LIPUS for 20 consecutive days post-injury (dpi) (300mW/cm2, 20% pulsed at 1 MHz for 5 minutes per day) can potentiate remyelination and sustain long-term regenerative effects at full recovery. We now hope to identify the cellular and molecular

mechanisms activated in response to LIPUS in repairing Schwann cells and to draw support and attention to LIPUS as a compelling regenerative treatment for peripheral nerve injury in humans.

Intraspinal Microstimulation Intended for Motor Rehabilitation Modulates Spinal Nociceptive Transmission

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Spinal cord injury (SCI) leads to radical and detrimental physiological changes at and below the level of the lesion. For example, SCI often results in dysregulation of spinal reflexes, impaired bowel and bladder function, respiratory distress, reduction of voluntary motor output, and neuropathic pain. Spinal stimulation-based therapies are a promising approach to treating each of these issues. However, spinal stimulation can also cause off-target effects.

Spinal stimulation for motor rehabilitation relies on the recruitment of sensory afferent pathways and interneuron networks that provide excitatory synaptic drive to spinal motor pools. Some of these pathways are also implicated in the development and persistence of SCI-related neuropathic pain. Yet, spinal stimulation-based therapies intended to restore motor function after SCI often fail to consider these potential off-target effects.

Here, we used spike train analyses to characterize the effects of intraspinal microstimulation (ISMS) intended to enhance voluntary motor output on spinal nociceptive neural transmission. All experiments were approved by the Institutional Animal Care and Usage Committees of Florida International University and Washington University in St. Louis and were conducted in adult male Sprague-Dawley rats under urethane anesthesia (n=14). After T13-L2 laminectomy, microelectrode arrays were implanted at the L5 dorsal root entry zone. Electrode locations for spinal stimulation targeted motor pools of the ventral horn, whereas their locations for quantifying neural transmission in sensory pathways spanned the superficial and deep dorsal horns. Prior to, during, and after ventral ISMS (vISMS), we mechanically stimulated the L5 dermatome by applying controlled forces ranging from non-painful to painful. Our primary outcome measure was change in maximum firing rate [Hz] of nociceptive specific (NS) and wide dynamic range (WDR) neurons during nociceptive transmission. These changes were quantified by comparing the average maximum instantaneous firing rates prior to, during, and after 2, 10, or 30min of vISMS.

We found that even short periods of vISMS (≤10min) modulated neural transmission in nociceptive pathways of the dorsal horn. Indeed, ~58% of NS (n=94) and ~60% of WDR (n=349) neurons reduced their maximum firing rate during induced nociceptive transmission after vISMS compared to before vISMS. And surprisingly, in neurons that were depressed by vISMS, the magnitude of depression continued to increase after vISMS was discontinued. For example, NS neurons exhibited a 40.86% decrease in firing rate after vISMS (relative to baseline) compared to a 26.76% decrease during vISMS.

Our results demonstrate that vISMS for motor rehabilitation simultaneously modulates transmission in spinal nociceptive pathways, with off-target effects persisting beyond the

duration of vISMS. Although future work is required to elucidate the mechanisms underlying these neuromodulatory actions, our results suggest that it may be possible to optimize the stimulation paradigm to deliver multi-modal therapeutic benefits.

Resolving the molecular basis for the translocation of a fungal pathogen from blood to brain

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Cryptococcus neoformans (Cn) is an encapsulated yeast whose spores are commonly found in our environment. Once spores are inhaled, life-threatening infections can develop. Infections begin in the lung, but the neurotropism of Cn promotes dissemination to the central nervous system (CNS) resulting in cryptococcal meningoencephalitis (CM). Normally, the entry of most pathogens into the CNS is prevented by the blood brain barrier (BBB) – a highly specialized structure that regulates the entry of molecules and preserves homeostasis within the microenvironment of the brain. A network of brain microvascular endothelial cells (BMECs) makes up the BBB and restricts transcellular traffic and paracellular diffusion into the CNS. The association of pericytes and astrocytic end feet with BMECs help to further maintain a functional barrier that protects the brain. Cn is the leading cause of fungal meningoencephalitis in adults and despite its devastating effects, the molecular mechanisms underlying the translocation of Cn from blood to brain are not fully resolved. Studies from our lab have demonstrated that EphA2-RTK (receptor tyrosine kinase) plays a critical role in the translocation of Cn across the BBB. Furthermore, EphA2-RTK has been shown to alter the cytoskeleton when activated. This implies that the transcellular and paracellular movement of Cn may be coregulated through remodeling of the cytoskeleton via EphA2 activity. To determine if the two translocation pathways are co-regulated, I will elucidate the molecular mechanisms mediating transcellular and paracellular translocation of Cn. Due to the size of Cn and the reported ruffled morphology of BMECs during a Cn infection, macropinocytosis is likely the process that Cn uses for transcellular transport, however, this has never been confirmed. Macropinocytosis is primarily an actin driven process and involves the ruffling of the plasma membrane to internalize large amounts of extracellular material. To determine whether Cn uses this endocytic pathway, I will examine Cn crossing of BMECs using an in vitro transwell model of the human BBB, while also monitoring common markers of macropinosomes, performing pharmacological disruption of macropinocytosis and measuring the uptake of dextran molecules in Cn infected cells. Determining if macropinocytosis is a path taken by Cn to translocate from the blood to brain will provide further evidence that the cytoskeleton of BMECs is altered during infection and could therefore alter junction proteins involved in the paracellular movement of Cn. Ultimately, identifying biomarkers/targets of Cn crossing will lead to the development of new therapeutics for CM.

Increased nuclear DNA damage and activation of the DNA damage response in LRRK2 G2019S Parkinson's disease models

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Previous research from our laboratory demonstrated that LRRK2 G2019S-induced mitochondrial DNA (mtDNA) damage is LRRK2 kinase dependent and inhibition restores mtDNA integrity in Parkinson's disease (PD) models. However, how LRRK2 kinase activity modulates genome integrity is currently unknown. We hypothesized that increased LRRK2 kinase activity alters signaling pathways involved in the DNA damage response (DDR), compromising DNA repair and global genome integrity.

We measured nuclear DNA damage and activation of the DDR in CRISPR/Cas9-edited LRRK2 G2019S and wild type HEK293 cells. Additionally, we tested if inhibiting LRRK2 kinase activity (or the kinase activity of DDR master regulators) could reverse DDR markers in vitro. Our data demonstrates an increase in nuclear DNA damage in LRRK2 G2019S mutant cells compared to controls by alkaline comet assay. We also observe activation of the DDR master regulators ATM and DNA-PKcs, and phosphorylation of downstream substrates CHK2 (pT68) and P53 (pS15). Markers of DNA double-stranded breaks, histone H2A.X pSer139 (γH2A.X) and 53BP1 nuclear foci, were also elevated. Preliminary in vivo data supports these findings. In vitro kinase inhibition experiments show a decrease in γH2A.X foci after inhibition of LRRK2, ATM, or DNA-PK kinase activity.

This work provides evidence of nuclear DNA damage accumulation and sustained DDR in LRRK2 G2019S PD in vitro and in vivo models. In addition, our data suggests that this DDR activation is pathogenic, as inhibition of ATM or DNA-PKcs kinase activity reduces the number of γH2A.X foci, a marker of DNA double stranded breaks. We also demonstrate that inhibition of LRRK2 kinase activity decreases γH2A.X foci in a LRRK2 G2019S cellular model, implicating LRRK2 function in nuclear genome maintenance. Experiments in progress aim to further characterize these findings in vivo and in iPSC-derived dopaminergic neurons.

The Effect of an Antioxidant Coating on the Acute Recording Performance of Planar Silicon Intracortical Microelectrode Arrays

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Intracortical Microelectrode Arrays (MEAs) can record neural activity from the brain and are used to develop brain-machine interfaces and to advance understanding of brain circuitry. However, implantation of these devices initiates a neuroinflammatory cascade, potentially resulting in reactive oxygen species that accumulate around the implant site, resulting in neuronal dysfunction. This process may contribute, at least in part, to the failure at the interface because the ability of MEAs to record is related to the proximity of viable neurons around the implant. Our group has developed a sustained antioxidant coating based on the immobilization of mimetic superoxide dismutase – a protective enzyme that degrades harmful superoxide anions into less reactive elements – to improve the longevity of recording MEAs by attempting to modulate the accumulation of reactive oxygen species around the implant. Here, the goal was to investigate the effect of these coatings to the MEA acute recording performance in-vivo. Animal procedures were approved by the University Texas at Dallas IACUC. Sprague Dawley rats were implanted with coated or uncoated (control) MEAs targeting the primary motor cortex (M1) of the brain. Neurophysiological data were recorded weekly for 10 minutes under anesthesia at a sampling rate of 40 kHz for weeks after implantation and then filtered between 300-3000 Hz. Individual spike waveforms were extracted using a -4σ threshold and sorted using K-means. The active electrode yield was then calculated as the proportion of total electrode sites that captured at least one single unit. A two-proportion z-test was used to calculate statistical differences between groups with $p<0.05$ considered as significant.

Coated and uncoated MEAs showed similar proportion of active electrodes upon implantation (approximately ~43%). Both groups experienced an almost two-fold increase in the number of active electrodes after 1 week (approximately ~77%); however, there was no statistically significant difference between groups. Whereas the uncoated MEA group demonstrated a constant loss of active electrodes, the coated group experienced fluctuations in yield of ~10% thereafter. Despite these fluctuations, eight weeks after implantation, there was a statistically significant difference in the proportion of active electrodes between the two groups ($p < 0.05$).These results suggest that coating MEAs with an antioxidant can significantly improve the reliability of these devices to acutely record neural activity. Future studies should evaluate the effect of these coatings on the chronic performance of MEAs.

Mitochondrial Trafficking as a Target for GBM Therapy

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Glioblastoma (WHO Grade IV glioma) is the most aggressive brain cancer. The current standard of care treatment includes surgery, radiation, and chemotherapy. Tumor recurrence is almost inevitable as less than 50% of patients survive more than two years. The low survival rate poses a dire need to develop an effective therapy for GBM patients. GBM cells are resistant to treatment, as they activate their DNA damage response mechanisms to overcome the effects of radiation and temozolomide (TMZ) chemotherapy treatments. Recurrent tumors can arise from slow cycling and self-renewing glioma stem/tumor-initiating cells resistant to radiation and TMZ. No second-line therapy was proven to prolong survival after TMZ failure.

Magmas (Mitochondria-associated protein involved in granulocyte-macrophage colony-stimulating factor signal transduction) is a subunit of the TIM23 complex regulating precursor protein trafficking into the mitochondrial matrix. Magmas is encoded by pam16, known to be upregulated in human pituitary adenomas, prostate cancer, and GBM. Previous studies have demonstrated that Magmas negatively regulates the stimulatory activity of Pam18, which stimulates the ATPase activity of mitochondrial heat shock protein 70 (mtHsp70). No small molecules targeting MAGMAS are in clinical use. We developed a novel small molecule inhibitor (BT9) that has been specifically designed to inhibit Magmas binding to Pam18. BT9 induces apoptosis through cleavage of caspase-3, reduced mitochondrial respiration, and glycolysis. Our recent findings also demonstrate that BT9 treatment reduced protein trafficking of Lon protease into the mitochondrial matrix. Pretreatment of glioma cells with BT9 sensitizes cells to radiation treatment and enhances the TMZ activity. BT9 can cross the blood-brain barrier and improve survival in intracranial glioma PDX models. BT9 has potential therapeutic value by directly dysregulating mitochondrial function in GBM, enhancing radiation and chemotherapy response, and improving survival in a relevant animal model.

Sex-dependent cognitive dysfunction following repeated mild TBI in adolescent animals may be dependent on alterations in acetylcholine and corticotropin releasing factor expression

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Sports-related concussions (SRC, a subset of mild TBI) are a leading cause of long-term cognitive deficits in adolescents. Either moderate TBI or chronic stress in animals lead to alterations in expression of choline acetyltransferase (ChAT) and corticotrophin releasing factor (CRF) within the medial septum (MS), contributing to impairments in hippocampal-dependent memory. Previously, we had reported that repeated mild injuries in adolescent male and female rats resulted in spatial memory deficits at 1-and-4-weeks post-injury in the novel object location task; only male brain-injured animals exhibited significant deficits at 1 week whereas both male and female brain-injured animals showed impairment at 4 weeks. We hypothesized that disrupted cholinergic transmission between the MS and hippocampus may be the mechanistic basis for these deficits, and that CRF expression in the amygdala regulated ChAT expression in the MS. Following behavioral assessment at each time point, rats were sacrificed for quantitative real-time PCR and immunohistochemistry. Our data show that there is a decrease in ChAT immunoreactivity in the MS of male brain-injured animals at 1-and-4-weeks, but fewer ChAT(+) cells were observed in female brain-injured animals only at the 4-week time point. Whereas CRF immunoreactivity within the MS was only increased in female brain-injured animals, and CRF immunoreactivity in the amygdala showed no change in any brain-injured animal, CRF mRNA within the amygdala was increased 1-and-4-weeks post-injury only in male brain-injured animals. These results provide novel sex-dependent associations between ChAT and CRF expression and cognitive impairments post-injury, offering further insight into a potential mechanism of action.

Oncostatin M induces nociceptive signaling and satellite glial activation in human dorsal root ganglia

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Oncostatin M (OSM) is one of the least studied cytokines in the interleukin-6 family especially considering that its expression correlates with hallmarks of chronic itch, rheumatoid arthritis, irritable bowel syndrome, and more recently neuropathic pain. This gap in knowledge is attributed to numerous species differences in the protein structure of OSM, and its receptor usage both of which affect physiological function. Here we uncover some of these discrepancies across mouse, rat, and human models, further underpinning the importance of studying OSM in human context. We characterized the receptors expression profile of OSMR in human dorsal root ganglia (hDRG) from healthy organ donors and confirmed its presence in small-diameter neurons and surrounding glial-like cells via RNAScope in situ hybridization. To investigate OSM-mediated signaling in hDRG, we treated acutely sliced explants with 10ng/ml OSM for 30min and immunoassayed with markers of translation regulation via the Mitogen activated protein kiNase interacting Kinase (MNK) pathway and its downstream target eukaryotic translation Initiation Factor 4E (eIF4E). We noted significant increases in the p-eIF4E intensity signal in small-diameter neurons and glial-like cells suggesting that OSM activates MNK-eIF4E signaling in these cell types. Our findings cumulatively suggest that blocking OSM signaling in hDRG may attenuate nociceptive hyperexcitability and presents a viable therapeutic target for the treatment of pain.

Genipin rescues sensory neuron defects in Familial Dysautonomia

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Familial Dysautonomia (FD) is a devastating genetic disorder that affects the peripheral nervous system (PNS) without an available cure or treatment. It is caused by a mutation in the ELP1 gene leading to developmental and degenerative defects in the sensory and autonomic lineage that are associated with loss of pain perception, heart-rate instability and dysautonomic crisis. Current FD treatments are mainly supportive and do not address the disease mechanism. Thus, there is a need for a model system that allows discovery and testing of novel therapeutics on FD-derived cells. We previously employed human pluripotent stem cell (hPSC) technology to show that peripheral sensory neurons (SNs) are not generated efficiently and degenerate over time in FD. Using this platform, we conducted a chemical screen to identify compounds able to rescue this SN differentiation inefficiency. We discovered genipin, a compound with neurogenic and neuroprotective properties that is prescribed in the form of a Traditional Chinese Medicine (TCM) for neurodegenerative disorders. Genipin is further used to crosslink extracellular matrix (ECM) proteins. We found that genipin rescues neural crest and SN development deficiencies and prevents neurodegeneration observed in FD. Additionally, genipin has activity in vivo, promoting development of SNs in FD mouse models. Finally, we found that these effects are exerted via ECM crosslinking. Our results suggest genipin as a promising drug candidate for FD and potentially for other, common PNS neuropathies.

Developing a Collaborative Community Intervention to Train Chronic Kidney Disease Family Caregivers in providing Decision Support

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Problem: Patients with chronic kidney disease (CKD) often rely on the support of family caregivers to help cope with having a serious illness and make health-related decisions (e.g., renal replacement therapy, self-care, self-management, end of life). CKD patients and caregivers often report being unprepared to make health-related decisions, which has been attributed to a lack of CKD knowledge, not having discussed prognosis, and unclear expectations of treatment, especially before dialysis initiation. Consequences: Patients and caregivers underprepared to make health-related decisions as a dyad may experience poorer coping and heightened distress when facing decisions. Knowledge gap: There is a critical need to train CKD caregivers to effectively partner with their patients in health-related decision making; however, interventions focused on optimizing health-related decision-making in CKD have focused exclusively on the patient and have not included the caregiver, particularly in historically marginalized populations. The purpose of this study was to explore the needs, challenges, and experiences of CKD patients and their caregivers. In addition, an advisory group of CKD patients and caregivers and a second Clinical and Policy Advisory Group provided insights into recruitment protocol, interview guide development and our work continues will collaborate in developing a decision-partnership program. Approach: 15- CKD patients (stages 3 – 5) and 6 CKD caregivers to date have participated in one-on-one, semi-structured, telephone-based interviews. Thematic analysis was used to analyze the interviews and to identify common themes. Preliminary Themes highlight challenges to decision-making, factors that influence decision-making and protective factors to decision making. Conclusion: There is a disconnect with information sharing between diagnosis and end-stage disease , the middle is missing and in the middle is where patients and caregivers make decisions. Future programs must empower dyads with the skills needed to navigate clinical encounters successfully and with confidence.

Race/Ethnic Disparities in Insulin and Sulfonylurea Use in Older Patients with Type 2 Diabetes

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Background

Risk of severe hypoglycemia increases markedly with age in type 2 diabetes (T2D). For many older patients, discussion of safe de-prescribing strategies is indicated to reduce iatrogenic overtreatment. We examined differences in insulin and sulfonylurea (SU) prescription prevalence among older adults (age ≥ 75 years) by race/ethnicity. Because race/ethnic disparities among Black patients have been attributed to doctor-patient communication barriers, we tested the hypothesis that older Black patients were more likely to be prescribed medicines that can induce severe hypoglycemia.

Methods

We studied 18,149 adults (≥75 years) with T2D and a last measured HbA1c ≤ 8.0% between 2019-2021 in Kaiser Permanente Northern California. Electronic health records were used to identify prescription of insulin, SUs, or both. Medication treatment prevalence was stratified by race/ethnicity (Non-Latino White [NLW], Black, Latino, Asian, Other) and compared using chi-square tests.

Results

Mean age was 80.5 (±4.8) years; 54% were women; mean HbA1c was 6.9% (±0.7%), with 42% prescribed insulin, 72% prescribed SUs and 13% prescribed both. Elderly Black and NLW patients had a higher insulin prevalence (both at 45%, p<0.001), while Asians had the lowest (31%, p=<0.001). Conversely, Blacks had the lowest SU prevalence (66%, p<0.001), and Asians had the highest (79%, p<0.001). A similar pattern was observed in patients with the tightest HbA1c control (< 7.0%; n=8,794): Black patients had the highest insulin (39%, p<0.001) and lowest SU prevalence (69% p<0.001), while Asians had the lowest insulin (26%, p<0.001) and highest SU prevalence (81%, p<0.001).

Conclusion

We found significant variation in use of high-risk medicines by race/ethnicity. Older Black patients had higher insulin use while older Asian patients had the highest SU use. Efforts to promote safe de-prescribing practices in older adults may need to be tailored to the unique physiologic and cultural needs of different patient groups.

Stress Accumulation and Sleep among Black Adults Living in the Rural South

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Centuries of systemic racism in the United States have led to Black Americans being disproportionately burdened by multiple life stressors. These stressors occur across multiple ecological levels—individual, interpersonal, and community—and adversely impact health, contributing to health inequities. The current study used longitudinal data from a sample of 692 Black adults (Mage = 38.32) living in the rural Southern United States to examine the effects of three stressors stemming from systemic racism—neighborhood stress, financial strain, and interpersonal racial discrimination—on sleep problems. We examined both the unique effects of each stressor and how they operate in tandem with each other to impact sleep problems. Findings provide support for both univariate and multivariate effects of multisystemic stress on the sleep of Black Americans. Specifically, financial strain and interpersonal racial discrimination were both significantly associated with sleep problems in univariate models. In multivariate models, both of these stressors were independently associated with sleep problems, consistent with an additive model. There was also a significant interaction between stressors, consistent with a multiplicative model, such that having higher levels of both financial strain and interpersonal racial discrimination exacerbated sleep problems. Results from this study demonstrate how multiple stressors rooted in systemic racism can adversely impact the health of Black Americans. Further, these findings foreground the need for more research on factors that promote positive health and well-being in the face of these stressors and for systemic change that reduces the amount of stress experienced by Black Americans throughout the lifespan.

Session D
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Identifying Distinctions and Commonalities between Elder Mistreatment and Late-life Intimate Partner Violence: A Study of ADRD Caregiving Dyads

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With the increase of older adults living with Alzheimer's disease and related dementias (ADRD), the need for caregivers will increase. Conflict and abuse are particular concerns when the care receiver has ADRD, as behavioral problems associated with dementia may arise during caregiving, such as delusions, hallucinations, and aggression. The web of violence describes abuse and trauma as co-occurring in different patterns across the lifespan and points to the strong "interconnections among different forms of interpersonal violence". Based on this framework, those caregivers who have lifespan patterns of abuse may be at greater risk for experiencing or perpetrating abuse once caregiving obligations begin. Living arrangement is associated with occurrence of violence within caregiving dyads with ADRD. Spousal and intimate partner (S/IP) caregiving dyads are most likely to live together, so it is probable that aggression and abusive behaviors are likely to be present in these dyads. This study used descriptive statistics and t-tests to describe and compare S/IP caregivers to other types of caregivers, focusing on those characteristics that are conceptually related to abusive behavior across their lifespans and across their relationship, including experience with adverse childhood experiences (ACEs), relationship quality, relationship closeness, caregiving burden, behavioral problems, and physical and psychological health conditions. ACE score and relationship quality were significantly different between S/IP caregivers and other types of caregivers. More caregivers of non-intimate dyads reported 4 or more ACEs (21.2% vs. 6.82%), psychological intimate partner violence (26.8% vs. 11.1%), and psychological abuse in the past year (17.9% vs. 6.5%). More S/IP caregivers reported physical intimate partner violence (13.3% vs. 11.1%) and physical abuse in the past year (8.7% vs. 1.5%).

Cognitive Reserve Moderates the Association Between Cerebral Blood Flow and Language Performance in Older Adults with Mild Cognitive Impairment

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Background: It has consistently been demonstrated that individuals with higher cognitive reserve (CR) exhibit decreased risk for dementia, and differences in cerebral blood flow (CBF) among those with higher CR may offer a protective mechanism against the cognitive changes associated with dementia. We sought to investigate CR as a moderator of the effect of CBF on memory and language performance in patients with mild cognitive impairment (MCI) and those who are cognitively unimpaired (CU).

Method: Older adults from the Alzheimer's Disease Neuroimaging Initiative (ADNI; N=46 MCI, 101 CU) underwent neuropsychological evaluation and arterial spin labeling MRI, which was used to quantify CBF in a priori regions. Estimated VIQ from the American National Adult Reading Test was used as a CR proxy. Multiple linear regressions assessed for (1) effect of CR on cognition, (2) effect of CR on CBF, and (3) interaction between CR and CBF on cognition. Cognitive outcomes included memory (word list) and language (category fluency, naming). All models adjusted for age, gender, APOE e4 allele frequency, and education, and interactions additionally adjusted for baseline FDG and pulse pressure.

Result: VIQ was not associated with memory but was positively associated with fluency and naming in both groups (ps<0.005). VIQ was not associated with CBF in either group. Individuals with MCI showed a significant interaction between VIQ and CBF on fluency across several regions, including the bilateral hippocampus (p=0.003), bilateral inferior temporal gyrus (p=0.008), superior frontal gyrus (p=0.005), and inferior frontal gyrus (p<0.001). For all models, as CR increased, the magnitude of the association between CBF and performance increased. These interactions were not significant in the CU group. Models with naming or memory as the outcome variable were not significant in either group. Results remained similar when education was not adjusted for.

Conclusion: These results suggest that among individuals with MCI, the association between CBF and category fluency is dependent on CR, even after adjusting for several factors. Specifically, higher CR may serve as a protective factor against the negative effect of CBF reductions on fluency. Further research is needed to clarify the role that CR plays as a moderator of CBF and cognition.

An Epithelial Organoid Approach to Understanding Stemness in The Intestine

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The intestine is a rapidly cycling regenerative tissue fueled by crypt base intestinal stem cells (ISCs) marked by expression of Lgr5. Lgr5+ ISCs are multipotent stem cells which repopulate the intestinal epithelium every three to five days. Paracrine signaling is necessary for maintenance, repair, and self-renewal of Lgr5 ISCs. It is postulated that, upon lineage commitment, Lgr5+ ISCs transit through a so-called transit amplifying (TA) cell state prior to multilineage differentiation. However, the TA cell is not molecularly defined. Here, we utilize in vitro grown intestinal epithelial organoids (IEO) to elucidate signaling pathways important to TA cell biology.

Understanding Barriers to the Digital Collection of Mobile and Wearable Device Data to Monitor Health & Cognition in Older Adults: A Scoping Review

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Background: Continued advances in health-related technologies have made continuous and remote health monitoring possible which can enable early detection and intervention of cognitive and health problems.

Objective: This study conducted an initial scoping review of the literature aimed at understanding barriers to the digital collection of mobile and wearable device data by older adults with and without mild cognitive impairment (MCI) to monitor health and cognition and support adherence.

Methods: The framework for this review was based on the Arksey and O'Malley methodology for scoping reviews. Selected articles were US based articles that were focused on the experienced or perceived barriers to the digital collection of mobile and wearable device data by older adults ages 55 years and older with and without MCI.

Results: 14 studies met the study's inclusion criteria and were published between 2010 and 2021. Six were qualitative studies, three were mixed methods studies, another three were quantitative studies, one was a usability study and one was a round table discussion. Identified themes included barriers related to usability, the users' health experience with technology, first and second level digital divide, aesthetics, comfortability, adherence and attitudinal barriers. Elicited barriers were observed to influence the output quality, ease of use, intention to use, perceived usefulness, result demonstrability and usage behavior of older adults as it relates to the use of mobile and wearable technology data to monitor their health and cognition.

Conclusions: Collecting these data effectively with little or no barriers among older adults can facilitate efforts to improve older adults' health status, quality of life and improve adherence to protocols that can help to predict and prevent cognitive decline through early detection.

The Effect of APOE on Lipid Droplet Dynamics in Microglia

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Background: The microglial immune response is a significant contributor to Alzheimer's disease (AD) pathophysiology and neurodegeneration. Aging microglia accumulate lipid droplets (LDs), have high levels of reactive oxygen species, secrete pro-inflammatory cytokines, and are defective in phagocytosis. The E4 allele of Apolipoprotein E (APOE) is the strongest genetic risk factor for late-onset AD, and is associated with heightened neuroinflammation and increased LD formation. We hypothesize E4 microglia have increased LD formation under basal conditions and a higher capacity to form LDs under stress, resulting in greater pro-inflammatory cytokine production. We characterized LD development in microglia in the context of APOE genotype and analyzed LD surface proteins and lipid content from control and lipopolysaccharide (LPS) stimulated ApoE3 and ApoE4 mice.

Methods: Primary microglia were isolated from mice expressing human ApoE3 and ApoE4. Microglia were exposed to 250uM oleic acid (OA), 10ug/mL LPS, OA+LPS, dead N2A cells, or dead N2As+LPS. ApoE3 and ApoE4 expressing mice were injected with saline (control) or LPS (5mg/kg) and perfused at 24h. Livers were extracted, the LD enriched supernatant fraction was collected after centrifugation, and proteomic and lipidomic analyses were performed.

Results: Primary microglia from ApoE4 mice accumulated more LDs at baseline, with exogenous OA, LPS stimulation, and N2As as a percentage of E3 control across multiple experiments (E3 v E4 p values: baseline, 0.0317; LPS, 0.0032; OA, 0.0277; N2A, 0.0192). Western blots on LD fractions confirm LD enrichment by surface protein, PLIN2, along with increased expression of PLIN2 (i.e. more LDs) in E4 LPS mice. Proteomics reveal LD fractions from E4 mice are enriched for proteins involved in innate immunity, while E3 LDs are enriched for proteins involved in lipid □-oxidation.

Conclusion: E4 microglia accumulate more LDs compared to E3 microglia under all conditions tested. The proteomic profile of E4 liver LDs support the hypothesis that E4 expression increases inflammation under basal conditions, and upon stimulation, causes a more robust response. Increased LD formation is present in non-aged, non-diseased E4 cells, suggesting preclinical dysfunction associated with the highest risk APOE genotype. A better understanding of LD dynamics within these cells and their functional implications can provide targets to improve E4-related outcomes.

The Neighborhood Context and All-Cause Mortality among Older Adults in Puerto Rico

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Objective: The aim of this study was to identify neighborhood characteristics that have multi-level influences on all-cause mortality among older Puerto Rican adults. Two measures of the neighborhood context are considered due to varying patterns of social (dis)advantage and methodological considerations.

Methods: Data from the 2000 US Census were linked to the longitudinal Puerto Rican Elderly Health Conditions Project (PREHCO) to examine the relationship between the neighborhood context and all-cause mortality from 2002 to 2021 using a multilevel mixed-effects parametric survival model with a Weibull distribution.

Results: Older adults residing in neighborhoods classified as "Urban High Deprivation" and in neighborhoods with a "low socioeconomic position" in Puerto Rico were at higher risk of death over the 19-year study period.

Conclusions: Results show that there are differences in material and social living conditions between places that increase the risk of mortality and must prioritize structural determinants of these disparities. Moreover, this study offers an opportunity to generate future hypotheses in observational and clinical research, identify priorities and targets for public policy and screening, and promote preventive care.

Short-term high-fat diet consumption impairs long-term potentiation in the aged hippocampus

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More Americans are consuming diets higher in saturated fats and refined sugars than ever before. These trends could have serious consequences for the older population because high-fat diet (HFD) consumption, known to induce neuroinflammation, has been shown to aggravate and accelerate memory declines. Because obesity is a complex disease with many comorbidities, making the study of underlying mechanisms difficult and confounded, we employ a short-term diet manipulation protocol. We have previously demonstrated that short-term consumption (3 days) of a HFD among aged rats produced profound impairments to contextual and emotional/fear memories, which depend on an intact hippocampus and amygdala. These impairments were precipitated by increases in proinflammatory cytokines, primarily interleukin-1 beta (IL-1B), in both brain regions. Here, we explore the extent to which HFD consumption amongst aged rats disrupts hippocampal long-term potentiation (LTP), the form of synaptic plasticity thought to underlie long-term memory consolidation in mammals. Furthermore, we explore whether these effects could be reversed with an IL-1 receptor antagonist (IL-1ra). Young adult (3 months old) and aged (24 months old) F344xBNF1 rats were assigned to either chow or HFD for 3 days. Using transverse hippocampal slices, we examined the individual and combined effects of age and diet on several forms of synaptic activity. Specifically, excitatory post-synaptic potentials were induced in the stratum radiatum of CA1 and LTP expression was triggered with a theta-burst stimulation protocol. Our preliminary data demonstrate that late-phase LTP was particularly compromised by the combination of aging and HFD while LTP maintenance was robust in chow-fed young and aged rats. Results of IL-1ra treatment on these effects will be presented. These findings suggest that the previously observed neuroinflammation-mediated hippocampal memory impairments in aged HFD-fed rats occurs, at least in part, through deterioration of synaptic plasticity, as measured by LTP, in the hippocampus.

Optimizing Induced Neuron Cultures From Aged Human Fibroblasts for Electrophysiology Studies

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Aging results in a greater risk for neurodegenerative diseases, therefore it is crucial to have a cell culture model that reflects this biological decline. The current induced pluripotent stem cell (iPSC) model provides a powerful method for studying the genetics of neurological disease. However, there are limitations due to loss of epigenetic modifications acquired throughout one's life and therefore they are not suited for disease modeling of healthy aging. In comparison to the iPSC model, the induced neuron (iN) model, where dermal fibroblasts are directly converted into neurons, more accurately reflects aging as it preserves epigenetic modifications. Indeed, the iN model can generate a highly enriched population of glutamatergic neurons, yet preliminary functional studies of iNs suggest that this method generates electrically immature neuronal cultures with limited action potential firing ability. In order to optimize the iN model to study ion channel function during aging, we tested the hypothesis that iNs need to be co-cultured with other cell types in order to form functional networks and support electrogenic maturation. We tested whether co-culturing iNs derived from aged human subjects with human astrocytes and other neuronal culture preparations can improve synaptic transmission and action potential firing. We found that co-culture conditions resulted in a more electrically mature phenotype of neurons, displaying increased action potentials, however it was unclear if these effects were simply due to prolonged survival when neurons were cultured with astrocytes. Continued optimization of the current iN culture protocol will allow further insight into the study of functional changes due to aging.

The Moderating Effect of Race and Mediating Role of Systemic Inflammation on Racial Disparities in Incident Dementia: A Decomposition Analysis

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Background: Exposure to systemic racism is linked to increased dementia burden among racialized minority groups. To assess systemic inflammation as a potential pathway linking exposure to racism and dementia disparities, we investigated the moderating and mediating roles of high sensitivity C-reactive protein (hsCRP), a systemic inflammation marker, on racial disparities in incident dementia.

Methods: In the US Health and Retirement Study (n=5,143), serum hsCRP was measured at baseline (2006, 2008 waves). Incident dementia was classified by cognitive tests over a six-year follow-up. Self-reported racialized categories were a proxy for exposure to racism. We decomposed racial disparities in dementia incidence (non-Hispanic Black vs. non-Hispanic White; Hispanic vs. non-Hispanic White) into: 1) the mediated effect of hsCRP, 2) the moderated portion attributable to the interaction between race/ethnicity and hsCRP, and 3) the controlled direct effect (other pathways through which racism operates).

Results: The 6-year cumulative incidence of dementia was 15.5%. Higher concentrations of hsCRP were observed among non-Hispanic Black (6.39□g/mL) and Hispanic (4.40□g/mL), relative to non-Hispanic White (3.94□g/mL) participants. Among non-Hispanic Black relative to non-Hispanic White participants, the mediating effect of hsCRP on the racial disparity in dementia was 2% (95%CI:-1%,8%), the portion attributable to interaction was 8% (95%CI:-5%,21%), and the controlled direct effect of racism was 92% (95%CI:78%,104%). Analyses for Hispanic relative non-Hispanic White participants showed moderation, but not mediation. Findings were robust to potential violations of causal mediation analysis.

Conclusions: Systemic inflammation is a moderating and mediating factor implicated in the production of racial disparities in incident dementia.

Fall Prevention Through Wearable Robotics

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Falls are the leading cause of fatal and non-fatal injuries in older adults (ages 65+). Injuries due to falling result in 2.8 million emergency visits annually, and 25 % of falls result in very serious injuries (such as fractures or traumatic brain injury). This work proposes to develop a wearable smart-belt device that can alert the wearer to the risk of falling and potentially prevent falls. The first aim of this work is to conduct focus groups with older adults on the wearable smart-belt device, such as style and comfort. The second aim will test the wearable smart-belt device by collecting visual and sensor data from older adults while walking on various surfaces at slow and brisk paces. The data will be used to train the falls prevention model utilizing machine learning methods. The ultimate goal is for the model to be able to calculate fall risk by predicting potential walking trajectories of the wearer and imbalance and alert the wearer via vibrotactile response if the risk of falling is probable. By preventing falls from occurring in the first place, the proposed device has the potential to greatly increase the overall well-being, long-term health, and independence of older adults as well as costs associated with hospital visits and caregiving.

A machine learning-based brain age biomarker more sensitive to chronic pain

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Machine learning (ML)-based predicted brain age have shown to be sensitive to underlying pathologies and mortality risk [1]. Literature has shown significantly larger predicted age differences (PAD - predicted minus chronological age) estimations when using a supervised statistical learning method called Gaussian Process Regression (GPR) in individuals with chronic pain [2,3]. However, this is still the matter of controversy and debate [4].

Given the nature of ML architectures and their differences we hypothesize a variation in the PADs between models and expect the pathologies of pain to have different effects based on morphological changes. Here we use a different ML method, a novel Convolutional Neural Network (CNN [5]) for predicting brain age as a biomarker of chronic pain.

Asymmetric Predominance of Neuronal Atrophy in Primary Progressive Aphasia due to Pick disease

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Background: Primary progressive aphasia (PPA) is a dementia syndrome that is characterized by an isolated and progressive language impairment with atrophy localized to left-hemispheric language regions. Our prior work has shown concordance between the asymmetric (leftward) distribution of pathology in language regions of the brain and the salience of the language disorder in PPA. The objective of this study was to determine whether neuronal atrophy (i.e., neuronal size) shows similar concordance with the aphasic phenotype in PPA.

Methods: Nine right-handed cases with PPA and PiD as the sole pathologic diagnosis were identified from the Northwestern Alzheimer's Disease Research Center Brain Bank. Paraffin-embedded sections of bilateral middle frontal gyrus (MFG) and inferior parietal lobule (IPL) were stained with 1.0% Cresyl Violet to visualize neurons. Digital images of 3 slides per region/hemisphere were obtained at 20x, and cortical layers III and V were annotated using the QuPath digital pathology software (V.0.3.1). Neurons were manually traced and area (μm^2) of each neuron was calculated. Parametric t-tests were used to compare average neuronal size (area) between hemisphere, regions, and cortical layers.

Results: Neuronal atrophy between hemispheres was asymmetric, with both the MFG and IPL demonstrating statistically smaller mean neuronal size (more atrophy) in left regions compared to right ($p < 0.0001$). Bilateral (mean L & R) IPL also showed significantly smaller neurons than those in bilateral MFG ($p < 0.0001$). In left MFG, layer 5 neurons were significantly smaller than layer 3 neurons ($p < 0.01$).

Conclusions: Preliminary findings suggest that asymmetric (leftward) predominance of neuronal atrophy in PPA-PiD, particularly in the IPL, is consistent with the aphasic phenotype characteristic of PPA. The finding of relatively smaller layer V neurons compared to layer III neurons in MFG is worth further investigation as it may provide insight into the mode of disease spread between cortical regions. Future directions will examine the relationship between neuronal shrinkage and other pathologic markers including Pick bodies, microglia, and synaptic integrity.

Chronic Psychosocial Stress Mediated Cerebrovascular Dysfunction In Novel Hepatic Xanthine Oxidase Knockout Mice Model

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Overview: Chronic psychosocial stress is a major risk factor for cognitive impairment. Xanthine oxidase (XO) generates reactive oxygen species (ROS), but it is unclear if XO is involved with chronic stress.

Hypothesis: Chronic stress leads to an upregulation of XO leading to cerebrovascular dysfunction.

Methods: The unpredictable chronic mild stress (UCMS) protocol was performed on mice at 4 months of age for a period of 8 weeks. In study 1, the mice were treated with regular or Febuxostat treated water (50 mg/L) to pharmacologically inhibit XO production. In Study 2, we genetically knockout XO using the novel hepatic liver-XO (HXO) knockout C57BL/6 mouse model. The mice were separated into wild-type and HXO for control and UCMS groups. For both studies, mice were euthanized at 6 months of age and tissues were harvested. The middle cerebral artery (MCA) was isolated to study the vessel function via ex vivo pressure myography.

Results: In study 1, the UCMS group showed a 50% deficit in MCA dilation to Acetylcholine compared to the control group due to reduce nitric oxide dilation, with no differences in SNP dilation indicating the cerebrovascular dysfunction was endothelial dependent. Study 2 concluded that the HXO UCMS group showed an 80-85% restoration of MCA function due to restoration of nitric oxide dilation, again no differences in SNP dilation were noted between groups.

Conclusion: XO plays a role in the stress induced cerebrovascular dysfunction.

Modulation of TIMP2 mediates transcriptomic changes within hippocampus associated with plasticity

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Aging is the strongest risk factor for the development of Alzheimer's disease, of which there is currently no known cure. Recent work exploring age-related cognitive decline identified changes in blood-CNS communication across aging in mice and humans. These studies established age-associated changes in the systemic environment and the potential of young blood to "rejuvenate" the CNS and peripheral tissues. TIMP2, tissue inhibitor of metalloproteinases 2, is a youth-associated blood-borne protein that sufficiently revitalizes the aged brain and restores hippocampal function. It is not yet known, however, the mechanism by which TIMP2 is capable of mediating these phenotypes. Our current results suggest aged mice exposed to young blood or recombinant TIMP2 experience transcriptional changes in the hippocampus while complete knock-out of TIMP2 in mice further induces changes to gene expression and pathway enrichment analyses. Further characterization of local TIMP2 in altering hippocampal function may highlight the role of TIMP2 as a modulator of CNS health during aging or in the context of neurodegenerative disease.

Rural-Urban Disparities in Allostatic Load in the United States

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Allostatic Load (AL) is a measure of cumulative physiological "wear and tear" resulting from adaptation to life demands. In the current study we explored (1) rural-urban disparities in AL, and (2) whether age patterns in AL differed by rural-urban residence among adults (ages 18+) in the United States. We used data from 17,268 respondents to the National Health and Nutrition Examination Survey, 1988-1994 (NHANES III) who participated in the Medical Examination component of the study. To calculate AL, we determined how many out of ten biomarkers exceeded clinically determined thresholds. For the age-pattern evaluation respondents were classified into one of seven age-groups based on their age at time of interview. Weighted means and regression models were used to ascertain whether AL patterns differed by rural-urban residence. Significantly higher AL levels were found among rural than urban populations (mean=2.11 vs mean=1.90). This disparity observed across six of seven age groups. The exception being the oldest (80+) age group. Regression models with statistical controls confirmed a rural disadvantage in AL and across most age groups and no difference (convergence) at older ages. The findings call attention to rural-urban differences in AL and by age, and the role of this difference in shaping persistent rural disparities in health and mortality.

Future research will establish the contextual factors that shape the rural-urban differences described in this study.

Addressing Disparities in ADRD Through More Inclusive Research: A Community Engagement Pilot During COVID-19

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Background

Inclusivity of diverse populations in Alzheimer's Disease and Related Dementias (ADRD) research is crucial to better understanding ADRD ethnoracial disparities. To recruit for inclusive research, relationships and trust must be established between researchers and communities, yet the most efficient methodology to build relationships remains an enigma. Even less is known about this process during the challenges of a pandemic. Here we detail the methodology and results of a pilot to build relationships with the African American community in the San Francisco Bay Area (SFBA) during the COVID-19 pandemic with the goal of more inclusive ADRD research.

Methods

Between 02/3/21 and 02/28/22, the University of California San Francisco Alzheimer's Disease Research Center engaged in a pilot to build relationships with the African American community in the SFBA, with an emphasis on engaging vulnerable neighborhoods. We defined vulnerability by the Minority Health Social Vulnerability Index (SVI) and the Area Deprivation Index (ADI) (Figure 1).

Results

We engaged 22 (17 local, 5 non-local) community-based organizations and sustained relationships with 14. Median ADI of block and SVI of tract where local organizations were located was 3.75 and 0.76, respectively. African Americans represented 0-40.5% of the population in the tracts (Figure 1-2, Table 1). Through 17 collaborative events we engaged 788 community-dwelling individuals, 383/788 (49%) through events with health and healthcare-based organizations. We contacted organizations 117 times outside of events (1.2-1.4 times per organization per month) and time needed for relationship building until we held first collaborative event with an organization varied widely (26-126 days) (Table 1). To assess efficiency of our efforts, we developed a scoring scheme and estimated we were able to engage 1.2-5.5 community dwelling individuals through each engagement effort with a community-based organization (Table 1).

Conclusions

Partnership with community-based organizations is an effective means of engagement with community dwelling individuals to promote ADRD research involvement and can be performed effectively even in a pandemic with limited opportunities for in-person engagement. Partnership with certain types of organizations may yield engagement with larger numbers of community

dwelling individuals but additional studies are needed to further establish the most effective methodology to build the high yield relationships.

A Developmental Perspective on Bilingualism and Musical Expertise

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Cognitive advantages have been associated with bilingualism and musical expertise. These advantages have been noted in performance of measures tapping into different cognitive domains. For example, an enhanced performance in executive function and control of nonverbal spatial tasks in both musicians and bilinguals was noted compared to monolinguals (Bialystok and DePape, 2009). Additive benefits have also been observed in measures of melody discrimination and rhythm synchronization in bilingual musicians in comparison to monolingual musicians (Vaquero et al., 2020). In regards to language measures, musical expertise has been associated with enhanced perception and production of speech sounds from foreign languages (Chobert and Besson, 2013). However, these are cross-sectional studies that were conducted with adult cohorts. Might similar cognitive advantages be observed in comparisons between monolingual and bilingual children with and without music experience?

A study focusing on bilingualism and musical training in childhood found enhanced prosody discrimination using phonetically identical French sentences, a foreign language for the participants, for bilingual and musician participants compared to monolingual and nonmusician controls (Stephanov, Pavlic and Stateva, 2018). Even though this latter study does target developing populations, longitudinal studies of music and bilingualism in developing populations are more scarce. Furthermore, rhythm is a subcomponent of music that also remains largely understudied (Jungblut et al., 2012), even though it is significant to the central organizing structure of music (Thaut, Trimarchi, and Parsons, 2014).

Therefore, in the current study we want to address if there are performance-based differences in language and rhythm abilities between children and adolescents who are bilingual or monolingual, with and without musical training. Participants were recruited for the Pediatric Longitudinal Imaging, Neurocognition, and Genetics (PLING) Study focusing on brain and behavior developmental trajectories, with a subset of participants enrolling in a nested study focusing on music cognition and skills: Studying the Influence Music Practice Has on Neurodevelopment in Youth (SIMPHONY). Data were acquired for four years with one time point per year from 2012 to 2015. Language-related assessments were conducted from the Comprehensive Test of Phonological Processing (CTOPP; i.e., Blending Words, Sound Matching, Elision and Rapid Object Naming) and the Test of Word Reading Efficiency (TOWRE; i.e., Sight Word Efficiency (SWE) and Phonetic Decoding Efficiency (PDE)). Music assessments included the Beat Alignment Test (BAT) and the Gordon Intermediate Measures of Music Audiation (tonal and rhythm). Using linear mixed effects models, we conducted likelihood ratio tests of goodness of fit using a full model that includes fixed effects (bilingualism, musical experience) and a constrained model excluding these variables, with age, maternal/guardian education, and household income as covariates.

Significant effects of linguistic status were observed with higher performance of monolinguals on a measure of morphosyntactic development and working memory (Chi-Square: 5.064 , p = 0.024). No musical training was associated with better performance on measures of rapid object naming (Chi-Square: 6.213, p = 0.013) and musical training was associated with enhanced performance of rhythm discrimination (Chi-Square: 8.742, p = 0.003). Given the current findings, we will consider the effects of socioeconomic factors, proficiency and degree of language use, and effects associated with development for bilingual participants and participants with music experience in light of the results for recalling sentences and rapid object naming. We want to further understand the interplay that language and music processing undertake during development and to find potential evidence of domain-general processing (Fedorenko and Thompson-Schill, 2014) occurring during these earlier life stages.

References

Bialystok E. and DePape, A.M., (2009). Musical Expertise, Bilingualism and Executive Functioning. Journal of Experimental Psychology: Human Perception and Performance, Vol. 35, No. 2, 565-574. DOI: 10.1037/a0012735.

Chobert, J., and Besson, M., (2013). Musical Expertise and Second Language Learning. Brain Sciences, 3(2), 923-940. https://doi.org/10.3390/brainsci3020923.

Fedorenko, E., and Thompson-Schill, S., L., (2014). Reworking the Language Network. Trends in Cognitive Sciences, Vol. 18, No. 3. http://dx.doi.org/10/1016/j.tics.2013.12.006.

Jungblut, M., Huber W., Pustelniak, M., and Schnitker R. (2012). The Impact of Rhythm Complexity on Brain Activation during Simple Singing: An Event-Related fMRI Study. Restorative Neurology and Neuroscience, 30, 39-53. DOI 10.3233/RNN-2011-0619.

Stephanov, A., Pavlic, M., and Stateva, P., (2018). Children's Early Bilingualism and Musical Training Influence Prosodic Discrimination of Sentences in an Unknown Language. The Journal of Acoustical Society in America, Special Issue: Additive Manufacturing and Acoustics, 143 (1),

E1-E7.

Thaut, M., H., Trimarchi, P., D., and Parsons, L., M., (2014). Human Brain Basis of Musical Rhythm Perception: Common & Distinct Neural Substrates for Meter, Tempo, and Pattern. Brain

Sciences, 4, 428-452. doi:10.3390/brainsci4020428.

Vaquero, L., Rousseau P.N., Vozian D., Klien D., et al. (2020). What You Learn and When You Learn It: Impact of Early Bilingual and Music Experience on the Structural Characteristics of

Auditory-Motor Pathways. NeuroImage, 213 (116689), 1-10. https://doi.org/10.016.

Insula to BNST circuit adaptations following restraint stress and alcohol withdrawal associated negative affect

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Mental illnesses such as anxiety and depression impact almost 50 million adults in the United States (US). An estimated 95 million adults in the US meet the criteria for Alcohol use disorder (AUD), which is highly comorbid with anxiety and depression. Stress and negative affective states are both thought to contribute to AUD. Research to elucidate the precise neural changes that occur during negative affective states, and their regulation by alcohol is greatly needed. Our previous work in mouse models has implicated a projection from the insular cortex to the bed nucleus of the stria terminalis (BNST) in active coping during restraint stress, and in the emergence of negative affective behaviors during alcohol abstinence. Here, we assess the impact of restraint stress, as well as chronic drinking forced abstinence (CDFA) on synaptic and excitable properties of insular neurons that control the BNST using C57BL6/J mice. Using whole cell patch clamp electrophysiology, we find a sex-specific increase in inhibitory drive onto mid- and BNST-projecting, but not anterior insula cells in male mice following acute restraint stress. These data suggest that interneurons within the insular cortex may be more active during acute restraint stress. Using the calcium indicator GCaMP virally expressed in either parvalbumin (PV), somatostatin (SST) or vasoactive intestinal peptide (VIP) expressing interneurons and fiber photometry, preliminary data suggests that the activity of PV and SST but not VIP insular interneuron activity is correlated with active struggling bouts during restraint stress. We next investigated Insula to BNST circuitry 24hrs or 2 weeks following CDFA in female mice. We find that BNST-projecting insula cells are transiently more excitable following acute ethanol withdrawal. In future experiments we will be assessing the mechanisms underlying the observed hyperexcitability of this neuronal population. Furthering knowledge on how acute and chronic stress reorganizes neural circuitry will provide insight into how negative affective states may lead to relapse.

Cocaine induced neurophysiological alterations to reward predictive cues following outcome devaluation in the infralimbic and dorsolateral striatum

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The ability to alter behavior in response to changes in consequences is necessary for navigating an ever-changing environment. Substance use disorders (SUDs) are characterized by a continuation of maladaptive behavior despite negative consequences. Thus, characterizing the underlying processes that modulate the ability to change or stop behavior in response to updated expected outcomes is critical for understanding the neurobiological alterations in SUDs. We investigated how cocaine withdrawal leads to aberrant, differential patterns of neural activity in subregions of the rat frontal cortex (infralimbic, IL) and striatum (dorsal lateral striatum, DLS) to reward predictive cues and how this is linked to impaired ability to suppress behavior towards a reward predictive cue after updates in expected outcome value. Mildly water deprived Long-Evans rats were split into two groups. Cocaine rats underwent self-administration for cocaine (i.v., 1 mg/kg/press) and control rats underwent self-administration for saline (i.v.) and water (0.2 ml into a receptacle). The self-administration protocol was daily 2-hour sessions for 14 days. After 3 weeks of abstinence, rats underwent Pavlovian conditioning for 10 days. They were presented with two distinct cue light patterns as conditioned stimuli (CS+), each predicting a different reward. One CS+ predicted a sugar pellet and the other CS+ predicted a food pellet (10 trials each). Two other light patterns did not predict a reward (CS-). After 10 days of conditioning, rats underwent a devaluation procedure for the sugar pellets to induce a conditioned taste aversion (LiCl, i.p., 0.3M; 7.5 ml/kg). After devaluation, rats were tested on the same Pavlovian task to evaluate their ability to avoid the devalued outcome and associated CS+ in the absence of the rewards (under extinction). IL and DLS electrophysiological recordings showed neuronal populations that were phasic to the CS+ [excited, EXC; or inhibited, INH] or nonphasic (no response to the CS+). Rats in the cocaine group showed higher % of excited phasic neurons compared the control group in the DLS in response to the devalued CS+ (control: 14% of total DLS neurons; cocaine: 37% of total DLS neurons) and nondevalued CS+ (control: 14% of total DLS neurons; cocaine: 26% of total DLS neurons). We also observed a similar pattern in the IL in that the cocaine group showed higher % of excited phasic neurons compared to the control group in response to the devalued CS+ (control: 3% of total IL neurons; cocaine: 18% of total IL neurons) and nondevalued CS+ (control: 7% of total IL neurons; cocaine: 18% of total IL neurons). Thus, we show elevated neural activity in the IL and DLS after a history cocaine to reward predictive cues which may contribute to the deficits observed in the ability of rats to shift behavior. Future studies will determine whether anxiety-like behavior during early withdrawal predicts behavioral or neural deficits in rats with a history of cocaine.

The Association Between Perceived Discrimination and Suicidal Thoughts and Behaviors in ABCD: Does Race and Ethnicity Moderate Findings?

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Objective: Describe the moderating effects of race and ethnicity on the relationship between discrimination and suicidal thoughts and behaviors (STBs) in youth aged 9-13 in the Adolescent Brain Cognitive Development℠ Study (ABCD Study®).

Background: Significant ethno-racial differences of STBs exist among youth. However, mechanism research characterizing the relationship between discrimination experiences and STBs is less understood. This study examined discrimination¬ (i.e., race, national origin, sexual orientation, weight) and STBs (i.e., suicidal ideation, suicidal attempt) associations using the Cultural Phenomenological Stress Diatheses framework.

Methods: Youth/caregivers enrolled in the ABCD study completed questionnaires at Years one (ages9-12) and two (ages10-13) follow-up time points assessing demographics, discrimination experiences within the past 12 months, and lifetime STBs. The longitudinal data were analyzed with logistic regression, implemented with generalized estimating equations and moderation models, with multiple imputations for missing data.

Results: The sample consisted of N=11,225 youths and their caregivers (52.3% male; 65.3% White. Positive endorsement of race-based discrimination was associated with a 1.34 increase in odds of reporting STBs across all ethno-racial groups (95%CI 1.06-1.68, p=.013). Youth who experienced discrimination due to sexual orientation were ~4 times as likely to report STBs (95%CI 3.56-5.53/3.44-5.08, p=<.001), with Black and Hispanic/Latinx youth having greater odds of sexual orientation-based discrimination than White youth. Youth who experienced weight discrimination were 2.70 (2.26-3.23, p=<.001) times as likely to report STBs.

Conclusion: Youth who experienced race, weight, and sexual orientation discrimination are more likely to report STBs, and ethno-racial minoritized youth are more vulnerable than their White counterparts to the effects of discrimination. Future prospective research in the ABCD cohort should probe culturally relevant associations between discrimination and STBs.

The Role of Family Functioning and Race/Ethnicity on the Efficacy of an Opioid Misuse Prevention Videogame Intervention for Adolescents

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Background: Family functioning factors such as parent-child communication, parent-child conflict may operate as risk or protective factors in the development of opioid misuse suggesting that these factors may influence how adolescents respond to prevention interventions designed to address risk for substance misuse. In addition, risk and protective factors for the development and maintenance of opioid misuse differ by racial/ethnic groups. However, no prior studies have investigated the role that family functioning and race/ethnicity may play in how adolescents respond to a digital intervention developed to prevent opioid misuse among adolescents.

Aims: The objectives of this study are to investigate whether adolescent report of family functioning and race/ethnicity moderates the effect of a videogame intervention in preventing opioid misuse outcomes among adolescents aged 16-19 years old.

Methods: Adolescents aged 16-19 years are being recruited from ten high schools in CT for a larger NIDA HEAL funded study. Eligible adolescents must 1) attend a high school that has a school-based health center and be 16-19 years (grades 9-12); 2) report not having engaged in any prior opioid misuse; 2) be at "high-risk" based on their report at baseline of past 30-day use of cigarettes, e-cigarettes, Juul, alcohol, marijuana (including synthetics), amphetamine, cocaine, benzodiazepines, ecstasy, bath salts, or any other misuse of non-opioid prescription drugs or use of non-opioid illicit drugs AND have a score of >3 on the PHQ-2149 or a score of >10 on the GAD-7150 (both screening tools used by SBHA); 3) be willing to sit for 60 minutes/session to play the game; and 4) be able to provide assent/parental/guardian consent (if under 18 years). Adolescents who complete HEAL baseline are informed about the supplement study and consent is obtained from parents of adolescents who express interest in the study.

Results: Institutional Review Board approval was obtained in Oct, 2021. As of July 21, 38 adolescents have completed baseline assessments and 23 have completed 3 month follow up assessments. Racial/ethnic distribution of enrolled participants are as follows: 13 Hispanic adolescents (35%), 7 non-Hispanic white adolescents (19%) and 17 non-Hispanic Black adolescents (46%). Sex distribution of enrolled participants is as follows: 19 adolescent girls (51%), and 18 adolescent boys (47%).

Conclusions: The findings from this study will inform about how family level factors influence the efficacy of videogame interventions and help us develop video-game interventions that are culturally informed and address family level risk factors for substance misuse among adolescents.

Game-Based Digital Biomarkers of Cognitive Risk for Adolescent Substance Misuse

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Background: Up to 90% of adolescents who need treatment for substance use disorders do not receive it. A predominant reason is that most adolescents at risk are not identified. The current standard for identifying adolescents at risk rely on primary care providers who are not screening half the time due to time and other constraints. Also, adolescents are not always forth coming about their use, in part because their perception of risk of harm is low and also due to fear of getting into trouble. Novel objective automated methods are needed for identifying at-risk adolescents. Games can be used to collect objective data in the form of performance metrics which in turn can be used to measure cognitive function. Cognitive deficits such as poor working memory and inhibitory control are risk factors for substance misuse and as such could be used to identify adolescents at risk for future substance misuse.

Objective: The objective of this proof-of-concept study is to determine whether specific in-game performance metrics – digital biomarkers – can be used to identify adolescents at risk for substance misuse. Aim 1 will identify game-based digital biomarkers of adolescent substance misuse. Aim 2 will determine the association between identified digital biomarkers and adolescent executive function.

Methods: We will use data collected previously in an earlier randomized controlled trial (RCT) of a videogame intervention, PlayForward to develop a predictive model that identifies adolescents at high risk for substance misuse. During a prior RCT, in-game data (data collected by the game software while an adolescent was playing the game) as well as self-report data on drug use were collected at baseline, 6 weeks, 3, 6 and 12 months. Baseline report of drug use and self-efficacy to refuse drugs was used to categorize adolescents into high risk versus low risk. Specific in-game performance metrics of PlayForward will extracted and tested to determine which in-game metrics – digital biomarkers – are predictive of adolescents in the high-risk group compared to the low-risk group. We will then develop a prediction model using identified digital biomarkers and derive a digital biomarker score for each individual that ranges from 0 to 1 using logistic regression where an individual with a higher score has higher substance misuse risk. Aim 2: We will determine the association between identified digital biomarker score and executive function. Hypothesis: Digital biomarker scores (derived in Aim 1) will negatively correlate with executive function scores. We will conduct a pilot study using a high school sample of 30 adolescents aged 14-15 years. At baseline, adolescents will complete a validated self-report measure of executive function (EF) and two tasks of EF (the n-back task and the go/no-go task). Participants will then play 4 hours of the PlayForward game.

Results: In-game data extraction is underway, and our protocol is currently under Institutional Board Review.

Conclusions: Findings from this study will help us determine which specific in-game performance metrics can serve as digital biomarkers of cognitive risk for substance misuse and inform the development of a game-based screening tool that can identify adolescents at risk for substance misuse.

Meth, Guilt, and Spiritual Awakening

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The rates of meth use in the US have steadily increased over the last decade, and meth is now the leading cause of stimulant overdose deaths in the US. Meth use is particularly prevalent among SUMSM (stimulant-using men who have sex with men), putting them at greater risk of contracting HIV. One of the more widely accessible substance use treatment programs in the US is Alcoholics Anonymous (AA), which follows the 12-step method to achieve recovery. The last step is spiritual awakening, and the goal is to remain engaged in the 12-step program until spiritual awakening is achieved and recovery is subsequently reached. This study examined whether guilt is a predictor of 12-step engagement and spiritual awakening. Data were derived from a community-based study of HIV+ MSM who use methamphetamines. The study found that guilt was a significant predictor of 12-step engagement and that 12-step was an indirect pathway between guilt and spiritual awakening. This indicates that guilt may have an adaptive function that can be ethically harnessed by clinicians to motivate people who begin substance-use treatment with AA to remain engaged in the program throughout their recovery.

Mechanistic Insight into Microbial Regulation of Psychostimulant Abuse

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The abuse potential and psychomotor stimulant properties of amphetamines (AMPHs) have been associated with their ability to increase extracellular dopamine (DA) levels. This increase is mediated, at least in part, by the reversal of DA transporter (DAT) function, which causes non-vesicular DA release (DA efflux).

Recent studies suggest that imbalances in the gut microbiome participate in the pathogenesis of substance use disorders. Microbial products such as short-chain fatty acids (SCFAs), are suspected to play a fundamental role in this process. Among SCFAs, butyrate is known to cross the blood-brain barrier and directly act on neurons and glial cells. Fusobacterium nucleatum (F. nucleatum) is a bacterial species that secretes butyrate and whose abundance is increased by AMPH abuse in both rodents and humans. It is important to note that butyrate is a potent inhibitor of histone deacetylases (HDACs) and that inhibition of HDACs robustly increases expression of both DAT mRNA and protein levels in cell culture systems.

Here, we report that colonization of the intestinal tract of gnotobiotic Drosophila with F. nucleatum significantly enhances AMPH-induced DA efflux and associated behaviors. This potentiation of AMPH actions by F. nucleatum was paralleled by oral administration of butyrate. Further, both pharmacological inhibition and genetic knockdown of HDAC1 increased AMPH-induced DA efflux and locomotion as well as DAT expression. These data demonstrate that F. nucleatum modulates AMPH-induced behaviors through secretion of butyrate, inhibition of HDACs, elevation of DAT expression, and increased DA efflux. These findings suggest modulation of the gut microbiome, or their downstream targets, as a therapeutic approach for substance use disorders.

All In? A Qualitative Exploration of Diversity, Organizational Stress, & Buy-In among MAT Staff in Justice Populations

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There is mixed empirical support regarding how correctional employees impact justice outcomes. This study explores the relationship between staff diversity, organizational stress and buy-in concerning medication assisted treatment (MAT) programs in justice settings. Specifically, this research examines if/how correctional staff race, ethnicity, gender, and class as well as perceptions of organizational stress impact their attitudes towards, understanding of, and commitment to MAT treatment in justice settings. Considering the challenging conditions of the carceral environment and the heightened strain brought on by the COVID-19 pandemic, this work also considers the role of organizational stress in shaping staff perceptions. Research findings are expected to inform organizational recruitment and retention efforts as well as facilitate the implementation of innovation programs – such as MAT – that consider the perceptions and diversity of staff as critical. The study is currently in the beginning of data collection phase; the poster reflects the current status and some challenges the research faces.

Activated CD4+ T cells induce epithelial cell death in an IFN-γ dependent mechanism

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Inflammatory Bowel Disease (IBD) is an umbrella term that describes chronic autoinflammatory disorders of the digestive tract, specifically ulcerative colitis (UC) and Crohn's disease (CD). Traditionally, UC is described as driven by Th2 CD4+ T cells, while CD is caused by Th1 CD4+ T cells {1,2,3}. However, a more sophisticated view of IBD pathogenesis also implicates cytolytic CD8+ cells {4,5} and the less well studied T cell subset bearing the γδ T cell receptor (TCR) {6,7}. Previous studies of T cell-mediated inflammation in IBD have not compared the immunological contributions by T cell subtype and compartment as they interact with intestinal epithelial cells.

Blood-derived CD4+ or CD8+ T cells were isolated from healthy human donors using negative magnetic separation. These cells were co-cultured with colonic epithelial stem cell spheroids in Matrigel from healthy donors. A subset of T cells was activated with anti-CD3/CD28 tetramer for 24 h prior to coculture. Activated CD4+ T cells co-cultured with spheroids from healthy donors caused significant epithelial cell death, as measured by brightfield microscopy and flow cytometry. Other T cells (CD8 with and without activation and non-activated CD4) had no effect. We next investigated if potential soluble mediators were causing cell death. Using a luminex cytokine multiplex we found that IFN-γ was prominently secreted by CD4 T cells cocultured with spheroids. Neutralizing IFN-γ antibody was able to significantly inhibit CD4+ T cell induced death. However, upon addition experiments adding protein to spheroid cultures, IFN-γ at high concentrations did not significantly induce spheroid cell death, indicating that it is necessary but not sufficient to kill spheroids. Future work is aimed at uncovering the differences between CD4+ T cell and CD8+ T cell cytokine secretion or other potential factors such as cell-cell contact leads to the differences in spheroid death. Additionally, the mechanism of spheroid death is under active investigation.

Emotion Dysregulation and Nicotine Consumption: Assessing the Moderating Role of Physical Activity Among Female, Daily Smokers

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Emotion dysregulation has been found to result in a susceptibility to drug-use (e.g., smoking) in order to down-regulate distressful emotional states (Gonzalez et al., 2008). In addition to being more likely to be diagnosed with anxiety and mood disorders, both of which are characterized by disturbances in emotion regulation, women have also been found to be less successful than men in quitting smoking (McLean et al., 2011; Smith et al., 2015). In the interest of informing future interventions tailored to the needs of women, the current observational study examined if increased moderate-to-vigorous physical activity (MVPA), an adaptive way to cope with stressors, moderated the relationship between baseline-level emotion dysregulation and daily nicotine consumption over the course of a menstrual cycle in female, non-treatment-seeking smokers (Bernstein & McNally, 2018). We hypothesized that there will be a weaker relationship between emotion dysregulation and daily nicotine consumption on days in which participants do more MVPA than their average. Emotion dysregulation was measured at baseline using the Difficulties with Emotion Regulation (DERS) scale, daily cigarette consumption was measured using Ecological Momentary Assessment (EMA), and daily amount of time spent doing MVPA was measured using wrist-worn Actigraph accelerometers. Emotion dysregulation was not a significant predictor of subsequent cigarette consumption on the daily level. However, an increase in the amount of time per day engaged in MVPA predicted a higher daily number of cigarettes consumed on average ($p < 0.05$). While an increase in moderate-to-vigorous physical activity did not reduce cigarette consumption in the current study, experimental research is needed in order to further test the effectiveness of physical activity, as well as to better understand the relations between these variables. Future research should examine the efficacy of lower-intensity physical activity as an intervention as well, in addition to other health-behaviors.

Positive Emotion as a Risk and Protective Factor for Substance Use Among Indigenous Youth

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Substance use rates among indigenous youth in the US have historically been greater than national averages. Positive emotional functioning is a leading risk/protective mechanism for substance use in youth. Meanwhile, youth from historically marginalized communities are at greater risk for having lower trait positive affect. Despite this, no studies have examined positive affect as a risk/resilience factor for substance use in indigenous youth. The current study sought to characterize the positive emotional profile of youth living on or near reservations in the contiguous United States and its association with substance use. Multilevel models predicting trait positive affect indicated that engaging in spiritual practices and identifying as male were associated with increased positive affect. Schools with lower proportion of students that live on the reservation and higher proportion of indigenous students exhibited higher school average positive affect. Regarding substance use, being non-indigenous, older, engaging in less spiritual practices, and having lower levels of positive affect were association with increased alcohol use over the previous 12 months and schools with a lower average trait positive affect among its student was associated with a higher proportion of students that drank alcohol in last year. In contrast, being indigenous, older, engaging in less spiritual practices, and having lower levels of positive affect were association with increased cannabis use over the previous 12 months and schools with a lower average trait positive affect among its student was associated with a higher proportion of students that used cannabis in the last year. This pattern of results highlights positive affect as a cross-cultural risk/protective factor for substance use at both the individual- and school-levels. These results suggest that school-based emotion literacy programs that focus on positive emotion identification, links between antecedents and emotional consequences, and the natural time-course of emotional arousal present an opportunity to better support young people.

Opioid withdrawal as a catalyst for positive and negative outcomes

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Background: Opioid withdrawal is common among people who regularly use illegal opioids and is associated with risk behaviors and serious health outcomes, including overdose. At the same time, withdrawal experiences are also increasingly recognized as important junctures during which people who use illicit opioids may be more receptive to treatment initiation or cessation. To investigate the role of withdrawal in medication for Opioid Use Disorder (MOUD) treatment initiation and retention as well as its role as a catalyst for risk-involved behavior, this mixed methods study builds upon the capacity of a parent R01 that is investigating changes in opioid overdose risk behavior over time.

Methods: We use a mixed-methods approach that incorporates baseline and monthly survey data from 575 participants who use illegal opioids to explore potential relationships between individual characteristics, such as age, race, and gender, and whether or not participants are engaged with MOUD treatment, on peoples' experiences with withdrawal, as well as how opioid withdrawal impacts peoples' MOUD treatment choices over time. To generate more specific data about the context and sequence of events between withdrawal experiences and treatment decisions, and to aid in theory-building and interpretation of longitudinal survey analysis, we are also conducting a qualitative inquiry with a theoretical subsample of study participants designed to provide in-depth information about how MOUD patients conceptualize the relationship between withdrawal, their treatment decisions, and their risk of negative health outcomes, including overdose.

Results: Preliminary findings demonstrate the importance of withdrawal in the lives of people who use illegal opioids, both as a catalyst for engaging in risk-involved behavior and as a primary motivator for initiating and engaging with MOUD. 70% of those not receiving MOUD and 37% of participants receiving MOUD reported experiencing withdrawal in the past 30 days. Participants also strongly linked their willingness to engage in risk-involved activity to the importance of alleviating or avoiding withdrawal. For example, participants reported readily engaging in behaviors such as buying drugs from unknown sources, using drugs in public, or using in ways that increase the risk of HIV and HCV transmission such as sharing syringes, when in withdrawal. Participants generally characterized their concern for negative outcomes, such as arrest, overdose, or disease transmission, as secondary to the importance of alleviating the immediate pain and anxiety withdrawal. Similarly, participants framed their participation in MOUD as a temporary or permanent solution to withdrawal. Most described the periods immediately preceding treatment initiation as being defined by the near-constant threat of withdrawal and saw MOUD as a means of reducing or eliminating it. MOUD's ability to ameliorate withdrawal was seen as its primary benefit and most participants framed it in this way rather than as a form of 'substance use treatment'. However, the highly regulated, top-down

structure of MOUD programs often created barriers to this goal by forcing participants to come in daily and refusing to allow dose increases to patients who continued using illegal drugs.

The role of municipal policies and practices in the sustainability of community-adopted interventions

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Title: The role of municipal policies and practices in the sustainability of community-adopted interventions

Background: Local governments and community members play an important role in responding to the opioid epidemic. Scholarly literature and best practices around municipal drug strategies frequently name the importance of these stakeholders in destigmatizing drug use, providing access to treatment, and championing public health policies.

Objective: I sought to understand the perceptions of HEALing Communities Study, Massachusetts (HCS MA) community team members, Wave 1 coalition partners, and community members regarding the impact municipal policies would have on the interventions introduced in Wave 1 communities.

Methods: I conducted semi-structured interviews with 10 HCS MA community team members, HCS MA coalition partners, and Wave 1 community members. Interviews were recorded and transcribed verbatim. They were then analyzed using thematic analysis to identify emerging themes.

Results: All of the HCS MA community team members, HCS MA coalition partners, and Wave 1 community members named knowledge of substance use-related issues, misinformed understanding about harm reduction, internal policies (human resources related), funding policies, and municipal structure (town manager vs. mayor; dedicated staff person) as having an impact on the sustainability of the HCS MA interventions. For many, they recognized that HCS MA provided a level of coordination and shared language that did not exist internally; and when there were no shared definitions around issues such as harm reduction that local policymakers struggled to implement programs and services that could address the opioid epidemic.

Conclusions: This initial study provides insight into the policies and practices that may have an impact on the sustainability of HCS MA interventions in Wave 1 communities. Understanding existing policies and practices is crucial to changing municipal responses to the opioid epidemic.

Parameters influencing food intake in a conditioned overconsumption task

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Overconsumption past homeostatic needs is a behavior associated with both binge-eating disorder and obesity. Associative conditioning to food cues is one underlying cause of overconsumption (van den Akker et al., 2018). Our lab recently designed a behavioral task in which mice are trained to overconsume past homeostatic needs in response to food-associated-cues (Stern et al 2020) in order to identify neural mechanisms that underlie this type of associative learning (Stern et al 2021). This task requires just two or three training sessions and can be completed in as little as 5 days, an improvement over previous training protocols which required up to one month of training. Due to the novelty of this training paradigm, there is little known about the parameters that can influence the task's ability to properly train mice to overconsume.. Obesity and binge-eating disorder are prevalent in both females and males, but the task was originally tested only in male mice. We therefore first repeated the task in a cohort of male mice to replicate the original conditions. We then tested the paradigm with female mice and found the levels of overconsumption comparable to male mice. Due to the obesogenic environment that may contribute to obesity and binge-eating, we also used highly palatable food (Ensure) as the conditioned food source, which also revealed significant overconsumption behavior in the food-associated context compared to the neutral context. In contrast, animals trained to consume 60% high-fat diet in the food-associated context did not show significant overconsumption behavior. Future studies will investigate the mechanisms that differentiate the response to Ensure and high-fat diet in both male and female mice.

Simultaneous & sequential cocaine+alcohol PSU alters neurocircuitry of cocaine seeking

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Background: While many medications reduce the reinstatement of cocaine-seeking in animals, these agents show little clinical efficacy at preventing relapse in humans. This is possibly due to the fact that an estimated 60-90% of cocaine users also use alcohol, but animal models of polysubstance use (PSU) are seldom used. Here we developed a rat model of simultaneous cocaine+alcohol self-administration for the investigation of the neurobiology of drug seeking in a PSU condition.

Methods: Male and female rats underwent IVSA of cocaine alone (n=21) or a cocaine+alcohol solution (n=21) for 2 hr/day for 5 consecutive days, then 6 hr/day, 2 days/week, for 5 weeks. Two doses of cocaine were tested (0.25 and 0.5 mg/kg/infusion) with and without alcohol (12.5 or 25 mg/kg/infusion). At the conclusion of IVSA, rats were tested on a progressive ratio schedule for 2-3 days, followed by tests for cued cocaine-seeking (relapse) at 1 and 30 days of abstinence.

Results: For the low dose of cocaine, the addition of alcohol to the intravenous solution had no effect on cocaine intake during self-administration, breakpoint for self-administration, or cued relapse after 30 days of abstinence. For the high dose of cocaine, the addition of alcohol to the intravenous solution resulted in increased cocaine intake during self-administration ($p=0.0201$), increased breakpoint for self-administration ($p=0.0026$), and increased cued relapse after 1 day of abstinence ($p=0.0359$).

Conclusions: Simultaneous cocaine+alcohol self-administration increases cocaine intake and the motivation to seek drug and drug-associated cues in a dose-dependent manner. Ongoing work is examining the effects of alcohol on the neurocircuitry of cued cocaine-seeking.

Increased probability of fentanyl adulteration in cocaine decreases cocaine demand

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In recent years, the increased presence of fentanyl adulteration in cocaine has drastically increased cocaine-related overdose deaths, yet there is limited research on how people who use cocaine perceive fentanyl adulteration. This online study developed the Adulterated Cocaine Purchase Task (ACPT), a novel modification of the original Cocaine Purchasing Task, to quantify how people respond to fentanyl adulteration in cocaine. In the ACPT, participants indicated how much cocaine they would purchase when cocaine had no (0%) vs. some (10%) probability of fentanyl adulteration. This study aimed to determine how possible fentanyl adulteration affects cocaine demand (intensity and elasticity). Participants (N = 32) consisted of self-reported cocaine users recruited via Amazon Mechanical Turk. Two mixed effects models (one per index) suggested that greater probability of fentanyl adulteration (10%) lowered cocaine demand, but only for intensity (an index of how much participants consume when cocaine is free). We did not see an effect on elasticity (an index of how sensitive cocaine use is to escalating prices). Overall, our findings indicated that fentanyl adulteration did reduce demand for cocaine in some but not in all participants, suggesting this is a potentially sensitive way to identify individuals at risk from fentanyl adulterated cocaine.

Mitragynine Reverses Paclitaxel Chemotherapy-Induced Peripheral Neuropathy and is Mediated via Opioid Receptor Involvement

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Chemotherapy-induced peripheral neuropathy (CIPN) is a problematic side effect in patients receiving chemotherapeutic cancer treatments. Clinical use of approved analgesic drugs often does not adequately control the pathological pain arising from CIPN and does not account for potential abuse with opioid therapeutics. Mitragyna speciosa (kratom) contains the alkaloid mitragynine, which exhibits analgesic properties. However, the underlying pharmacological mechanisms that underlie these analgesic properties are complex and not completely understood. Male and female C57bl/6 mice received 8 mg/kg intraperitoneal injections of paclitaxel, a taxane class chemotherapeutic, every other day over the course of 7 days. To confirm the development of CIPN, the von Frey assay was utilized to determine the onset mechanical allodynia, which arises when a previously non-painful stimulus is perceived as painful. Intraperitoneal mitragynine and the prototypical opioid agonist morphine both dose-relatedly reversed CIPN-induced mechanical allodynia. Effective doses (ED)50 were as follows – morphine: 7.02 (6.56 – 7.51) mg/kg, mitragynine: 109.80 (104.27 – 115.62) mg/kg. Pretreatment with the opioid antagonist naltrexone 0.032 mg/kg, intraperitoneally produced a rightward shift in both morphine and mitragynine dose-response curves. Effective doses (ED)50 were as follows – naltrexone + morphine: 27.93 (24.84 – 31.40), naltrexone + mitragynine: 245.41 (211.76 – 284.39), resulting in a 3.98 and 2.24 fold shift of dose response curves, respectively. Here we show that mitragynine reverses mechanical allodynia associated with paclitaxel CIPN in a manner likely mediated through opioid receptor activity. Mitragynine may be an effective analgesic treatment option for patients experiencing painful CIPN.

Leveraging Qualitative and Administrative Data to Characterize Delivery of Substance Use Services for Youth Involved in the Juvenile Justice System in Rural Indiana

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Youth who are involved in the juvenile justice system (YJJ) are at particular risk for opioid-related harms, given their high rates of mental health and substance use disorders (SUDs) compared to youth who have never been arrested. Although evidence-based interventions (EBIs) for SUDs exist, few youth in general, and YJJ specifically, access EBIs. This is a particular difficulty in rural settings, where shortages of behavioral health specialists, limited access to transportation and other resources, and recruitment and retention challenges present additional barriers to behavioral health care. This difficulty is compounded during the opioid crisis, as adolescent use of opioids is more prevalent in rural areas where opportunities for substance use treatment are severely lacking. The funded parent grant, Alliances to Disseminate Addiction Prevention and Treatment (ADAPT), employs a Learning Health System model to develop alliances between the juvenile justice system and community mental health centers (CMHCs) in 8 rural counties and implement a bundled treatment approach to improve use of EBIs for SUDs. This Diversity Supplement proposes to expand this work in two ways. First, we will characterize EBIs for SUDs in rural CMHCs, including the services provided and the workforce involved. We will conduct qualitative interviews with staff and leaders at rural CMHCs to identify specific strategies used to implement EBIs for SUDs in rural settings, describe the providers involved and their roles in implementing these tasks, and define the rationale for the identified service-delivery model. Second, we will compare the youth SUD services provided and the workforce involved across more rural and less rural settings. Using linked administrative data from juvenile justice agencies, CMHC visits, and Medicaid insurance claims in participating counties, we will describe service and workforce variables relevant to the implementation of EBIs for SUDs, and compare SUD services between less rural and more rural areas. The results of this project will provide a fuller picture of current SUD treatment practices and guide future EBIs in rural settings. Specifically, results will inform future task-shifting model of care research to leverage lay health workers, case managers, and peer recovery coaches in the delivery of EBIs with fidelity in rural CMHCs.

Prenatal Tobacco Exposure on Brain Morphometry and Cognitive Measures in the Adolescent Brain Cognitive Development (ABCD) Cohort

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Prenatal tobacco exposure (PTE) affects cognitive and brain developmental outcomes. However, specific brain regions and sex-specific effects associated with PTE are not well defined. We used baseline data from 9-and-10-year-old children with (n=620) and without (n=10,989) PTE in the Adolescent Brain, Cognitive Development study. We assessed parent-reported socio-demographics, prenatal exposures to tobacco, alcohol, and marijuana, and structural MRI and neurocognitive measures. Linear mixed models were used to compare groups and assess for possible sex-specific effects associated with PTE. Significance was defined as $p< 0.05$, corrected using the false-discovery rate. Compared to unexposed children, PTE-children had poorer performance (corrected-$p\leq0.01$) on executive function, working memory, episodic memory, reading, crystallized intelligence, fluid intelligence and overall cognition. PTE-children also had thinner parahippocampal cortices, smaller surface areas in the posterior-cingulate and pericalcarine cortices, the lingual and inferior parietal gyri, and smaller volumes of the thalamus, globus pallidum and nucleus accumbens (all corrected-$p\leq0.001$). Furthermore, we found that only PTE-girls had smaller surface areas in the superior-frontal (interaction-$p=0.03$), precuneus (interaction-$p= 0.02$) and postcentral gyrus (interaction-$p=0.01$), while only PTE-boys had smaller putamen's (interaction-$p=0.02$). Our findings suggest that PTE may lead to poorer cognitive and structural brain development that remain evident in early adolescence.

Preventing Substance Misuse and Substance Use Disorder by Examining Service Provider Interactions and Ethnic Identity

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Experiencing homelessness is often accompanied with negative interactions and discrimination from others, and both stressors are associated with increased substance use and the potential development of substance use disorders. An important component of exiting homelessness and reducing substance misuse is engaging with social services (e.g., going to medical appointments or the DMV to obtain a state ID). However, young adults experiencing homelessness (YAEH) encounter negative and discriminatory interactions with service providers that reduce social service utilization and likely decrease the chance of exiting homelessness and reducing substance misuse. While negative and discriminatory interactions are documented in the extant literature (i.e., service provider discrimination), relatively less is known about interpersonal and behavioral strategies that contribute to positive interactions with YAEH. Moreover, discriminatory experiences are associated with increased substance use. Past literature has demonstrated that psychological factors, such as Ethnic Identity, can reduce discrimination's association with substance use. However, securing basic needs (e.g., safety, food, and shelter) may supersede efforts to explore and develop one's ethnic identity among young adults experiencing homelessness, thereby missing the opportunity to develop protective psychological resources. As such, the current multi-method study proposes to investigate (a) positive interpersonal strategies with YAEH by interviewing service providers (N = 10), advocates, and Black or African American YAEH and (b) the extent to which a randomized clinical trial (N = 240) offering six-months of housing fosters the development of ethnic identity and its moderating effect on substance use and the development of substance use disorders.

Prenatal Cannabinoid Exposure Leads to an enhanced GABAergic Signaling Resulting in Learning and Memory Deficits in Adolescent Rat Offspring

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Cannabis is the most abused drug by pregnant women. Its use is expected to increase, with 39 states having medical marijuana laws and 19 having recreational marijuana laws. Also troubling, the concentrations of tetrahydrocannabinol (THC) in cannabis have doubled worldwide over 40 years, and higher concentrations are likely to increase associated problems. Clinical data showed that when pregnant women use cannabis, their offspring have learning and memory deficits. However, no studies have investigated cellular and molecular mechanisms of memory loss due to prenatal cannabinoid exposure (PCE). Based on our preliminary data, we hypothesize that PCE leads to an increase in GABAergic signaling, resulting in deficits in synaptic plasticity, leading to learning and memory deficits.

The first aim of our study will assess how pre- and post-synaptic GABAergic signaling are altered in PCE rodents. To determine the effects of PCE on GABAergic signaling, we will measure the synaptic expression of the proteins involved in GABAergic neurotransmission using western blot analysis. To investigate the single-channel properties of GABAA receptors, we will use isolated synaptosomes reconstituted in "tip-dip" bilayers. In addition, presynaptic GABA release will be examined using ceramic-based microelectrode arrays (MEAs). The second aim will test the hypothesis that PCE-associated GABAergic alterations will lead to deficits in synaptic plasticity mechanisms required for learning and memory. Pharmacologically blocking the activity of hippocampal GABAA receptors will help determine the effects of GABAergic modulation on synaptic plasticity. The third aim will investigate the effect of altered GABAergic signaling in PCE rodents' learning and memory performance. We will use behavioral assays, such as Morris Water Maze (MWM) and Trace Fear conditioning (TFC), in the presence or absence of GABAAR antagonist gabazine. The experiments described using biochemistry, electrophysiology, and behavioral assays will elucidate how an increase in GABAergic signaling will lead to deficits in synaptic plasticity mechanisms, leading to impairment in learning and memory performance in PCE rodents.

Measurement of Nature Contact: The Influence of Cultural Practices on Sleep Health and Chronic Disease among Rural and Urban American Indians

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Researchers have called for a deeper understanding of how social, cultural and environmental factors influence chronic disease and sleep health outcomes. The parent grant –American Indian CHronic disEase RIsk and Sleep Health (AI- CHERISH; R01MD014035)—aims to estimate the prevalence of sleep problems and their associations with specific cardiovascular and metabolic risk factors, as well as the qualitative characterization of cultural factors related to sleep health among participants recruited from American Indian (AI) participants previously enrolled in the Strong Heart Family Study (SHFS). This Diversity Supplement uses secondary analysis of quantitative data to develop a geographical variable (RUCA), and to test associations

and interactions between this variable, sleep health, and chronic disease risk factors. It also uses secondary qualitative data to develop a measure of cultural practices involving nature contact. This new measure will be tested for interaction effects on associations between the RUCA variable and sleep problems. This Supplement will enhance the parent study's ability to meet each of its specific aims through deepening understanding of geographic variations in sleep health as well as through enhancing understanding of specific cultural features key to sleep health by urbanicity and rurality, characterized by the creation of a rural-urban commuting area (RUCA) variable. Findings will enhance our understanding of geographic variation in sleep health and chronic disease outcomes, as well of culturally appropriate psychometric instruments that may help identify pathways for prevention and treatment. Findings will also contribute to a limited body of literature on the health benefits of nature contact among AIs.

Exploring Usability and Acceptability of a Mobile Tool to Report Inpatient Safety in Racially and Ethnically Diverse Families

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Background:

Patients and caregivers are the closest observers of patient safety. However, Safety reporting differences have been noted by ethnicity, which may lead to worse patient outcomes in patient-centered safety efforts. Our objectives were: to assess the usability and acceptability of a mobile-phone based tool for patients and families to report inpatient safety events; to explore differences in usability and acceptability by race and ethnicity.

Methods:

The Family Input for Quality and Safety (FIQS) mobile-phone tool allows patients and families to report inpatient safety events regarding: medications and infusions; hospital room and equipment; communication; infection prevention; procedures, tests, or other care; other; and what went well. The tool is available in English and Spanish. To evaluate tool usability and acceptability, we conducted 3 simulation activities with English and Spanish-speaking family members of inpatients, and patients ≥13 years in a pediatric inpatient unit, assessing usability. We asked open-ended questions about tool acceptability, including comfort with reporting positive and negative experiences.

Results:

Of 23 participants,17 were English-speaking and 6 were Spanish-speaking, including 17 parents/caregivers and 6 patients (5 patient-caregiver dyads), with 17 female-identifying and 6 male-identifying. There were 3 AAPI, 3 Black/African American, 9 Hispanic/Latinx, 2 Mixed-race, and 6 White/Caucasian participants. All participants were able to complete the simulation exercises successfully. Most participants agreed that the wording of the tool was clear, all categories they would like to see were listed, and that they would be willing to complete the tool every day of their hospital stay. Half of the participants expressed preference to make safety reports anonymously. All participants were comfortable reporting positive experiences. The majority (87%) stated they would feel comfortable reporting negative experiences. Three of nine Hispanic/Latinx (1 English-speaking, 2 Spanish-speaking) participants reported not feeling comfortable reporting negative experiences, whereas no English-speaking non-Hispanic/Latinx participants expressed concerns about reporting negative events.

Conclusions:

The FIQS tool was usable and acceptable to most hospitalized patients and their caregivers for reporting safety concerns. However, only Hispanic/Latinx Spanish-speaking patients felt uncomfortable reporting negative experiences. This gap raises concern for disparities in

reporting, and therefore disparate impact of improvement work. Additional work to address disparities in acceptability for Hispanic/Latinx Spanish-speaking patients is underway, using a revised script informed by these findings. When developing new strategies to improve quality of care and patient safety, healthcare providers should ensure that they do not exacerbate existing disparities due to disparate reporting.

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Single-cell RNA-seq Analysis of CD34+ Cells and Characterization of the Bone Marrow Microenvironment in Sickle Cell Disease Patients

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In Sickle Cell Disease, the periodic vaso-occlusive episodes initiated by reactive sickle cells result in a chronic status of inflammation in multiple organs. Little is known about the impact of SCD-induced inflammation in the bone marrow (BM) of Sickle Cell Disease (SCD) patients. In this study, we characterized the hematopoietic stem and progenitor cells (HSPC) and the stromal cells from BM aspirates of nine SCD patients before transplant in comparison with age-matched BM aspirates from healthy individuals. Using multiparametric flow analysis, we observed a group of SCD patients (n=5) with CD34+ frequency similar to controls, and a distinct group of SCD patients (n=4) that exhibited lower average frequencies of CD34+, HSCs, multipotent, and lymphoid progenitors cells compared to controls. We examined the transcriptomic landscape of the HSCs in the BM of SCD patients by single cell RNA-sequencing (scRNA-seq). Our analysis revealed a common SCD signature including several upregulated genes related to cell proliferation and differentiation; we also found several downregulated genes related to cell-cell signaling in the HSC subset. Analysis of the BM stromal component showed a trend for increased non-hematopoietic cells including CD45-/CD31+ and CD45-/CD105+ subsets in SCD patients compared to controls, suggesting a change in their BM stromal niche. In vitro, SCD BM-derived stromal cells revealed distinct gene expression patterns including increased inflammatory cytokines and inflammatory regulators. BM biopsies revealed alterations in the stromal components as evidenced by an overall decrease in cellularity in some patients. These observations were accompanied by an increase in angiogenic factors in the BM plasma of most patients. Taken together, these preliminary data suggest that SCD can associate with molecular and functional changes of hematopoietic cells and their BM niche and that the intensity of these changes may be associated with disease severity.