NIH Diversity Supplement Professional Development Workshop

August 29th – August 30th 2023

Book of Abstracts

Agenda

Tuesday, August 29th

11:00a.m. – 11:05 a.m. ET	**Welcome Remarks and Meeting Goals** (Zoom) *Aria Crump, ScD, 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. – 11:50 a.m. ET	**Keynote Address** (Zoom) *Keith Whitfield, Ph.D. President University of Nevada, Las Vegas*
11:50 a.m. – 12:10 p.m. ET	**General Introduction to the NIH Grant and Review Process** (Zoom) *Rob Rivers, PhD, Acting Director, Office of Minority Health Research Coordination, National Institute of Diabetes and Digestive Kidney Diseases, National Institutes of Health*
12:10 p.m. – 1:20 p.m. ET	**Demystifying NIH Research Funding Opportunities and the Review Process: Breakout Sessions** (Zoom) **Breakout: Undergrad/Post-Baccalaureate** *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

Anahid Ebrahimi, Ph.D., Health Program Specialist, Office of Programs to Enhance Neuroscience Workforce Diversity, Division of Extramural Activities, National Institute of Neurological Disorders and Stroke, National Institutes of Health

Breakout: Predoctoral Student

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

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

Rob Rivers, PhD, Acting Director, Office of Minority Health Research Coordination, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

Andrew Louden, PhD, Program Director, Division of Clinical Innovation, Clinical and Translational Science Awards Program Branch, Education and Training Section, National Center for Advancing Translational Sciences, National Institutes of Health

Cendrine Robinson, PhD, Chief Diversity Officer, National Institute on Deafness and Other

Communication Disorders, National Institutes of Health

Jamie Lahvic, PhD, Program Officer, Office of Strategic Extramural Programs, National Institute on Aging, National Institutes of Health

Breakout: Postdoctoral Fellow

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

Kalynda Gonzales Stokes, PhD, Program Director, Division of Training, Workforce Development and Diversity, National Institute of General Medical Sciences

Breakout: Investigator/Faculty

Carolina Solis Sanabria, MD, MPH, FACS, Program Official, Division of Clinical and Health Services Research, National Institute on Minority Health and Health Disparities, National Institutes of Health

Carlos O. Garrido, PhD, MS, MPH Program Director, Division of Integrative Biological and Behavioral Sciences, National Institute of Minority Health and Health Disparities, National Institutes of Health

12:10 p.m. – 1:20 p.m. ET **SBIR/STTR Breakout Session** (Zoom)

Julia Berzhanskaya, PhD, Innovator Support Lead, Innovation and Commercialization Office, National Heart, Lung, and Blood Institute, National Institutes of Health

	Stephanie Davis, PhD, Small Business Program Coordinator, Innovator Support Coordinator, Innovation and Commercialization Office, National Heart, Lung, and Blood Institute, National Institutes of Health *Toyin Ajisafe, PhD, Program Officer and Small Business Point of Contact, National Center for Medical Rehabilitation Research/National Institutes of Child Health and Human Development, National Institutes of Health*
1:20 p.m. – 1:40 p.m. ET	**Lunch Break** (Zoom or Gather Town networking)
1:40 p.m. – 2:15 p.m. ET	**Meet with a Program Officer (PO): Small Group Breakout Session with POs from Institutes and Centers** (Gather Town)
2:15 p.m. – 3:15 p.m. ET	**Diversity Supplement Scholar Poster Session 1** (Gather Town)
3:15 p.m. – 3:30 p.m. ET	**Break**
3:30 p.m. – 4:55 p.m. ET	**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*

Toyin Ajisafe, PhD, Program Officer and Small Business Point of Contact, National Center for Medical Rehabilitation Research/National Institutes of Child Health and Human Development, National Institutes of Health

Panelists:

Bruno Lima, DDS, PhD, Assistant Professor at the University of Minnesota, School of Dentistry

Patricia Simon, PhD, Clinical Psychologist and Vice President of Clinical Affairs at Oui Therapeutics, Inc.

Andrea Gilmore Bykovskyi, PhD, RN, Associate Professor and Associate Vice Chair of Research, Berbee Walsh Department *of Emergency Medicine, Deputy Director, Center for Health Disparities Research; University of Wisconsin*

Christiane Voufo, PhD, World Bank EdTech Consultant

Emma M. Lessieur-Contreras, MD, Ph.D, Postdoctoral Scholar at the University of California, Irvine (UCI), 2023 MOSAIC Awardee

4:55p.m – 5:00 p.m. ET

Day 1 Closing Remarks/Q&A/Adjourn (Zoom)
Toyin Ajisafe, PhD, Program Officer and Small Business Point of Contact, National Center for Medical Rehabilitation Research/National Institutes of Child Health and Human Development, National Institutes of Health

Wednesday, August 29th

11:00 a.m. – 11:05 a.m. ET	**Day 2 Welcome Remarks (Zoom)** *Shakira Nelson, PhD, Program Director, Division of Training, Workforce Development and Diversity, National Institute of General Medical Sciences*
11:05 a.m. – 11:35 a.m. ET	**Keynote Address** (Zoom) *Bonnielin Swenor, Ph.D., M.P.H.* *Associate Professor, Johns Hopkins School of Nursing* *Director, Johns Hopkins Disability Health Research Center*
11:35 a.m. – 12:30 p.m. ET	**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. – 1:05 p.m. ET	**Lunch Break and Optional Meet with a Program Officer (PO): Small Group Breakout Session with POs from Institutes and Centers** (Gather Town)
1:05 p.m. – 2:05 p.m. ET	**Diversity Supplement Scholar Poster Session 2** (Gather Town)
2:05 p.m. – 2:10 p.m. ET	**Transition to Zoom**

2:10 p.m. – 3:00 p.m. ET	**Resubmissions, Resources and Resilience (R3)** (Zoom) *Jamie Doyle, PhD, Program Director, Division of Clinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health* *Aria Crump, ScD, 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* *Fred Tyson, PhD, Program Director, Genes, Environment, and Health Branch, Division of Extramural Research and Training, National Institute of Environmental Science, National Institutes of Health*
3:00 p.m. – 3:45 p.m. ET	**How to Choose a Mentor and Navigate the Relationship (Panel Discussion)** (Zoom) **Moderators:** *Lucia Hindorff, PhD, Lead Extramural Training Program Director, Training, Diversity, and Health Equity Office, National Human Genome Research Institute, National Institutes of Health* *Arundhati Gogineni, PhD, Program Officer, Division of Integrative Biological and Behavioral Sciences, National Institute on Minority Health and Health Disparities, National Institutes of Health*

Panelists:

Angela Brooks, PhD, Professor, Biomolecular Engineering, Director of Diversity for the Genomics Institute, UC Santa Cruz

Ronald Hickman, PhD, RN, ACNP-BC, FAAN, Associate Dean for Research, Center for Research and Scholarship, Frances Payne Bolton School of Nursing and

David Garcia, PhD, FACSM, Associate Professor, Public Health, Health Promotion Sciences Department, Mel & Enid Zuckerman College of Public Health, University of Arizona

3:45 p.m. – 4:55 p.m. ET **Science Careers Panel Discussion** (Zoom)

Moderators:

Julia Berzhanskaya, PhD, Innovator Support Lead, Innovation and Commercialization Office, National Heart, Lung, and Blood Institute, National Institutes of Health

Veronica Abraham, MD, Fellow, National Institutes of Health-American College of Medical Genetics and Genomics Fellowship Program, National Human Genome Research Institute, National Institutes of Health

Utibe Bickham-Wright, Program Official, Division of Integrative Biological and Behavioral Sciences, National Institute on Minority Health and Health Disparities, National Institutes of Health

Pamela Tamez, PhD, Health Science Policy Analyst, Chief Officer for Scientific Workforce

Diversity (COSWD) Office, National Institutes of Health

Panelists:

Zulmarie Pérez Horta, PhD, Program Manager, BioInteractive team, HHMI.

Peter Velazquez, PhD, Associate Director, Analytical Development at Poseida Therapeutics, Inc.

Maria Artunduaga, MD, MPH, CEO of Respira (Samay Health)

Nii Addy, PhD, Albert E. Kent Associate Professor of Psychiatry and Associate Professor of Cellular and Molecular Physiology, Director of Scientist Diversity and Inclusion, Yale School of Medicine, Director, Minority Organization for Retention & Expansion (MORE) Faculty Mentoring Program, Associate Director of Diversity, Biological Sciences Training Program (BSTP) in Psychiatry at Yale School of Medicine

4:55 p.m. – 5:00 p.m. ET

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

Session A

1 **Delineating Parallel Ascending Pathways onto the Secondary Auditory Cortex**
Michellee Garci, University of North Carolina at Chapel Hill
NIDCD

2 **Sound reward learning induces unique transcriptional landscapes within the auditory cortex**
Guan-En Graham, Rutgers University
NIDCD

3 **Olfactory processing thresholds in wild-type mice and a model of autism**
Eyerusalem Haile, University of Illinois at Chicago
NIDCD

4 **Is the Timed Up & Go a sensitive measure for predicting fall risk in urban communities?**
Brittani Morris, New York University
NIDCD

5 **Cancer Worry and Fatalism Among Deaf and Hard of Hearing Adults**
Emmanuel Perrodin-Njoku, Gallaudet University
NIDCD

6 **Increased Ventricular Volume in Autistic Individuals Exposed to Prenatal Hypoxic Conditions**
Cristian Preciado, University of California-San Francisco
NIDCD

7 **Transcriptome dynamics during vocal fold injury and repair.**
Kristy Wendt, University of Wisconsin-Madison
NIDCD

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

9 **Characterization of Pumilio family gene expression during early avian development**
Mariann Guzman-Espinoza, University of California, San Francisco
NIDCR

10 **Strategies for analyzing gene expression in embryonic bird jaw bone**
Claire Houchen, University of Missouri-Kansas City School of Dentistry
NIDCR

11 **Porphyromonas gingivalis tyrosine-phosphatase targets STAT3 for subduing IL-6 in gingival epithelial cells**
Jaden Lee, Medical University of South Carolina
NIDCR

12 **Porphyromonas gingivalis Invades Brain Microvascular Endothelial Cells and Impacts Glutamine Metabolism for Intracellular Survival**
Brianyell McDaniel Mims, Medical University of South Carolina
NIDCR

13 **Identification of Initial Signs of Osteoradionecrosis using Retrospective Electronic Health Record Review**
Jillian Rigert, The University of Texas MD Anderson Cancer Center
NIDCR

14 **Elucidating the role of keratin 75 in enamel using Krt75tm1Der knock-in mouse model**
Brent Vasquez, University of Pittsburg School of Dental Medicine
NIDCR

15 **Maternal weight trajectories and eating behaviors in the Study of Latinos Family Lifestyles Outcome Research (SOL-FLOR)**
Christina Cordero, University of Miami
NIDDK

16 **Perinatal high fat diet induces systemic inflammation during development and decreases expression of gut peptides in adulthood**
Lindsey Macias, Johns Hopkins University
NIDDK

17 **Trends in Diabetes Screening Before and After the SARS-CoV-2 Pandemic Shelter-in-Place Mandate among Adult Patients with Prediabetes in an Integrated Health Care System in Northern California.**
Luis Rodriguez, Kaiser Permanente Northern California, Division of Research
NIDDK

18 **Therapeutic Effects of Selenium for Cadmium Induced Effects on Vision and Morphology**
Myles Covington, University of Miami
NIEHS

19 **Peturb-seq to Identify Genes That Modulate the Transcriptional Phenotype of FRDA Lymphoblastoid Cells**
Christian Maugee, University of Florida
NIEHS

20 **Toxicity of the phthalate replacement di-2-ethylhexyl terephthalate (DEHTP) and its metabolite on the mouse ovary**
Courtney Potts, New Jersey Institute of Technology
NIEHS

21 **The Impact of Co-Exposures on Pediatric Obesity and Sleep Behaviors in Rural Appalachian Children**
Ketrell McWhorter, University of Kentucky
NCATS

22 **The Effect of Thyroid Stimulating Hormone on Brown Adipose Tissue in Humans**
Alina Gavrila-Filip, Beth Israel Deaconess Medical Center
NCATS

23 **Community-Academic partnerships with CTSI: Assessing the level of readiness for churches to engage in health promotion activities**
Jamila Kwarteng, Medical College of Wisconsin
NCATS

24 **Dual imaging of cytosolic and endoplasmic reticular Ca2+ dynamics in dendrites in vivo**
Justin O'Hare, Columbia University
NINDS

25 **Perinatal HIV infection and exposure is associated with saturated and monounsaturated fatty acid levels in 11-18-year-old Ugandan adolescents**
Vanessa Cardino, Michigan State University
NINDS

26 **Elucidating Regulatory Mechanisms of Lamin B1 Expression in Autosomal Dominant Leukodystrophy (ADLD)**
Sandy Rubio, University of Pittsburg
NINDS

27 **Intraspinal microstimulation simultaneously rebalances motor and nociceptive transmission in chronic spinal cord injury**
Maria Bandres, Washington University in St. Louis
NINDS

28 **Pathogenic ANKRD11 variant identified in an individual with intractable epilepsy and focal cortical dysplasia**
Elizabeth Chung, Brigham and Women's Hospital
NINDS

29 **Droplet-based forward genetic screening of astrocyte-microglia cross-talk**
Elizabeth Chung, Brigham and Women's Hospital
NINDS

30 **Huntingtin/HAP40 core complex is conserved in Drosophila and modulates Huntington's disease pathogenesis in vivo**
Stephen Farmer, University of Texas Health Science Center at Houston
NINDS

31 **Post-mitotic molecular consequences of Bak1 microexon 5 inclusion that contribute to neuronal loss**
Yi-Li Lam, University of California-Riverside
NINDS

32 **Increased PARP activation caused by the Parkinson's disease-linked G2019S LRRK2 mutation**
Jennifer Liu, Duke University
NINDS

33 **Effects of Repeated Head Injury on Circadian Response to Environmental Cues**
Kyli McQueen, University of Arizona
NINDS

34 **Characterization of Temporospatial Changes in Resident Microglia Following Traumatic Brain Injury**
Jatia Mills, Virginia Polytechnic Institute and State University
NINDS

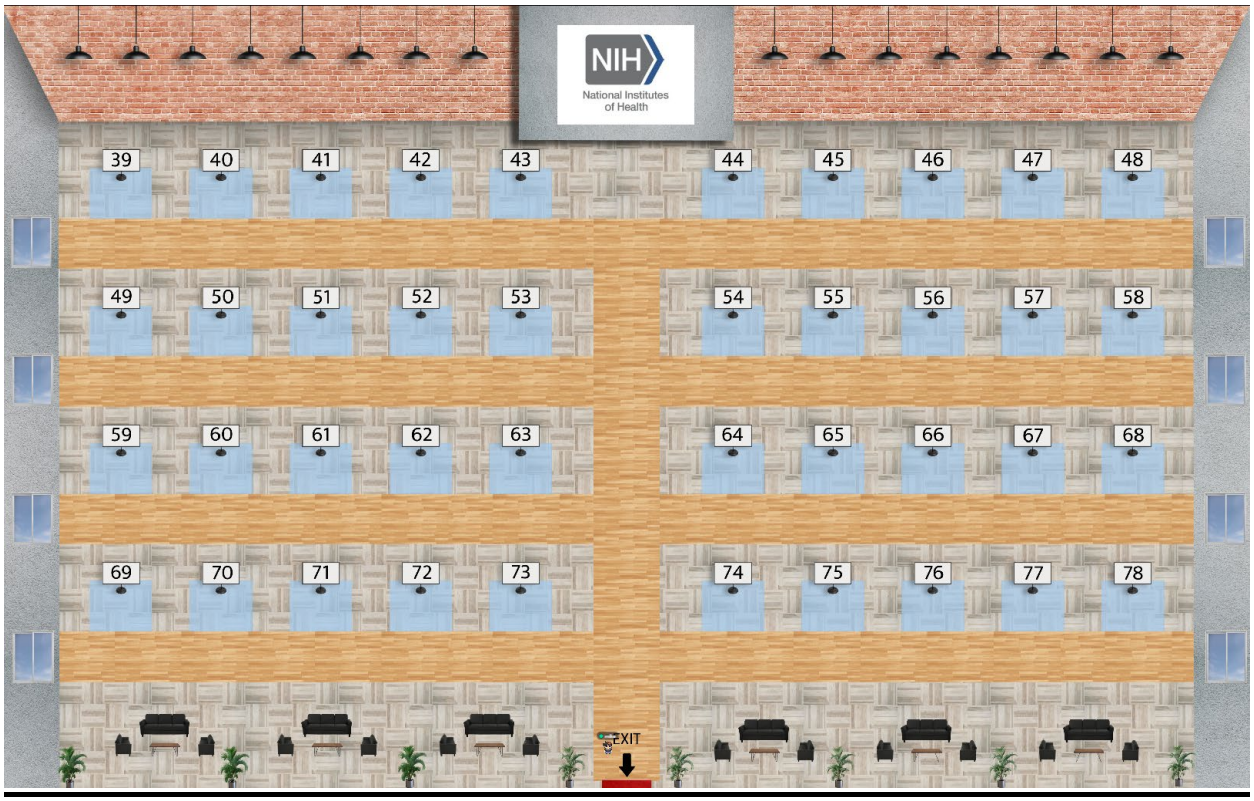
35 **To share or not to share? Research Participants Perspectives Regarding their Research Data Sharing Preferences**
Stephanie Nino de Rivera, Columbia University
NINDS

36 **Assessing NPC migration and neurite extension using a hiPSC-derived model of SCN3A encephalopathy**
Sarah Pham, University of Pennsylvania
NINDS

37 **Association between Neighborhood Disadvantage and Cognition in Older Adults with Focal Epilepsy**
Anny Reyes, University of California-San Diego
NINDS

38 **Neuronal and Behavioral Consequences of Early-life Seizures**
Sydney Townsend, The Ohio State University
NINDS

Session B



39 **No Advantage of Pragmatic Inference for Vocabulary Retention in Children with Autism**
Katherine Trice, Northeastern University

40 **Cell-Acquiring Fallopian Endoscope for early detection of ovarian cancer**
Dominique Galvez, University of Arizona
NCI

41 **A Dynamic Bayesian Network Approach To Modeling Engagement And Walking Behavior: Insights From A Year Long Mirco-Randomized Trial (HeartSteps II)**
Steven De La Torre, University of California-San Diego
NCI

42 **Characterization of Genetic Markers Involved in the Regulation and Composition of Follicle Cell Ring Canals**
Andreana Gomez, University of California-San Francisco
NIGMS

43 **Developing a Chemically-Controlled RAS Toolset**
Fernando Banales Mejia, University of Washington
NIGMS

44 **Pseudomonas aeruginosa Displays Enhanced Surface Motility Towards Staphylococcus aureus MRSA in Response to Elevated Calcium Levels**
Dulandika Malalage, Oklahoma State University, Department of Microbiology and Molecular Genetics
NIGMS

45 **Unraveling ECM Stromal Microenvironment Signaling in TNBC via the Sonic Hedgehog (SHH) Pathway**
Camilo Mora, University of Puerto Rico
NIGMS

46 **Anti-CRISPR Delivery for Precision Genome Editing**
Axel Vera, Massachusetts Institute of Technology
NIGMS

47 **Investigating interstitial fluid flow and increasing substrate stiffness on LECs permeability and morphology**
Aisha Abdulkarimu, University of Maryland
NIGMS

48 **Effect of Host Phylogenetics and Host Traits on Microbiome Assembly**
Taryn Broe, University of California-San Diego
NIGMS

49 **Integrative Analysis of Cardiac Isoforms of Drosophila Myosin**
Jared Rafael Camillo, San Diego State University
NIGMS

50 **Regulation of the Mitochondrial Pyruvate Carrier by the Wnt/beta-catenin pathway**
Luis Cedeno-Rosario, University of Utah
NIGMS

51 **Visualization and Immunoprecipitation of distinctively tagged PCH-2 strains in C.elegans**
Micaela Colmenarez, University of California-Santa Cruz
NIGMS

52 **Elucidating RhlR promoter selection mechanisms in Pseudomonas aeruginosa quorum sensing**
Nathalie Colon-Torres, Wadsworth Center, Ney York State Department of Health
NIGMS

53 **Modeling expectation-driven endogenous analgesia in mice**
Lindsay Ejoh, University of Pennsylvania
NIGMS

54 **Investigating the role of Rab GTPases in extracellular vesicle biogenesis and glial uptake**
Malek Elsayyid, University of Delaware
NIGMS

55 **Single Walled Carbon Nanotube (SWCNT) Detection of Interleukin 1 Î² (IL-1Î²)**
Atara Israel, The City College of New York
NIGMS

56 **Di-berberine conjugates as chemical probes of Pseudomonas aeruginosa MexXY-OprM efflux function and inhibition**
Logan Kavanaugh, Emory University
NIGMS

57 **TRIM9 modulates cytoskeletal dynamics, membrane remodeling, and motility in melanoma**
Kimberly Lukasik, University of North Carolina at Chapel Hill
NIGMS

58 **Synthetic Access to 4,5-Aminoglycoside Antibiotics**
Nainoa Norman Ing, Vanderbilt University
NIGMS

59 **The Role of BET Proteins in KDAC Transcription Regulation**
Sarah Olson, University of Arizona
NIGMS

60 **Biophysical Studies of a Novel Interaction Partner of Human Ribonucleotide Reductase**
Gerardo Perez Goncalves, Massachusetts Institute of Technology
NIGMS

61 **Investigating the cell and tissue-scale movements that drive avian foregut morphogenesis**
Olivia Powell, Columbia University
NIGMS

62 **Visible Light-Mediated, Diastereoselective Epimerization of Morpholines and Piperazines to More Stable Isomers**
Maria Vargas-Rivera, Yale University
NIGMS

63 **Examining Racial Discrimination, Insula Cortex Connectivity and Dissociation During Attention to Threat in A Trauma-Exposed Black American Women Population**
Aziz Elbasheir, Emory University
NCCIH

64 **The Intersection of Latinx MSMs' Cultural, Racial, and Sexual Identities on Friendship Network Dynamics, Mental Health, and PrEP Stigma, PrEP Awareness, and PrEP Discussions in South Florida**
Edda Rodriguez, University of Miami Miller School of Medicine
NIMH

65 **Exploring the influence of the socio-ecological environment on the Queen Club program for young women in Tanzania**
Camila Solorzano Barrera, University of California-Berkeley
NIMH

66 **Racial and Ethnic Disparities in Barriers to Mental Health Treatment in United States College Students**
Melissa Vazquez, Washington University Medical School in St. Louis
NIMH

67 **Exploring Neural Correlates of Reward and Punishment Learning in Depression using 7-Tesla MRI**
Jacqueline Beltran, Icahn School of Medicine at Mount Sinai
NIMH

68 **Using Thematic Analysis to Explore Barriers and Facilitators to mHealth Utilization: Preliminary Analysis**
Jacqueline Duong, University of Texas at Austin
NIMH

69 **Sex-differences in proteasome-dependent K48-polyubiquitin signaling in the amygdala are developmentally regulated**
Kayla Ferrell, Virginia Polytechnic Institute and State University
NIMH

70 **Neuroimmune mechanisms underlying chronic stress-induced reward deficits**
Rachel Fisher, Icahn School of Medicine at Mount Sinai
NIMH

71 **Associations of Posttraumatic Stress, Opioid Use, and Pain among Individuals with Probable PTSD and Chronic Pain: Investigating the Role of Health Literacy**
Shelby McGrew, University of Houston
NIMH

72 **Pursuit of a Reliable Working Memory Biomarker Characterizing the High Schizotypy Population**
Jenna Pablo, University of Nevada-Reno
NIMH

73 **Eating Disorder Prevalence, Related Psychopathology, and Treatment Need Among Rural, Suburban, and Urban Americans**
Jillian Shah, Washington University in St. Louis School of Medicine
NIMH

74 **Examining the Effects of Emotional Abuse from Family Members and Peer Victimization on the Development of Negative Inferential Style during Adolescence**
Auburn Stephenson, Temple University
NIMH

75 **Development of Suicide Ideation and Attempts among LGBTQ Youth of Color: An Integrative Data Analysis**
Alberto Valido, University of North Carolina at Chapel Hill
NIMH

76 **Molecular compensation by neurodevelopmental-risk genes Foxp1 and Foxp2 in the striatum**
Rachael Vollmer, University of Texas Southwestern Medical Center
NIMH

77 **Systematic Review of Research among Black Queer Cisgender Women Living with HIV**
Mya Wright, University of Miami
NIMH

78 **Parental Incarceration and Children's Health**
Sabrina Gebreselassie, Northwestern University Feinberg School of Medicine
NIMHD

Session C

79 **Congenitally blind and sighted adults use causal object history to assign colors**
Zaida McClinton, Johns Hopkins University
NEI

80 **Elucidating the role of Ankyrins during synapse formation in the outer retina**
Ross Perez, Baylor College of Medicine
NEI

81 **Analyzing the CHARGE-like phenotype caused by mutations of chd7 in Danio rerio**
Tasha Swenney, University of Kentucky
NEI

82 **TEM-seq: an ultrasensitive multiomic platform for epitope-targeted DNA methylation mapping**
Allison Hickman, EpiCypher
NHGRI

83 **Utilizing short sequences missing from the genome to identify and functionally characterize gene regulatory cancer driver mutations in liver cancer**
Jasmine Sims, University of California, San Francisco
NHGRI

84 **Intervention Mapping to Adapt an Evidence-Based Physical Activity Program for Rural Latinas**
Jackelyne Garcia, San Diego State University
NHLBI

85 **Log2Lose: Incenting weight loss and dietary self-monitoring in real time to improve weight management among adults with obesity**
Chloe Mayfield, University of Wisconsin-Madison School of Medicine and Public Health
NHLBI

86 **DCTN4-018 on Lung Epithelial Cells Impacts on Alveolar Immunity**
Blandine Victor, Cedars-Sinai Medical Center
NHLBI

87 **Overview: Structural racism and cardiovascular disease risk in pregnant women and their infants-Supplement to the Mother and Infant Determinants of Vascular Aging Study (MIDAS)**
Forgive Avorgbedor, University of North Carolina at Greensboro
NHLBI

88 **The Role of Sex Chromosome Complement and Gonadal Hormone in Lung microRNA Expression in a Mouse Model of Allergic Inflammation**
Sarah Commodore, Indiana University
NHLBI

89 **CaMK4 is a Novel Regulator of Myeloid Phenotype and Function in Atherosclerosis**
Azuah Gonzalez, Vanderbilt University
NHLBI

90 **Sex-specific Valvular Myofibroblast Activation in Response to Nano-scale Stiffness Cues**
Rayyan Gorashi, University of California, San Diego
NHLBI

91 **Cathepsin K cleavage of Angiopoietin-2 creates Tie2 antagonist fragments in sepsis**
Erik Loyde, University of Texas, Southwestern Medical Center
NHLBI

92 **Serum IgA and Gd-IgA1 levels associate with pulmonary phenotypes. The Multi-Ethnic Study of Atherosclerosis (MESA) Lung Study**
Tess Pottinger, Columbia University
NHLBI

93 **Coparenting Quality is Associated with Healthier Home Food Environments in Preschool-Aged Children**
Carmen Ramos, University of Michigan
NHLBI

94 **Cardiac Fibroblast-MHCII Contributes to Cardiac Pathophysiology in Doxorubicin-Induced Cardiomyopathy**
Maria A. Zambrano, Tufts University
NHLBI

95 **The association between anxiety and Alzheimer's disease plasma biomarkers across stages**
Mark Bernard, New York University Grossman School of Medicine
NIA

96 **Associations between Neighborhood Environment & Computerized Cognitive Performance among Black Adults**
Alexa Allen, Pennsylvania State University
NIA

97 **More Exposure to Everyday Discrimination Increases the Odds of Chronic Pain Among Hispanic and Non-Hispanic but not Non-Hispanic Black Middle-Aged and Older adults**
Antoinette Spector, University of Wisconsin-Milwaukee
NIA

98 **Functional Decline among Nursing Home Residents with ADRD during the COVID-19 Pandemic**
Ana Montoya, University of Michigan
NIA

99 **Parent factors associated with BMI, diet, and physical activity of adolescents with intellectual and developmental disabilities**
Amy Bodde, University of Kansas Medical Center
NIA

100 **Survival Methods in Electronic Health Records Data in the Presence of System Migration**
Kyle Conniff, University of California-Irvine
NIA

101 **Midlife Neuropsychological Profiles and Associated Vascular Risk: The Bogalusa Heart Study**
Ileana De Anda-Duran, Tulane University
NIA

102 **NLRP3 Gene Loss Exacerbates LV Hypertrophy and M2 Polarizes Left Ventricle Resident Macrophages in the DAHL/SS-Nlrp3em2Mcwi Rat Model**
Cesar De Jeronimo Diaz, Pennsylvania State University
NIA

103 **Action Plans Increase Advance Care Planning Documentation and Engagement Among English and Spanish-speaking Older Adults**
Clarissa Ferguson, University of California-San Francisco
NIA

104 **Facing Trustworthiness: Age-group differences in trust-related decision-making & learning**
Marilyn Horta, University of Florida
NIA

105 **Exploring Sex-Specific Impact of Alzheimer's Disease-Related APP Mutations on Colitis-Associated Colon Cancer**
Takako Ishida-Takaku, University of North Dakota
NIA

106 **Senescence-Based Drug Screening for Alzheimer's Disease Using Multi-Organ Models**
Joshna Jude Jose, University of California-Irvine
NIA

107 **Neuronal Integrity of Hippocampal Granule Cells in Primary Progressive Aphasia due to FTLD-tauopathies**
Vivienne Lubbat, Northwestern University
NIA

108 **Internal Mechanical Stress May Cause Stochastic Release of Large Neuronal Vesicles**
Andras Morera, Rutgers, The State University of New Jersey
NIA

109 **Wide Field of View Non-Mydriatic Biomarker Detection Portable Fundus Camera**
Diego Palacios, University of Miami
NIA

110 **Estrogen replacement therapy restores cerebral glucose uptake and attenuates cognitive decline in ovariectomized Bri-AÎ²42 mice**
Nicole Schartz, University of Arkansas for Medical Sciences
NIA

111 **Falls and Cognition in Adults Aging with Down Syndrome**
Selena Washington, Saint Louis University / Washington University
NIA

112 **PhD Graduate student and Research assistance**
Marie (Tamara) Adonis-Rizzo, Florida Atlantic University
NIA

113 **Investigating sex differences in the neuroanatomy and behavioral roles of neurons expressing the neuropeptide cholecystokinin (CCK) in the bed nucleus of stria terminalis (BNST)**
Haniyyah Sardar, Stanford University
NIAAA

114 **RNA-seq analysis reveals prenatal alcohol exposure is associated with increases in placental inflammatory cells and gene expression**
Randy Williams, Harvad University
NIAAA

Session D

115 **Hem1, Autoimmunity, and B Cell Tolerance**
Andreas Anderson, University of Washington
NIAID

116 **Development of New Oxysterol-Binding Protein (OSBP)-Targeting Antiviral and Anticancer Compounds**
Jorge L. Berrios-Rivera, University of Oklahoma Health Sciences Center
NIAID

117 **Delta-8-Tetrahydrocannabinol ameliorates colitis through suppression of inflammatory myeloid cells**
Jeffrey Garcia-Sanchez, University of South Carolina
NIAID

118 **Interactions between mycobacterial cell wall regulators may control peptidoglycan metabolism**
Karen Tembiwa, University of Texas at Arlington
NIAID

119 **Uncovering the Mechanisms of Transcriptional Regulation of ECA Biosynthesis in E. coli K-12**
Haley Bennett, the Agricultural and Mechanical College of Texas (Texas A&M)
NIAID

120 **Learning from Nature: Pyrococcus furiosus Thioredoxin (PfTrx) Scaffolds Displaying Extracellular Loops of the Treponema pallidum FadL Outer Membrane Proteins in the Search for a Syphilis Vaccine**
Kristina Delgado, University of Connecticut Health Center
NIAID

121 **ZnuABC is not essential for Klebsiella pneumoniae virulence**
Taylor Garrison, University of Louisville
NIAID

122 **Temperate phage encode regulators of the bacterial SOS response to control phage induction**
Nancy Haro-Ramirez, University of California-Irvine
NIAID

123 **Dissecting early molecular interactions between typhoidal and non-typhoidal Salmonella with human intestinal cells**
Joy McKenna, Stanford University
NIAID

124 **Use of Peroxisomal Targeting Sequences in Drug Delivery**
Sabrina Pizarro, Clemson University
NIAID

125 **Skin dendritic cell positioning and trafficking regulated by Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2)**
Hanna Taglinao, University of California, San Francisco
NIAID

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Delineating Parallel Ascending Pathways onto the Secondary Auditory Cortex

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How our brain integrates information across parallel sensory channels to achieve coherent perception remains a fundamental question in neuroscience. In the auditory system, sound information reaches the cortex via two parallel pathways. The “primary”lemniscal pathway relays fast and accurate sound information to layer 4 (L4) of the primary auditory cortex (A1). Conversely, the “secondary”non-lemniscal pathway is considered a slow integrator of multisensory information relayed indirectly from cortical areas. Recent anatomical and physiological findings, however, challenge this simple dichotomy. These include our discovery of short-latency (<10ms) sound input onto L4 and layer 6 (L6) of the secondary auditory cortex (A2), comparable in speed to the “fast”lemniscal input to A1. Here, we examined the hypothesis that this short-latency input is conveyed via non-lemniscal pathways by conducting cortical area- and layer-targeted retrograde tracing. We found that A2 L4 and L6 receive inputs from distinct medial geniculate nucleus (MGN) subdivisions; specifically, A2 L6 receives input from the medial division of MGN (MGm) while A2 L4 is innervated by the caudal part of the ventral division of MGN (MGv). Interestingly, further MGN subdivision-specific retrograde tracing revealed that MGm and caudal MGv receive inputs from overlapping but distinct domains of the shell of the inferior colliculus, which in turn receive direct input from the cochlear nucleus. These findings demonstrate a non-lemniscal origin of parallel ascending pathways that bypass A1 and directly reach both the superficial and deep layers of A2. Moreover, our results suggest that caudal MGv, but not rostral MGv, belongs to the non-lemniscal pathway, despite the conventional view of MGv as a homogeneous lemniscal structure. Ongoing electrophysiology and optogenetic manipulation studies aim to investigate the sound response properties of these non-lemniscal pathways and explore how parallel ascending pathways are integrated in the cortex to shape perception.

Sound reward learning induces unique transcriptional landscapes within the auditory cortex

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Forming long-lasting memories requires experience-dependent neurophysiological plasticity. Lasting changes to auditory system processing relies on learning-induced gene expression. Epigenetic mechanisms are powerful regulators of experience-dependent gene that can control long-lasting effects on neuronal function and strengthen learned behaviors. One such epigenetic regulator, histone deacetylase 3 (HDAC3) works with transcriptional machinery to influence activity-dependent de novo DNA transcription. The results presented here are the first to identify genome-wide changes in learning-induced gene expression in the auditory cortex thought to underlie the formation of auditory memory. We also report that the inhibition of HDAC3 during sound discrimination learning can either amplify or blunt the magnitude of transcript levels when compared to learning alone. Bioinformatic analyses of molecular pathways involved in auditory learning identified the cholinergic synapse (in learning without epigenetic manipulation) and the extracellular matrix (ECM)-receptor and neuroactive ligand receptor interactions (with epigenetic manipulation during learning). Ongoing work in the lab examines transcriptional changes in a cell-type specific manner through utilizing single molecule fluorescent in situ hybridization (smFISH) to visualize select transcripts in auditory cortical anatomy. Additionally, bilateral delivery of a mutated HDAC3 to the auditory cortex further investigates the role of HDAC3 in the auditory cortex during learning and memory. Together, the findings characterize key candidate effects underlying changes in cortical function that support the formation of long-term auditory memory in the adult brain and the regulatory role of HDAC3 on genetic targets that may be key for neurophysiological plasticity within the auditory cortex.

Olfactory processing thresholds in wild-type mice and a model of autism

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The olfactory system enables individuals to identify, interpret and discriminate between numerous odorants in their environment. Individuals with autism spectrum disorder (ASD) exhibit sensory processing deficits, namely odor hypersensitivity (hyperosmia). In this study, we explored olfactory perceptual capabilities and innate odorant preferences in healthy wild-type (WT) mice and in a mouse model of autism (Fmr1-KO). Fmr1 encodes for the fragile X mental retardation protein (FMRP) and its deletion results in Fragile X syndrome, a severe mental retardation associated with the development of the brain and synapses. Previous behavioral studies have established atypical sensory processing in the visual, auditory, and somatosensory systems using this model, but there is less direct evidence of olfactory processing deficits. A two-chamber spatial place preference assay was used to measure spatial preferences prior to and following odorization of one chamber. We also measured exploratory and locomotive behavior across four different odorants at five concentrations each. Our results indicated that WT mice spent less time in the odorized chamber at lower concentrations compared to Fmr1-KO mice. In Fmr1-KO animals, odorant aversions developed at higher concentrations and no distinctive preference was observed for positively valenced odorants such as peanut oil. While the Fmr1-KO mice had similar locomotor activity as WT mice across the five odorant concentrations, WT mice exhibited more sustained bouts of freezing behavior upon odorant delivery, indicating negatively-valant odor perceptions. These observations suggest an inherent disruption in the circuitry of olfactory perceptual processing in Fmr1-KO mice. Our future work will evaluate the neural basis of how autism spectrum disorders disrupt olfactory perceptual capabilities at successive stages of the olfactory processing hierarchy.

Is the Timed Up & Go a sensitive measure for predicting fall risk in urban communities?

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Background: The Timed Up & Go test (TUG), a valid test of basic functional mobility, is used clinically to predict fall risk in community dwelling older adults.1 Older adults living in urban communities who use public transportation have been shown to walk faster than those who do not.2 Although Normative data for average TUG scores across the lifespan have been established,3,4 these may not be applicable to urban dwelling individuals. In addition, there has been speculation that the TUG may not be a sensitive measure to detect mobility difficulties or fall risk in patient populations with known balance deficits, specifically vestibular hypofuntion.5 Furthermore, there is no literature supporting the TUG as a sensitive measure for individuals with other sensory deficits, such as those with hearing loss.

Purpose/Hypothesis: We aimed to answer 2 questions:

1: Is the TUG sensitive to vestibular hypofunction in patients who live in New York City?

2: Do established norms represent older adults with or without sensory loss in New York City?

Participants: Participants in this study included 52 individuals with Bilateral Hearing Loss (B) (Females=16, Mean Age=70.05 years, SD=10.46), 57 individuals with Unilateral Hearing Loss (U) (F=31, Mean Age=51.26 yrs , SD=17.45), 41 individuals with Unilateral Vestibular Hypofunction (V) (F=20, Mean Age = 54.61 yrs, SD=17.82) and 42 controls (C) (F=24, Mean Age = 51.6 SD=17.57 yrs).

Materials and Methods: Participants performed the TUG twice. We recorded the fastest of the 2 performances. We compared TUG scores between groups with a

multiple linear regression. We then used descriptive statistics to compare all groups combined to age-adjusted norms.

Results: No significant differences were found between groups. Mean TUG scores per group were as follows: C: 8.61 s, SD= 1.45, B: 9.53 s, SD= 2.57, U: 8.75 s, SD=1.58, V: 9.39 s, SD=1.97.

After combining the 4 groups, our sample mean TUG scores were faster than normative TUG scores for their age intervals across all age groups. For example, TUG scores for the 75-79 age group for females was 9.8 s, SD=1.5 and males was 9.9 s, SD=2.0 compared to the normative score for females of 13.1 s and males of 12.2 s. Likewise, TUG scores for our oldest adults aged 80+ for females were 9.2 s, SD=2.3 and males was 11.0 s, SD=0.1 compared to the normative score of 14.3 s for and 13.2 s, respectively.

Conclusions: In this study, TUG score for individuals with hearing loss and vestibular hypofunction were not significantly different than a control group. Additionally, our sample performed the TUG faster than established norms for older adults. The results of this study suggest that urban dwelling adults ambulate faster than established TUG norms.

Clinical Relevance: In urban settings such as New York City, the TUG may not be an adequate measure of mobility and fall risk. Use of alternative validated and reliable outcome measures, potentially instrumented6, may need to be considered when determining functional mobility deficits and fall risk in individuals living in urban communities.

Cancer Worry and Fatalism Among Deaf and Hard of Hearing Adults

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Background: The deaf and hard of hearing (DHH) community experiences lower cancer screening rates than the general population. Cancer worry and fatalism can impact cancer screening and the interaction with DHH health behavior needs to be investigated.

Objective: The study examines the association of the intersection of race and hearing status with cancer worry and fatalism.

Interventions/Methods: This cross-sectional study analyzed survey data from NCI HINTS-ASL (for DHH adults) and NCI HINTS (for hearing adults). Multivariable logistic regression models were used to assess i) the association of race-hearing status intersection with cancer worry and fatalism, as well as ii) the relationship between hearing status and outcomes within each race.

Results: The study found that the interaction between race and hearing status was not significantly associated with high cancer worry ($p=0.37$), but it did significantly affect high cancer fatalism ($p<0.001$). DHH White respondents had lower odds of cancer worry [OR (95% CI): 0.74 (0.57, 0.97); $p=0.03$] and fatalism [0.59 (0.45,0.77); $p<0.001$] compared to hearing White respondents, while DHH African American/Black respondents had lower odds of cancer fatalism only [0.44 (0.20, 0.95); $p=0.04$] compared to their hearing counterparts. No significant differences were observed for other groups.

Conclusions: Limited access to cancer information in American Sign Language (ASL) negatively impacts health literacy, which in turn affects the experience of cancer worry and fatalism. African American/Black and White DHH individuals have different odds of experiencing cancer worry and fatalism compared to their hearing counterparts.

Implications for Practice: To promote equitable healthcare for the DHH community, health systems should disseminate cancer information in ASL and facilitate cancer discussions. The integration of Community Health Navigators proficient in ASL could empower DHH individuals who are lagging in cancer screenings, enabling them to make informed decisions.

Increased Ventricular Volume in Autistic Individuals Exposed to Prenatal Hypoxic Conditions

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Background: The ventricles and subventricular zones (SVZ) play a crucial role in neurodevelopment and are implicated in various neurological disorders. Previous studies have provided evidence linking ventricular abnormalities and altered SVZ neurogenesis to prenatal hypoxia, however, their precise characteristics and potential as neuroanatomical markers remain unclear. Additionally, investigations from our laboratory have found a significant difference in a cohort of individuals with autism spectrum disorder (ASD) and controls. The results revealed increased prevalence of prenatal hypoxia in the ASD group, highlighting the potential involvement of hypoxia in the etiology of ASD. This research project aimed to investigate the quantitative analysis of lateral ventricles using MRI T1 images, with the objective of elucidating their morphological features and their potential implications in autism spectrum disorder.

Methods: A case control study design was employed, involving the acquisition of high-resolution MRI T1 images of 56 participants (36 ASD, 20 TDC). The images were preprocessed, including skull stripping, bias field correction, and spatial normalization. Automated segmentation techniques were applied to delineate the boundaries of the lateral ventricles. Region of interest (ROI) analyses were conducted to measure ventricular volume, shape features, and position within a standard anatomical space. Statistical analyses, including group comparisons and were performed using t-tests.

Results: Preliminary results demonstrated the successful segmentation of the lateral ventricles in the MRI T1 images. Ventricular volumes and shape features were quantified, and their associations with hypoxia were explored. Independent-samples t-test were conducted to compare lateral ventricular volume (mm3) between ASD+ individuals (exposed to hypoxic conditions) and ASD- groups (not exposed to hypoxic conditions). Typically developing controls (TDC) were also compared. There was a significant difference in left lateral ventricular volume for

the ASD+ group (M=7026.67, SD= 2695.47) and the ASD- group (M=4854.22, SD= 1588.17). There was also a significant difference in right lateral ventricular volume for the ASD+ (M=6503.51, SD= 2840.69) and the ASD- group (M=4702.26, SD= 2094.99). The t-tests indicated mean ventricular volume was significantly higher in ASD+ individuals. See Figure 1 for p-values. No significant difference was found within the TDC group.

Conclusion: Our study reveals a significant difference in lateral ventricular volume between individuals with and without exposure to prenatal hypoxia, suggesting a potential association between prenatal hypoxia and ventriculomegaly in autism spectrum disorder (ASD). The results of our study align with previous research indicating that hypoxic conditions during pregnancy can lead to alterations in brain structures, specifically ventricular enlargement. When split by diagnostic group the only significant differences found were in the ASD group bilaterally. This may be due to our relatively low sample size of the TDC group and future research with a larger sample size can more comprehensively investigate these differences. Future research should further investigate whether specific clinical presentations are associated with these anatomical differences. Further research into the underlying mechanisms and clinical implications of these associations could pave the way for targeted interventions and improved outcomes for individuals with ASD. The quantitative analysis of ventricular morphology may contribute to improved diagnostic approaches, personalized treatment strategies, and our overall understanding of the underlying neurobiology, ultimately enhancing health outcomes in affected individuals.

Transcriptome dynamics during vocal fold injury and repair.

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Vocal fold (VF) scarring is the single greatest cause of poor voice after vocal fold surgery and can have a significant negative impact on quality of life. VF scarring is characterized by disorganization in the extracellular matrix (ECM) of the lamina propria as well as alterations in the integrity of the surrounding epithelium and an overall reduction in VF pliability. There is no treatment that can eliminate formed scars or reverse scar formation. Research specific to vocal fold scarring has been limited by a lack of understanding of the contributing subpopulations of cells during injury and repair. The overall goal of this work is to elucidate the subpopulations cells that contribute to the VF injury response and repair in vivo and generate a single cell transcriptional atlas.

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/âˆ†h, where ???? (s-1) is the shear rate, ???? is the fluid velocity (m/s) obtained from the PIV experiment and Î”â„Ž (m) is the thickness of the artificial saliva film above the rim of the sponge. Then, shear stress was calculated as Tw=6Î¼S, where ???????? (dynes/cm2) is the shear stress, ???? is the dynamic viscosity (cP) and ???? (s-1) is the shear rate. Porosity of the sponge was assessed through SEM and image analysis.

Results: For the humanized tissue model, the final flow rate was set at 1 mL/min, corresponding to a shear stress of 0.40 dynes/cm2. To further validate the dimensionality and chosen parameters of the bioreactor, we profiled velocity, pressure, and vorticity in the simulated (FEATool Multiphysics - Finite Element Analysis Toolbox for Multiphysics; MATLAB) sections of the bioreactor perpendicular to the inlet-outlet axis. This 2D section is the portion of the sponge that most directly receives flow from the inlet; it follows that the resulting values are the upper limits. The computational simulation replicated the bioreactor geometry, the artificial saliva properties, the liquid height above the rim of the sponge, the permeability of the sponge, and the inlet flow rate. The velocity was profiled along the width of the sponge, at 4 mm above the rim of the sponge, and used to compute shear rate, viscosity, and shear stress. The results showed that all the parameters were within the physiological range. In addition, we computed the pressure at the two sides of the sponge and obtained the vorticity profile at different liquid heights along the width of the bioreactor. As expected, the pressure on the inlet side of the sponge was greater than that on the outlet side. Little vorticity was found except on the liquid above the sponge and near the outlet. Numerical discrepancies between the DPIV data and the simulation may be stemming from the approximations adopted including, laminar and Newtonian fluid flow, continuous flow media, 2D rather than 3D geometry, and pressure at the outlet set at 0 kg/m2. Despite those limitations, the numerical simulation was found in agreement with the experimental results.

Conclusion: Overall, the computational model was critical to finalize the bioreactor design, as a closed-loop circuit with circulating artificial saliva with a force profile compatible with native forces. The simulation supported the choice of geometry and parameters to approximate physiological values within the oral cavity.

Characterization of Pumilio family gene expression during early avian development

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Neural crest cells are a multipotent stem cell population found in vertebrate embryos that differentiate into a wide array of cells that contribute to skin pigment, facial skeleton, and the enteric and peripheral nervous system, to name a few. These cells become specified at the dorsal neural tube then migrate extensively prior to differentiation through a process called the epithelial-mesenchymal transition (EMT). To exit the neural tube and complete EMT, neural crest cells must downregulate their epithelial characteristics and upregulate mesenchymal characteristics. Defects in these processes can cause a myriad of birth defects; thus, a thorough understanding of the regulation of neural crest development and EMT is essential to identify the etiology of congenital malformations. Our lab has recently shown that neural crest EMT is regulated via post-transcriptional regulation, though the precise mechanism controlling this process is still unclear. Using single cell RNA-sequencing (scRNA-seq) data, we identified members of the Pumilio family of RNA-binding proteins (Pum1 and Pum2) as potential post-transcriptional regulators of neural crest development and EMT. Using hybridization chain reaction (HCR) technology in the avian embryo (Gallus gallus), we have characterized the spatiotemporal localization of Pumilio family gene expression during early stages of neural crest development. Here we show that Pum1 and Pum2, though expressed broadly across the three germ layers, were differentially enriched in ectodermally-derived tissues. We found that Pum1 was expressed more uniformly throughout the forming neural tube and neural crest, whereas Pum2 was enriched in the medial hinge point during neural tube closure as well as neural crest cells poised to undergo EMT. We thus hypothesize that these RNA-binding proteins have separable but important roles during both neural and neural crest development. Our future studies will seek to characterize the roles of Pum1 and Pum2 in early avian development.

Strategies for analyzing gene expression in embryonic bird jaw bone

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Our research investigates craniofacial bone development using an embryonic bird model. Birds are more distant evolutionary cousins to humans than other model organisms like mice, but because birds develop outside the uterus in an egg, we are able to easily make experimental manipulations and watch in real time as craniofacial bone develops. Specifically, we contrast the embryonic development of short-, pointy-beaked Japanese quail (Coturnix japonica) and long-, broad-beaked white Pekin duck (Anas platyrhynchos) to answer questions about which cellular and molecular factors influence lower jaw bone length. Bone cells can be difficult to study; for example, osteocytes are embedded in concrete-like mineralized bone and bone-resorbing osteoclasts tend to be very large cells. Due to the challenging nature of studying bone cells, we developed two strategies for investigating bone-related research questions in bird embryos. First, to compare osteocyte gene expression in quail and duck, we isolated quail and duck lower jaw bones and placed them in a collagen digestion to remove cells from the surface of the bone, then extracted RNA from the remaining bone chips, which allows the osteocytes to remain in their natural extracellular matrix. RT-qPCR analysis of expression of osteocyte-related genes showed the bone chip reflected an enriched osteocyte population. In our upcoming experiments, we plan to compare quail and duck bone chips containing an osteocyte enriched population using bulk RNAseq with short reads. Second, to compare gene expression of osteoclasts in a duck jaw versus in a quail jaw, we designed a chorioallantoic membrane transplantation assay. Quail and duck lower jaws are placed on a ubiquitous GFP chick chorioallantoic membrane in ovo, therefore the GFP-positive osteoclasts in the transplanted quail and duck lower jaws originate from the circulation of the GFP chick. Using the collagen digest mentioned above and filtering for the large osteoclasts removed from the surface of the bone, we plan to compare isolated GFP-positive chick osteoclasts from both quail and duck lower jaws using RNAseq to investigate how the bone cells in the host jaw (i.e. quail or duck jaw) influence gene expression of the osteoclasts from the donor chick. In summary, we

developed unique strategies for investigating the transcriptome of osteocytes and osteoclasts during bird craniofacial bone development.

Porphyromonas gingivalis tyrosine-phosphatase targets STAT3 for subduing IL-6 in gingival epithelial cells

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Objective: We recently characterized the ectonucleotidase-CD73 and its interaction with a key oral colonizer Porphyromonas gingivalis (P.g) significantly abrogates interleukin-6 (IL-6) in epithelial cells. While IL-6 is known as a critical pro-inflammatory cytokine involved in progression of periodontal disease, specific molecular mechanisms by which host IL-6 signaling is regulated during P.g incursion into gingival epithelia are poorly understood.

Methods: Human primary gingival-epithelial-cells (GECs) were infected by P.g 33277 at MOI100 over 24h. STAT3-phosphorylation ± CD73 siRNA-mediated depletion or CD73-overexpression in GECs was determined via Western-blotting. P-STAT3 dephosphorylation by active recombinant P.g tyrosine-phosphatase-0491 (r0491) was measured via Malachite-green-phosphate-assay. Invasion efficiency/intracellular survival of an isogenic P.g mutant strain lacking tyrosine-phosphatase (âˆ†PGN0491) was compared to Wild-type (WT) P.g via qPCR-based antibiotic-protection-assay. P-STAT3 dephosphorylation in r0491-transfected GECs was further studied via confocal-microscopy. IL-6 in GECs with PGN0491 strain or WT P.g was measured via ELISA. The statistical significance ($p<0.05$) was determined by one-way ANOVA and two-tailed Student's t-test.

Results: Infection by WT P.g significantly inhibited STAT3 phosphorylation in IL-6-pretreated GECs. However, PGN0491 strain, which showed comparable invasion efficiency to WT P.g, resulted in significant levels of STAT3 phosphorylation in GECs, pointing the dephosphorylating role of PGN0491. Further, PGN0491 strain showed intracellular survival deficiency while largely elevated IL-6 production was also detected in GECs. Confocal microscopy analysis demonstrated high levels of perinuclear co-localization of P-STAT3 and r0491 transfected into GECs resulting in time-dependent dephosphorylation. Lastly, CD73 enhanced P.g-mediated inhibition of IL-6/STAT-3 axis in infected GECs.

Conclusion: These findings together allude a highly sophisticated host-microbe adaptation mechanism by which P.g specifically targets host IL-6/STAT3 axis in GECs and aids in weakening the host pro-inflammatory IL-6. The depicted mechanism may have a direct impact on the dysbiotic presence of P.g in human gingival mucosa and could be applicable to other intracellular pathobionts.

Porphyromonas gingivalis Invades Brain Microvascular Endothelial Cells and Impacts Glutamine Metabolism for Intracellular Survival

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Chronic periodontitis affects over 60 million aging Americans and has emerged as a key facet in the development of vascular dysfunction-impairment and related-dementia (VCID/RD). The DNA of Porphyromonas gingivalis (P. gingivalis), the key pathobiont implicated in periodontitis, has been detected in the brains of Alzheimer's Disease patients indicating that P. gingivalis is capable of crossing the blood brain barrier and possibly invading brain microvascular endothelial cells (BMVECs), thus contributing to neurocognitive dysfunction. Because the role that BMVECs play in the formation of neurodegenerative diseases remains largely elusive, we wanted to determine if P. gingivalis was capable of replicating within BMVECs and the mechanism by which P. gingivalis infection contributes to the development of VCID/RD. Previous studies from our laboratory has shown that P. gingivalis can utilize host metabolic pathways, specifically glutamine metabolism as a means for survival in human gingival epithelial cells. We hypothesized that chronic P. gingivalis infection leads to progressive VCID/RD by also utilizing glutamine metabolism to invade and replicate within BMVECs. To test this hypothesis, we infected human BMVECs with P. gingivalis for varying hours (0h-24h) to determine if P. gingivalis was capable of invading and surviving within these cells. We observed a significant temporal increase in the intracellular levels of P. gingivalis within BMVECs ($p<0.005$). Next, we utilized a luminescence-based glutamine to glutamate conversion kit which is catalyzed by glutaminase- 1 (GLS1) to determine if P. gingivalis was dependent upon glutamine metabolism for survival. We observed a significant increase in the glutamate conversion levels during P. gingivalis infection when compared to uninfected cells which was aberrated by the addition of BPTES, a GLS1 inhibitor. These results signify that P. gingivalis is not only capable of invading BMVECs but is also able to convert host glutamine to glutamate through GLS1 to successfully replicate and prolong survival.

Identification of Initial Signs of Osteoradionecrosis using Retrospective Electronic Health Record Review

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Patients with head and neck cancer treated with radiation therapy have a lifetime risk of developing osteoradionecrosis (ORN). Several definitions of ORN require clinical signs of non-healing bone exposure, however, studies have shown that early bony changes may be detected on advanced imaging without clinical evidence of exposed bone. Early detection of radiation-associated bony changes may increase the opportunity to intervene with conservative measures and reduce ORN progression. Thus, the primary aim of this study is to determine if initial signs of ORN are present on imaging (primarily CT) without clinical findings of exposed bone. A retrospective electronic health record review of 91 patients treated with radiation for head and neck cancer at one institution who were suspected to have ORN based on initial screening of 1576 charts was completed. Charts reviewed by an Oral Medicine clinician confirmed presence of suspected ORN in 82/91 patients based on clinical and imaging findings, 31 (38%) of which had initial signs on advanced imaging (primarily CT) without clinical signs of exposed bone. This study revealed that early signs of bony changes consistent with ORN development may be evident on CT without clinical signs. Use of advanced imaging per cancer treatment and post-radiation cancer surveillance protocol may provide mechanism for early detection of ORN in high risk patients treated with radiation therapy for head and neck cancer and provide an improved mechanism for assessment of response to conservative interventions, such as pentoxifylline and vitamin E or hyperbaric oxygen therapy, compared to clinical examination and less sensitive dental x-ray imaging alone.

Elucidating the role of keratin 75 in enamel using Krt75tm1Der knock-in mouse model

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Keratin 75 (K75) was recently discovered in ameloblasts and enamel organic matrix. Carriers of A161T substitution in K75 present with the skin condition Pseudofollicullitis barbae. This mutation is also associated with high prevalence of caries and compromised structural and mechanical properties of enamel. Krt75tm1Der knock-in mouse (KI) with deletion of Asn159, located two amino acids away from KRT75A161T, can be a potential model for studying the role of K75 in enamel and the causes of the higher caries susceptibility associated with KRT75A161T mutation. To test the hypotheses that KI enamel is more susceptible to a simulated acid attack (SAA), and has altered structural and mechanical properties, we conducted in vitro SAA experiments, microCT, and microhardness analyses on 1st molars of one-month-old WT and KI mice. KI and WT hemimandibles were subjected to SAA and contralateral hemimandibles were used as controls. Changes in enamel porosity were assessed by immersion of the hemimandibles in rhodamine, followed by fluorescent microscopy analysis. Fluorescence intensity of KI enamel after SSA was significantly higher than in WT, indicating that KI enamel is more susceptible to acid attack. MicroCT analysis of 1st molars revealed that while enamel volumes were not significantly different, enamel mineral density was significantly lower in KI, suggesting a potential defect of enamel maturation. Microhardness tests revealed that in KI enamel is softer than in WT, and potentially less resilient to damages. These results suggest that the KI enamel can be used as a model to study the role of K75 in enamel.

Maternal weight trajectories and eating behaviors in the Study of Latinos Family Lifestyles Outcome Research (SOL-FLOR)

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Food addiction and reward-related eating are known risk factors for obesity. Whether weight changes in the reproductive years affect maternal eating behaviors is unknown. We aim to identify weight trajectory classes in a diverse group of Hispanic/Latina women and examine the association with eating behaviors. The Study of Latinos Family Lifestyles Outcome Research (SOL-FLOR; 2019-2022) is enrolling women from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) who became pregnant after the baseline visit (2008-2011) and their offspring (N=281). Among women eligible for SOL-FLOR (n=548), we identified weight trajectories using a latent class growth model of body mass index from up to 4 weights, self-reported for age 21, 1 year prior to baseline, baseline, and at a 6-year follow-up. Two weight trajectories were identified, Trajectory 1 (74%) started with lower BMI and had a slower rate of weight gain and Trajectory 2 (26%) started with higher BMI and had a higher rate of weight gain. The 13-item Reward-based Eating Drive scale was administered to women in SOL-FLOR, z-scores for each item were calculated and then averaged. Food addiction was measured through the modified Yale Food Addiction Scale 2.0; 7 items were summed and contribute to a diagnosis of food addiction. Linear and logistic regression models tested for the association between maternal weight trajectory and food addiction and reward-related eating behaviors, adjusted for education, income, Hispanic/Latina background, and nativity/years in the US. Compared to Trajectory 1, Trajectory 2 was associated with food addiction symptoms (OR=3.19 (95% CI 1.37, 7.42)). No significant associations were observed with reward-related eating (B=0.08 (95% CI -0.10, 0.26)). Hispanic/Latina women with a higher weight trajectory were more likely to report symptoms of food addiction. Screening for food addiction in women with higher weight gain may be beneficial.

Perinatal high fat diet induces systemic inflammation during development and decreases expression of gut peptides in adulthood

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Rat offspring of dams fed a high-fat diet during gestation and lactation have altered feeding behavior and are more susceptible to obesity. Previous data from our lab also show that maternal high-fat (HF) diet results in increased GI permeability and gut dysbiosis at weaning. Here we hypothesized that these changes result in increased systemic inflammation during development and could impact enteroendocrine cell (EEC) function and expression of gut peptides. EECs, which are specialized cells distributed throughout the gastrointestinal (GI) tract, are responsible for sensing luminal content and releasing hormones in response to nutrients. Timed-pregnant Sprague Dawley rats were fed either standard laboratory chow (CH) diet (13% kcal fat) or a purified HF diet (60% kcal fat) during gestation and lactation (n=6/diet). On postnatal day (P)1, litters were culled to 10 pups per litter (5 male, 5 female). On P21, plasma was collected from male and female pups for ELISA to measure C-reactive protein (CRP), a general marker of systemic inflammation. Cholecystokinin (Cck), glucagon like peptide 1 (Gcg), and peptide YY (Pyy) expression levels were assessed throughout the small intestine using qRT-PCR in adult offspring as measures of EEC function. There was an overall effect of maternal high fat diet increasing plasma CRP ($P<0.05$), in male (CH:

1044.0 ±156.5 vs. HF: 1521.0 ±124.1 ug/mL) and female (CH: 1074.0 ± 194.5 vs. 1578.0 ± 186.2 ug/mL) offspring. In adulthood, HF male offspring had significantly lower expression of Cck, Gcg, and Pyy in the small intestine. These findings suggest that exposure to maternal high fat diet induces peripheral inflammation in offspring during development and may have a long-term negative impact on EEC function.

Trends in Diabetes Screening Before and After the SARS-CoV-2 Pandemic Shelter-in-Place Mandate among Adult Patients with Prediabetes in an Integrated Health Care System in Northern California.

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BACKGROUND AND OBJECTIVES: The COVID-19 pandemic, its mandated shelter-in-place (SIP) orders, economic shutdowns, and related restrictions resulted in decreased utilization of preventive health care services. It is unclear how it may have affected screening for type 2 diabetes. This study evaluated the changes in type 2 diabetes screening associated with the SIP mandate, and whether disparities in type 2 diabetes screening among Medi-Cal patients and those from racial and ethnic minority groups were observed during the SIP mandate.

METHODS: Using electronic health record data, we identified 578,585 adults, ages 18-89 years old with a BMI ≥ 25 (or ≥ 23 if Asian American) and with prediabetes between January 1, 2019, and December 31, 2021, at Kaiser Permanente Northern California (KPNC), an integrated health care system. Prediabetes was defined as either a fasting plasma glucose value of 100-125 mg/dL or A1c of 5.7-6.4%. We used an interrupted time-series analysis of aggregated weekly type 2 diabetes screening rates per 10,000 patients to evaluate whether there was a decrease in screening associated with the start of the SIP mandate (March 19, 2020), and an increase in screening associated with the lifting of the SIP mandate (June 1, 2020). The interrupted times series analysis was operationalized by fitting segmented linear regression models using generalized least squares, with aggregated weekly rates of type 2 diabetes screening per 10,000 patients as the outcome. We also examined heterogeneous effects to evaluate if the changes associated with the SIP mandate varied by insurance status and race and ethnicity. We accounted for autocorrelation using autocorrelation function (ACF) and partial ACF plots along with likelihood ratio tests.

RESULTS: Screening for type 2 diabetes decreased significantly during the SIP

mandate. In the 14 months prior to the SIP mandate, on average 121/10,000 patients were screened for diabetes per week with an underlying stable trend. At the start of the SIP mandate, the model found a significant drop in screening of 122/10,000 patients per week, followed by a significant and steady increase in the trend of screening of 7.4/10,000 patients per week between March 19 and June 1, 2020. At the lifting of the SIP mandate on June 1, 2020, the model found a significant increase in screening of 50/10,000 patients per week, followed by a return to pre-pandemic trends. These findings were robust to sensitivity analyses which accounted for seasonal trends. The models that examined heterogeneous effects found that the association between the SIP mandate and diabetes screening was similar across insurance categories and racial and ethnic groups.

CONCLUSIONS: The COVID-19 pandemic SIP mandate was associated with an abrupt decrease in diabetes screening among adults with prediabetes at KPNC, followed by a rebound to prepandemic levels soon after the lifting of the SIP mandate. These findings demonstrate a worsening of testing rates during the COVID-19 SIP mandate on adult patients with prediabetes being investigated for type 2 diabetes.

Therapeutic Effects of Selenium for Cadmium Induced Effects on Vision and Morphology

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Cadmium (Cd) is a metal that is increasingly found in groundwater, soil, and drinking water due to industrial pollution. In humans, Cd-exposure is linked to cognitive impairments, macular degeneration, cancer and other pathologies. Selenium (Se) is a naturally occurring, nonmetallic element. In living organisms, Se is essential to maintain bodily function due to its presence in proteins, regulation of redox processes, and neuroprotective characteristics. Because of its narrow dose range, Se poisoning is common and detrimental, resulting in the decline of neurological performance. To assess the therapeutic potential of Se, we acutely and chronically exposed larval zebrafish to a combination of 10 ¼g/L Se, 2.5 and 5.0 ¼g/L Cd and system water (control). During a 5-day period prior to measuring photomotor responses and morphology in our preliminary study, we found that fish exposed to a mixture of Cd and Se had significantly fewer morphological deformities in comparison to cadmium-exposed fish. Selenium-exposed and control fish had a similar frequency of morphological defects. There is a tendency for acute selenium exposures to mitigate the adverse effects of Cd in the photomotor assay in a concentration dependent manner. Chronic exposure to selenium followed by acute exposure to Cd leads to a static response to photomotor stimuli. Thus, the therapeutic effects of Se may depend on the exposure timing and the concentration of Cd. Our study sets the foundation for using high-content screening with zebrafish to uncover the restorative effects of selenium.

Peturb-seq to Identify Genes That Modulate the Transcriptional Phenotype of FRDA Lymphoblastoid Cells

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Friedreich's Ataxia (FRDA) is a rare neurodegenerative disease most commonly caused by a homozygous trimeric repeat expansion in the first intron of the FXN gene. These repeats of GAA lead to repression of the Frataxin protein, an essential mitochondrial protein in iron sulfur cluster biosynthesis. The major cell types affected are those demanding the most energy: neurons, heart cells, pancreatic cells. FRDA patients, however, experience great phenotypic expressivity, and there is little to no explanation as to the molecular underpinnings of this variability. To address this, our lab plans to conduct a CRISPR screen with a single cell RNA sequencing read out (Perturb-seq) utilizing the Epigenome library on 6 lymphoblastoid cell lines; 3 FRDA and 3 WT. Here we report 405 differentially expressed genes (DEGs) between FRDA and WT with FDR corrected p-values <0.05 and fold change >2 or <-2. A total of 279 genes were over expressed while 126 genes were under expressed. The top 25 DEGs were also determined with FDR p-values <5E-7. Gene ontology utilizing KEGG identified the main enriched pathways being immune response, extracellular membrane, and gene expression. There are enough DEGs to create a gene expression profile (GEF) to continue with the screen; thus these findings will be utilized in the next steps of our project.

Toxicity of the phthalate replacement di-2-ethylhexyl terephthalate (DEHTP) and its metabolite on the mouse ovary

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Di-2-ethylhexyl terephthalate (DEHTP) is an alternative plasticizer for its structural isomer di-2-ethylhexyl phthalate (DEHP), a known endocrine disrupting chemical. DEHTP is considered safe for use by the chemical industry and is used as a plasticizer in various polyvinyl chloride products including children's toys and medical tubing. Exposure to DEHTP has raised concerns because its metabolites have been detected in humans at higher concentrations than the phthalates it replaces. This research tested the hypothesis that DEHTP is an endocrine disruptor due to its structural similarity to DEHP. To investigate these concerns, the impact of exposure to DEHTP and its metabolite mono-2-ethylhexyl terephthalate (MEHTP) was studied on the mouse ovary with a particular interest in the development of follicles, the structures that contains developing oocytes. Adult female CD-1 mice were dosed with vehicle (tocopherol stripped corn oil) or 10 µg/kg, 100 µg/kg, or 100 mg/kg of DEHTP for 10 days. Following treatment, ovaries and sera were harvested for histological assessment of follicle numbers and measurement of steroid hormone levels. For in vitro investigation, follicles were dissected from ovaries of adult female CD-1 mice and cultured with vehicle control (DMSO) or various concentrations of DEHTP ranging from 0.1-100 µg/mL to determine whether the presence of DEHTP has an adverse effect of follicle growth and development. Follicle growth was measured every twenty-four hours for five days. At the end of the culture period, media samples were collected to analyze steroid hormone concentrations We found that 1µg/ml DEHTP and 0.1, 10, and 100 µg/ml MEHTP significantly inhibited follicle growth in vitro compared to controls, which is similar to previous studies using DEHP and its metabolite monoethylhexyl phthalate (MEHP). In vivo, decreased primordial follicles and increased primary and abnormal follicles at 100 µg/kg and 100 mg/kg compared to control suggest accelerated follicle development. In contrast, we did not find statistically significance differences in hormone levels following exposure of DEHTP and MEHTP. Further study should be done on the toxicity of DEHTP to ensure it is a safe and sustainable chemical for use.

The Impact of Co-Exposures on Pediatric Obesity and Sleep Behaviors in Rural Appalachian Children

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Cardiovascular disease (CVD) remains the leading cause of death worldwide and has its origins in early life. Some hard-to-reach rural communities, Appalachian regions in particular, may be disproportionately burdened with higher rates of cardiovascular morbidity and mortality. Cardiometabolic health, including high BMI and hypertension (HTN) during childhood and adolescence, is an urgent public health concern. Obesity and cardiovascular risk factors in childhood increase the risk of CVD in later life. Secondhand tobacco smoke exposure (SHS) is a common early-life exposure that may contribute to future risk for obesity. Co-exposure to air pollution (e.g., metal mixtures) and SHS may also influence early-life pediatric obesity, but few studies have attempted to extricate their joint effects. This association may be stronger in children with poor sleep behaviors. Biomarkers of exposure to metals and SHS have been linked to self-reported sleep disturbances, such as insufficient sleep, nocturnal leg cramps (an indicator of poor sleep health), delayed sleep onset, frequent night awakenings, and a diagnosis of obstructive sleep apnea; however, this co-exposure has not been examined in children in relationship to obesity. Moreover, residents of Appalachia have the nation's highest rates of insufficient sleep, but the reasons for this are unclear. The central hypothesis is that co-exposure to SHS and heavy metals will result in a statistically significant increased prevalence of childhood obesity. We also hypothesize that poor sleep behaviors will moderate this relationship. To accomplish this, the study will accomplish the following specific aims: Aim 1. Examine the relationship between SHS exposure and BMI in a rural Appalachian pediatric cohort. Aim 2. Explore the relationship between co-exposure to SHS and metal mixtures on BMI. Elucidating the role of sleep, an upstream, modifiable health behavior, in the complex relationship between co-exposures to multiple environmental factors and pediatric obesity may lead to tailored interventions that target multiple health risk factors including poor diet, sedentary behavior, and tobacco use in children and families. These interventions are needed to prevent

adverse health outcomes related to tobacco use and obesity in a vulnerable, understudied population.

The Effect of Thyroid Stimulating Hormone on Brown Adipose Tissue in Humans

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Obesity and diabetes are two main public health concerns due to their high impact on global morbidity and mortality. In an effort to find new treatment strategies, highly active metabolic tissues such as brown adipose tissue (BAT) have been studied as potential targets. Animal studies have shown that TSH may have a direct local effect on BAT mass and activity by stimulating local T3 production via increased deiodinase-2 (DIO2) expression and also by increasing the expression of uncoupling protein-1 (UCP-1), the key molecule in BAT thermogenesis. We hypothesized that circulating TSH has a direct local effect on BAT mass and activity by increasing local TH production in adult humans.

Study Aims: Study 1. To evaluate whether recombinant human TSH (rhTSH) administration stimulates BAT mass and activity in adult humans.

Study 2. To evaluate correlations between circulating TSH and local tissue TH concentrations and thermogenic gene expression in subcutaneous and deep neck adipose tissue collected from patients undergoing thyroid surgery.

Methods: Study 1. We studied 7 patients with thyroid cancer who underwent total thyroidectomy and required rhTSH as part of their standard follow-up procedures. Each patient underwent 2 PET-CT scans for assessment of BAT volume and activity, first one day after the rhTSH administration (high TSH state) and then 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 and serum thyroid function tests, fasting glucose, insulin, HbA1c, and lipid panel were measured in the two TSH states.

Study 2. We collected neck adipose tissue samples from 16 patients who underwent thyroid surgery. Both deep (peri-thyroidal) and superficial (subcutaneous) neck

adipose tissue samples were collected from 15 patients, while only subcutaneous adipose tissue was collected from 1 patient. At the time of surgery, 2 patients had high TSH, 2 had low TSH, and 12 had normal TSH levels. We measured tissue TH (T3/T4) levels in the neck adipose tissue specimens using liquid chromatography/mass spectrometry. We also measured the expression of TH receptors (THR-alpha, THR-beta), TH transporters (SLC16A2, SLC16A10), TSH receptor (TSHR), as well as key thermogenic genes (UCP1, PPARG, CKTM2, ADRB3, DIO1, DIO2, DIO3) in the adipose tissue samples using RNA-sequencing analysis.

Results: Study 1. According to our study design, there was a statistically significant difference in TSH levels ($p = 0.0095$) with stable circulating T4 and T3 between the low and high TSH states. We found no statistically significant difference in the BAT volume and activity (SUVmax) between the high TSH and low TSH states. In addition, there was no significant difference in the muscle (erector spinae) SUV max and resting energy expenditure (REE) between the two states. Age was a significant predictor of BAT SUVmax ($p = 0.008$; coeff = -0.39, CI = -0.65-0.13), while BMI was a significant predictor for resting energy expenditure (REE) ($p = 0.04$; coeff = -203.95, CI = -396.12 - -10.78).

Study 2. We found no correlation between serum TSH and local adipose tissue T3/T4 levels, BAT activity or thermogenic gene expression, suggesting that systemic thyroid signaling may not directly affect local adipose tissue metabolism. We found a significant correlation between tissue T4 but not T3 levels and UCP1 and ADRB3 expression. The absence of a significant correlation between tissue T3 and thermogenic genes, specifically UCP1 may be due to a non-direct relationship between TH concentrations and thermogenesis in adipose tissue.
Conclusions: To get a deeper insight into the complex interaction between the thyroid axis and BAT function in humans, as a continuation of our studies, we plan to evaluate the relationship of TH concentrations in perithyroidal BAT with BAT activity, transcriptome and cellular composition and correlate with circulating TSH and TH levels and other metabolic parameters in 10 hyperthyroid and 10 hypothyroid patients who undergo thyroid surgery. As a control group, we will perform the same analysis in neck subcutaneous adipose tissue in addition to the deep adipose tissue samples collected from these patients.

Community-Academic partnerships with CTSI: Assessing the level of readiness for churches to engage in health promotion activities

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Community-Academic partnerships with CTSI: Assessing the level of readiness for churches to engage in health promotion activities

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Background: Church-academic partnerships are an effective way to address lifestyle risk factors related to cancer, yet few studies assess the level of readiness of churches to engage in cancer prevention and control research activities. Understanding a church's strengths and areas of needs can help with program planning and implementation. A readiness assessment can also determine the members' willingness (collective commitment) and capability (collective ability) to make a change towards cancer prevention and control activities.

A growing number of organizational capacity and readiness assessments are available to assess health promotion activities within churches, however, few focus specifically on cancer prevention and control. Therefore, the purpose of this presentation is to discuss the adaptation of a readiness assessment tool to assess the church's readiness to engage in cancer prevention and control research.

Methods: We followed the QUERI Roadmap for Implementation and Quality Improvement developed by the U.S. Department of Veteran Affairs. We began with the pre-implementation process that involves identifying a problem and a solution; engaging stakeholders; and developing measures and data.

Results: We adapted the organizational readiness for wellness promotion tool developed by Maxwell colleagues, for cancer prevention and control. Our adapted organization readiness tool will assess four domains within cancer prevention and control: community and organizational climate, attitudes and current efforts towards cancer prevention and control; commitment to change; and capacity to implement change. Multiple stakeholders were engaged to adapt the organizational readiness assessment tool for cancer prevention and control. Stakeholders included local church leaders, and experts in cancer prevention and control, religion and health; and community engagement. We developed a 12-page questionnaire to be delivered via REDCap to 20 pastors and leaders.

Conclusion: This tool has the potential to help us understand the degree to which faith-based communities are willing and prepared to address cancer prevention and control. It will also help us to understand the association between church readiness and past engagement in health promotion activities.

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Dual imaging of cytosolic and endoplasmic reticular Ca2+ dynamics in dendrites in vivo

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Synaptic plasticity is thought to support behavioral adaptation by updating how information propagates though neuronal circuits, but this process remains enigmatic due to its many levels of complexity. For instance, multiple circuits transmitting distinct streams of information often target anatomically- and functionally-specialized regions of a neuron's dendritic arbor. This combinatorial complexity is likely key to a neuron's ability to form complex receptive fields that faithfully represent the dynamic environments in which we live. Intracellular Ca2+ release (ICR) from endoplasmic reticulum (ER) was recently shown to operate at this circuit-subcellular intersection, acting preferentially in apical dendrites of hippocampal CA1 pyramidal neurons (CA1PNs) to shape the experience-dependent formation of spatial receptive fields commonly referred to as 'place fields'. However, it remains an open question as to when ICR is engaged: ER-resident Ca2+ has never been directly monitored in mammalian neurons in vivo. To address this foundational gap in knowledge, we simultaneously imaged ER Ca2+ dynamics and ongoing neuronal activity in radial oblique dendrites of individual CA1PNs as mice learned to navigate a series of novel virtual environments. We are now assessing ER Ca2+ dynamics to determine when ICR occurs relative to ongoing somatic and dendritic activity, place field formation, and animal behavior.

Perinatal HIV infection and exposure is associated with saturated and monounsaturated fatty acid levels in 11-18-year-old Ugandan adolescents

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Objectives: The objective of this study is to determine if baseline fatty acid levels differ by HIV status by performing a cross-sectional analysis of a cohort of 11-18-year-old Ugandan adolescents. We hypothesize that long chain polyunsaturated fatty acid (LCPUFA) levels will be lower in HIV-affected children because HIV exposure/infection may cause intestinal dysbiosis and affect fat absorption.

Methods: Perinatal HIV status was defined at enrollment for 383 Ugandan adolescents (aged 11-18 years) as perinatally HIV infected (APHIV), HIV exposed uninfected (AHEU), and HIV unexposed uninfected (AHUU). Serum samples were collected at enrollment, and serum fatty acids were extracted and methylated to fatty acid methyl esters (FAMEs). FAMEs underwent gas-chromatography mass-spectrometry (GC-MS) analysis, and the fatty acids were quantified from resulting chromatograms using TargetLynx. Fatty acid levels are presented as % of total fatty acids. Total and HIV-stratified mean and standard errors of the mean (SEM) were calculated for individual and calculated fatty acid measures using R. HIV-stratified means for each measure were compared using one-way ANOVA and Tukey HSD tests (statistical significance defined as $p<0.05$) using R.

Results: 126 APHIV, 133 AHEU, and 124 AHUU were included in this analysis. The mean age was 13.84 years (SD: 2.41), and APHIV were significantly older than AHEU and AHUU ($p=0.01$). Both APHIV and AHEU have lower palmitic, lignoceric, and total saturated fatty acid (SFA) levels than AHUU (all $p<0.05$). APHIV have higher oleic, eicosenoic, and total monounsaturated fatty acid (MUFA) levels than both AHEU and AHUU (all $p<0.05$). APHIV have higher levels of some omega-6 (n-6) PUFA levels and lower levels of omega-3 EPA (all 0.002).

Conclusions: HIV-affected adolescents had lower SFA levels than their AHUU

peers, while MUFA levels appeared to increase with perinatal HIV status severity. These results, in addition to lower n-6 PUFA levels in APHIV, suggest that Ugandan adolescents perinatally infected with HIV may have a healthier fatty acid profile than their uninfected counterparts.

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Elucidating Regulatory Mechanisms of Lamin B1 Expression in Autosomal Dominant Leukodystrophy (ADLD)

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Autosomal Dominant Leukodystrophy (ADLD) is an ultra-rare, adult-onset disease that primarily affects the white matter of the brain. Patients with ADLD have an increased expression of the LMNB1 gene, which ultimately results in CNS demyelination. The exact reasoning as to why overexpression of this gene causes CNS demyelination is not yet entirely understood. Although most ADLD patients acquire the disease due to tandem genomic duplications involving the LMNB1 gene, a select few acquire the disease due to upstream genomic deletions involving the LMNB1 gene.

Intraspinal microstimulation simultaneously rebalances motor and nociceptive transmission in chronic spinal cord injury

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Spinal cord injury (SCI) results in devastating physiological changes at and below the level of the lesion. For instance, SCI often leads to reduced motor output, dysregulation of spinal reflexes, impaired pelvic floor function and SCI-related neuropathic pain (SCI-NP). Electrical spinal stimulation (ESS) therapies are a promising approach to treat these issues. However, ESS approaches for SCI recovery are generally parametrized for motor rehabilitation only. Motor-targeted ESS approaches rely on the recruitment of sensory afferent pathways and interneuron networks that provide excitatory synaptic drive to spinal motor pools. Several of these pathways are also implicated in the development and maintenance of SCI-NP. Nonetheless, these approaches often fail to assess potential off-target effects regarding spinal nociceptive transmission.

Here, we used spike train analyses to characterize the effects of motor-targeted intraspinal microstimulation (ISMS) on spinal nociceptive neural transmission after SCI. Experiments were conducted in 15 adult male Sprague-Dawley rats with chronic incomplete SCI under urethane anesthesia. All experiments were approved by the Institutional Animal Care and Usage Committee of Washington University in St. Louis. Electrophysiological recordings were conducted 6-8 weeks after a midline T8 contusion. After a T13-L2 laminectomy, microelectrode arrays were implanted at the L5 dorsal root entry zone. ISMS was delivered to the motor pools in the ventral horn, and neural transmission in sensory pathways was characterized in the superficial and deep dorsal horns. Prior to, during, and after ISMS, we mechanically stimulated the L5 dermatome by applying controlled forces ranging from innocuous to noxious. Our primary outcome measure was the change in maximum firing rate [Hz] of nociceptive specific (NS) and wide dynamic range (WDR) neuron populations (analyzed at the animal level) during nociceptive transmission. These changes were quantified by comparing the average maximum instantaneous firing rates prior to, during, and after 30min of ISMS.

We found that only one session motor-targeted ISMS in the injured spinal cord modulated neural transmission in nociceptive pathways of the dorsal horn. Indeed, ~60% of NS and WDR neuronal populations, respectively, reduced their maximum firing rate during induced nociceptive transmission after ISMS compared to before ISMS. We also found the firing rate of WDR neurons progressively decreased with increasing ISMS duration before persisting at that depressed level after ISMS was discontinued.

Our results suggest that motor-targeted ISMS retains its ability to immediately modulate spinal nociceptive transmission and to induce neural plasticity in spinal sensory networks in the chronically injured spinal cord. These actions appear to result in a net decrease in spinal responses to nociceptive sensory feedback. Although future work is required to elucidate the mechanisms underlying these actions, our results suggest that it may be possible to optimize the stimulation paradigm to deliver multi-modal therapeutic benefits after SCI.

Pathogenic ANKRD11 variant identified in an individual with intractable epilepsy and focal cortical dysplasia

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Deep next-generation sequencing approaches have been highly successful in elucidating the role of genetic variation in neurological disorders of unknown etiology, including focal cortical dysplasia type II (FCDII). FCDII is characterized by disorganized or absent cortical lamination, loss of radial neuronal orientation, and the presence of dysmorphic neurons. FCDII has been associated with de novo pathogenic variants often arising post-zygotically that result in hyperactivation of the mTOR signaling pathway. In this study, we sequenced individuals with intractable focal epilepsy who have undergone brain resection, including individuals with FCDII, to identify pathogenic somatic and germline variants that may contribute to disease risk in the subset of genetically-unexplained cases. While many patients may have been found to harbor a disease-causing somatic (post-zygotically-acquired) genetic variant that can explain their condition, the possibility that these patients could have a causative or contributory germline mutation should not be dismissed. DNA isolated from epileptic resected tissue was analyzed in a cohort of 278 patients, including individuals with a diagnosis of hemimegalencephaly (n = 32), focal cortical dysplasia type I and related phenotypes (n = 126), focal cortical dysplasia type II (n = 98), or focal cortical dysplasia type III (n = 22). High depth exome sequencing from a female patient with FCDII revealed a de novo germline truncating mutation in ANKDR11 c.2197C>T (p.Arg733Ter). The ANKRD11 locus encodes an ankrin repeat-containing protein that inhibits ligand-dependent transactivation by recruiting histone deacetylases to the coactivator/nuclear receptor complex. Mutations in this chromatin regulator have been implicated in KBG syndrome, a rare autosomal dominant genetic disorder for which loss-of-function is a known mechanism of disease. KBG syndrome is characterized by distinctive craniofacial features, skeletal malformations, developmental delay, seizures and intellectual disabilities. Our patient displayed some phenotypic similarities to KBG syndrome with mild autism spectrum disorder, intractable neocortical temporal lobe epilepsy with

evidence of FCDII on pathologic review, and intractable nausea and vomiting of unclear etiology. The variant identified in our patient has been previously reported and interpreted as pathogenic by ClinVar. These results provide evidence that ANKRD11 could potentially be a novel gene in the pathogenesis of FCDII. While ANKRD11 has been shown to regulate neuronal migration and positioning during cortical development, additional studies are required to evaluate the role of ANKRD11 on neuronal mTOR signaling, along with finding additional cases supporting this association.

Droplet-based forward genetic screening of astrocyte-microglia cross-talk

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Cell-cell interactions in the central nervous system play important roles in neurologic diseases. However, little is known about the specific molecular pathways involved, and methods for their systematic identification are limited. Here, we developed a forward genetic screening platform that combines CRISPR-Cas9 perturbations, cell coculture in picoliter droplets, and microfluidic-based fluorescence-activated droplet sorting to identify mechanisms of cell-cell communication. We used SPEAC-seq (systematic perturbation of encapsulated associated cells followed by sequencing), in combination with in vivo genetic perturbations, to identify microglia-produced amphiregulin as a suppressor of disease-promoting astrocyte responses in multiple sclerosis preclinical models and clinical samples. Thus, SPEAC-seq enables the high-throughput systematic identification of cell-cell communication mechanisms.

Huntingtin/HAP40 core complex is conserved in Drosophila and modulates Huntington's disease pathogenesis in vivo

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Huntington's disease (HD) is a devastating brain degenerative disorder caused by amplifications of the poly-glutamine tract (polyQ) in Huntingtin (HTT). Aberrations in HTT's physiological functions due to polyQ expansion is one postulated factor in HD pathogenesis; however, the molecular function and regulation of HTT remain to be elucidated. Drosophila (fruit flies) is a powerful genetic model that allows convenient interrogation of evolutionarily-conserved pathways related to human diseases, including HD, given that HTT is conserved in fruit flies. Using a proteomics-based approach, we identified a novel 40kDa protein encoded by an uncharacterized fly gene CG8134 as a strong interactor of Drosophila HTT and further demonstrated it was the functional ortholog of human HAP40, an HTT-associated protein shown recently to modulate HTT's conformation but with unclear physiologic and pathologic roles. Validation experiments in flies and human cells supported conserved physical and functional interactions of HAP40 with HTT across the evolutionarily distant species, and HTT interacts with endosomal membranes in a HAP40-depedent manner. Corroborating with these observations, molecular modeling revealed conserved structural architectures between human and Drosophila HTT/HAP40 complexes, in particular a solvent-exposed membrane-association loop on HAP40. Moreover, genetic interaction assays showed that loss of HAP40 causes similar phenotypes as HTT knockout including a small lysosomal-related granule phenotype, which provides the first in vivo evidence on HAP's regulation of HTT. At the molecular level, HAP40 strongly affected HTT's protein stability, as depletion of HAP40 significantly reduced the levels of endogenous HTT while HAP40 overexpression markedly extended its half-life. Conversely, in HTT-deficient cells, the majority of HAP40 protein was degraded, potentially by the proteasome. Further, polyQ expansion did not significantly alter the affinity of the HTT/HAP40 complex based on computer modeling predictions and in vitro biochemical assays. Lastly, when tested in Drosophila models of HD, HAP40 modulated the neurodegenerative effects of full-length mutant HTT but not HTT exon 1 fragment. Taken together,

our study uncovers a conserved regulatory mechanism of HTT by HAP40, and demonstrates that HAP40 is a conserved partner of HTT, governing HTT's protein stability and normal physiological functions while also modulating the toxicity of mutant HTT, therefore being a potential therapeutic target against HD.

Post-mitotic molecular consequences of Bak1 microexon 5 inclusion that contribute to neuronal loss

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Alternative splicing is a highly dynamic and regulated post transcriptional diversification process vital to neurogenesis and implicated in conditions such as autism spectrum disorder and Alzheimer's disease when misregulated. Among the abundant splicing changes driving neurodevelopment, downregulation of mitochondrial and pro-apoptotic protein Bak1 through the neuron-specific, post mitotic exclusion of its microexon 5 has proven vital to neuronal apoptosis attenuation and early neonate survival. However, the interplay of neuronal and glial molecular response to microexon 5 mis-splicing beyond early post-natal development and in select neuronal subtypes remains poorly understood. Immunofluorescence and western blot studies with Bak1 microexon 5 (Bak1-e5) conditional knockout mice reveal a persistant neuronal loss coinciding with microglia activation and uncleaved caspase 1 and 8 upregulation, all of which are uncharacteristic of apoptosis. Elucidating the mechanisms of Bak1 microexon 5 alternative splicing could have implications in understanding neurological disease and cell death mechanisms.

Increased PARP activation caused by the Parkinson's disease-linked G2019S LRRK2 mutation

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Parkinson's disease (PD) is the most common movement neurodegenerative disorder, with over 10 million cases worldwide. Pathogenic mutations in leucine-rich repeat kinase 2 (LRRK2) cause PD. LRRK2 is a large and complex protein, with an enzymatic core consisting of a Ras-like GTPase and a serine-threonine kinase domain. The most frequent pathogenic LRRK2 mutation is the Gly2019Ser (G2019S) LRRK2 variant, which results in an increase in kinase activity. The molecular mechanisms by which G2019S LRRK2 causes PD are not well understood. Accumulation of DNA lesions and DNA repair defects have been linked to several neurodegenerative diseases, with increasing evidence for a role in PD. Preliminary studies from our lab suggest that the DNA damage response (DDR) is activated in G2019S LRRK2, specifically, the PARP and ATM-mediated DDR pathways. Preliminarily, we found that in G2019S LRRK2 knock-in HEK293 cells, LRRK2 causes increased activation of PARP compared to wild-type cells. Treatment with the PARP inhibitor olaparib rescues LRRK2-dependent PARP activation. In addition, our preliminary studies show that G2019S LRRK2 causes increased activation of ATM signaling than in wild-type cells, indicated by levels of ATM pS1981, CHK2 pT68, P53 pS15, and Î³-H2AX foci. Thus, we hypothesize that G2019S LRRK2 causes increased activation of PARP and ATM-mediated DDR pathways, which may drive Parkinson's disease pathogenesis. Future and ongoing work is focused on further characterizing the role of PARP and ATM-mediated DDR in LRRK2 PD. Overall, findings from these studies may provide increased understanding of the molecular mechanisms that drive PD pathogenesis and potentially identify a novel signaling axis for targeted therapy.

Effects of Repeated Head Injury on Circadian Response to Environmental Cues

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Sleep is critical to overall health and wellbeing, such that sleep disturbances can indicate underlying pathology. Acute head injury can disturb sleep, evidenced by post-traumatic sleep and circadian rhythm disruption. However, the cumulative effects on sleep-wake behavior of repeated closed head injury, such as those modeled by weight drop injury in the mouse, remain unclear. We hypothesized that closed head injury every other day would disrupt sleep-wake behavior, particularly at light cue transitions, and effect sizes would be cumulative with subsequent injuries.

Male C57BL/6J mice were randomly assigned to either sham (n=5) or weight drop head injury (Height: 94cm, Weight: 100g) that allowed free head rotation every other day for two weeks (6 total) in the hour before the active cycle (ZT=11); sham received anesthesia without injury. Sleep-wake behavior was recorded as percent sleep and mean bout length using non-invasive piezoelectric sensor sleep recording chambers every hour before and after each injury.

A main effect of injury on sleep-wake behavior was evident after the first injury, when head-injured mice slept less than sham during the light change 12 hours post-injury (ZT=0). No significant effect on total sleep in the light period suggested an injury effect on the circadian response to environmental cues. Subsequent head injuries had no effect on sleep-wake behavior or circadian responses. Thus, days between head injuries may impart conditioning or resilience with regard to sleep. Further studies will confirm conditioning versus cumulative effects on sleep and cerebrovascular permeability based on inter-injury intervals.

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Characterization of Temporospatial Changes in Resident Microglia Following Traumatic Brain Injury

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Traumatic brain injury (TBI) results in long-term cognitive deficits and is a risk factor for neurodegenerative disease such as dementia. Neuroinflammation is a key factor elicited by resident microglia and peripheral-derived myeloid cells. The aim of this study is to identify the various temporospatial microglial changes that occur following controlled cortical impact (CCI) injury and how these changes may be influenced by recruitment of peripheral immune cells. GFP chimeric mice were generated in order to identify resident vs infiltrating myeloid cells and histological evaluation was performed at 4hrs, 1d, and 3 days post-injury (dpi). Our findings indicate that there is a progressive increase in the quantity of both microglial and peripheral-derived macrophages (PDMs) in the lesion and peri-lesion cortex. Using Iba1 immunostaining, we find that both GFP+/Iba1+ and GFP-/Iba1+ cell types show an increase in proliferation at 3dpi compared to 1dpi or 4hrs. The morphological changes of microglia also indicate a greater presence of cells that are amoeboid near the lesion core compared to the adjacent or contralateral cortex, which were ramified. Arg1+ staining also revealed that there is a low percentage of microglia expressing this anti-inflammatory protein at 3dpi indicating that most cells were pro-inflammatory in nature. In addition, novel object recognition has been conducted to correlate the effects of microglial activation on memory function up to two months post-injury. These studies add to our understanding of the temporospatial activation state of microglia following TBI.

To share or not to share? Research Participants Perspectives Regarding their Research Data Sharing Preferences

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Background: Current national recommendations focus on making research data more accessible to other researchers and the larger public for transparency, reproducibility, and greater scientific advancement; however, the public's perspective remains inadequately understood. Participants who do not have a clear understanding that their data could be stored and shared for later uses can become hesitant to participate in research and may feel less trust in the research enterprise.

Objective: In this study, we evaluated participants' preferences on (1) the specific type of research health data they want to share with external groups and (2) evaluated how deciding what groups can access their data impacts their willingness to participate in research.

Methods: A cross-sectional survey was administered in March 2023 using Prolific to recruit a sample balanced on age, gender, and race to match the U.S. census. Differences in responses by self-reported race were assessed with the Wilcoxon rank sum test for continuous variables, and the Pearson's chi-squared test and Fisher's exact test for categorical variables.

Results: Among 610 participants, 50% were female, with an average age of 46 years (SD: 16). Overall, 78% of participants self-identified as White, and 7% self-reported identifying as Hispanic or Latino. There was variation by race in the degree to which participants were comfortable sharing specific data with external entities (Table 2). For example, when sharing with public platforms, a smaller proportion of Black/African American participants (37%) would share their biological data compared to White (54%), Asian (44%), and participants that identified as another race (52%). In addition, a lower proportion of Black and Asian participants would share their clinical symptoms, sexual health and fertility information, and imaging data with public health organizations and health policy institutions.

Discussion: A legacy of disenfranchisement and abuse that has led to distrust in the research community among racial minorities, and evidence persists of regarding re-identification risks for the de-identified medical data. It is thus important to ensure that data sharing policies safeguard patient autonomy and privacy. Participants who do not want their data to be shared for secondary uses may show reduced willingness to participate in research and diminished trust in the research enterprise. Our results highlight the importance of providing participants with control over data sharing decisions to not deter them from participating in research studies, especially in historically underrepresented communities. The informed consent process can serve as an avenue to increase willingness to participate in future research studies by explaining possible secondary uses of health data in clear language and allowing participants to specify the recipients of their data and the type of data shared.

Assessing NPC migration and neurite extension using a hiPSC-derived model of SCN3A encephalopathy

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Pathogenic variants in SCN3A, a gene encoding the voltage-gated Na+ channel ±-subunit Nav1.3, are associated with SCN3A-related neurodevelopmental disorder, which includes a clinical spectrum of epilepsy, developmental delay/intellectual disability (DD/ID), and malformation of cortical development (MCD). Affected patients present with some combination of these 3 core features. Interestingly, MCD is atypical of a channelopathy yet it is surprisingly a key characteristic phenotype in SCN3A encephalopathy. Although SCN3A is expressed highly in the brain during embryonic development, the mechanism(s) by which mutations in the Nav1.3 channel result in MCD remain unknown.

In order to address this question, we generated induced pluripotent stem cell (iPSC) lines from a patient with the SCN3A-I875T variant-which is associated with all 3 core phenotypes - and its CRISPR-corrected isogenic control. We perform a 2D directed cortical differentiation of these iPSC lines through an NPC stage to cortical neurons in order to model human cortical development in vitro.

To specifically examine early-stage neurodevelopment potentially relevant to SCN3A encephalopathy pathogenesis, we developed a neurosphere migration assay using 3D aggregates of NPCs. With this assay, neurospheres can be fixed, immunostained, imaged, and analyzed to assess NPC migration and neurite extension. The establishment of this hiPSC-derived model system will allow us to investigate the mechanistic underpinnings of cortical malformation in SCN3A encephalopathy.

Association between Neighborhood Disadvantage and Cognition in Older Adults with Focal Epilepsy

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Rationale: Epilepsy is associated with significant health disparities, including disparities in the prevalence and incidence of epilepsy, access to care, and epilepsy outcomes. Several social determinants of health (SDOH) have been identified as contributing factors to these disparities, including economic deprivation, education and occupational attainment, discrimination and stigma, and sociocultural factors. The area deprivation index (ADI) is a proxy measure for assessing neighborhood-level socioeconomic disadvantage and it has been shown to be associated with multiple adverse health outcomes. In young-to-middle aged patients with epilepsy, greater neighborhood disadvantage has been associated with worse cognition. Here, we examine the relationship between neighborhood disadvantage and cognitive, vascular, clinical, and sociodemographic profiles in older adults with epilepsy.

Methods: Thirty older adults (mean age= 66.9 years; mean education=15 years; 50% female; 78% Non-Hispanic White) completed cognitive testing including measures of learning and memory, language, processing speed, and practical judgement. A composite score was calculated for each cognitive domain by averaging across tests. Demographic, vascular, and sociodemographic information were obtained at a study visit. ADI deciles were calculated using the Neighborhood Atlas (UWSMPH) and classified as high (i.e., 6-10) or low (i.e., 1-5).

Results: Thirty-three percent of older adults with epilepsy had a high ADI (i.e., 6-10), suggesting significant socioeconomic disadvantage. Poorer performance on measures of learning ($r=-.371$, $p=.043$), memory ($r=-.402$, $p=.028$), and processing speed ($r=-.383$, $p=.037$) were associated with higher ADI (most disadvantaged); Figure 1. Those with higher ADI also demonstrated greater impairment in practical judgment ($r= -.382$, $p=.041$). Lower ADI (least disadvantaged) was associated with higher personal and family income ($p<.001$), and there was a trend towards greater years of education being associated with lower ADI ($p=.088$). There were no other

differences in demographic, clinical, and vascular variables between patients with high and low ADI.

Conclusions: These preliminary findings demonstrate an association between neighborhood disadvantage and patient individual sociodemographic factors (i.e., income, education) and cognitive performance in older adults with epilepsy. Neighborhood disadvantage has been associated with an increased risk for developing Alzheimer's disease and related dementias (ADRD). Given the bidirectional relationship between epilepsy and ADRD, the ADI metric can help identify patients that are at increased risk for progressive cognitive declines and developing dementia. Importantly, neighborhood disadvantage is a modifiable SDOH that could inform epilepsy-related policy aimed at identifying the most at-risk neighborhoods and developing system-level interventions focused on increasing access to epilepsy care, education, and resources.

Neuronal and Behavioral Consequences of Early-life Seizures

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The high comorbidity between epilepsy and autism may be related to the fact that both diseases are associated with developmental neuronal abnormalities, including structural, transcriptional, and epigenetic changes. Early-life seizures have been correlated with neuronal loss, memory impairment, and negative cognitive outcomes. Likewise, autism is associated with neuronal and synaptic abnormalities leading to deficits in communication and social behavior. While both etiologies are multi-factorial, there is clearly a link between the two diseases. Therefore, it is a critical need to understand how early-life seizures are affecting the trajectory of brain development. Here we aim to correlate early-life seizures with a comprehensive set of behavioral, transcriptional, and epigenetic outcomes in mice. We propose to develop an early-life seizure model in WT CD-1 mice that will induce changes to hippocampal-dependent behavior and neuronal activity. Pentylenetetrazol (PTZ), a GABA-A receptor antagonist, promotes archetypal histological, transcriptional, and epigenetic changes in the brain that induce autism-like social and cognitive impairments in mice. Mice were exposed to either Saline or PTZ to induce repeated seizures during a critical window of hippocampal development. Characterization of consequences of early-life seizures were tested beginning at 1 month of age using a suite of assays that test for autistic-like behavior. Lastly, by way of single-cell multiomics we will identify transcriptional and epigenetic signatures of early-life seizures by assaying both gene expression and chromatin accessibility at multiple timepoints. Mice were exposed to PTZ or Saline on postnatal day 7. Following seizure induction brains were then harvested at the 1hr and 24hr timepoints. From scRNA-seq data we observed an increase in neuronal activity as there was increased expression of Immediate Early Genes at the 1-hr timepoint. ï¿¼Together, these findings will elucidate the association between early-life seizures and neuronal/behavioral consequences, which may inform the connection between autism and epilepsy.

No Advantage of Pragmatic Inference for Vocabulary Retention in Children with Autism

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Immersed in a world full of potential referents, language learners often need to reason about other's mind and make pragmatic inferences for referential resolution (Tomasello, 2003; Stephan, 2021). Our previous research has found both adults and 6-to-9-year-olds retain words learned via pragmatic inference better than those directly mapped, but not 4-to-6-year-olds whose social cognition skills are still maturing (Saratsli et al., 2020; Trice et al., 2021). As social cognition skills are well-documented as both a critical gatekeeper for typical word learning and a central aspect of Autism Spectrum Disorder (ASD), such situations pose likely barriers to vocabulary acquisition in autistic children (Kuhl, 2011; Baron-Cohen, 1985). However, previous research has focused on the use of present social cues (e.g., eye gazes, joint attention) rather than pragmatic inferences (Tenenbaum, 2014). Furthermore, research beyond in-the-moment mapping is also lacking, with more ecologically valid measures like longer-term retention frequently excluded. Thus, we examine how autistic children learn and retain novel words in a pragmatic-inferential vs. direct-mapping context relative to their typically developing (TD) peers.

34 6-to-9-year-old autistic children from the SPARK registry with functional language abilities (11 girls; M_age=7.6, SD=0.9; M_SCQ=15.8, SD=6.8) and 34 age- and sex-matched TD children (16 girls; M_age=7.4, SD=0.8) learned eight novel words, four per condition, administered through Zoom. During learning in the Direct Mapping Condition (DMC), two distinct toys each held an identical novel object. Participants would hear a phrase such as "Look! I like this bink! It's on the dinosaur!" As such, the novel word could be uniquely mapped to the novel object in a one-to-one mapping. During learning in the Inference Condition (IC), two identical toys each held an identical novel object, and one of these an additional unique novel object. Participants would hear a phrase such as "Look! I like this dinosaur! It's holding a mel!" To resolve the referential ambiguity, children must assume that the speaker intends to be informative and thus the novel word must refer to a distinctive object – the unique novel object. Immediate word

recall was tested after each block. Word retention was tested approximately 20 minutes later for all eight words in a randomized order.

Both groups performed similarly for in-the-moment mapping during learning, showing above-chance performance in identifying the target object in IC (ASD: 0.70+0.05; TD: 0.65+0.05; p's<0.05). Higher SCQ scores in the ASD group correlate with lower learning IC accuracy (Rho=-0.35, p<0.05). For immediate recall, autistic children performed significantly worse than their TD peers (ASD: M_IC=0.48, M_DMC=0.38; TD: M_IC=0.61, M_DMC=0.56; estimate=0.85, z=2.27, p<0.03) with no group-by-condition interaction. Importantly, for retention, a significant advantage of the IC over DMC was only observed in the TD group (M_IC=0.51, M_DMC=0.46, estimate=0.87, z=2.24, p<0.03), but not in the ASD group (M_IC=0.45, M_DMC=0.47, p=0.99). Across immediate recall and retention, the ASD group showed significantly more stable word memory over time (M_ASD_Immediate=0.43, M_ASD_Retention=0.46) than the TD group (M_TD_Immediate=0.59, M_TD_Retention=0.48; timepoint*group: estimate=-0.68, z=-2.40, p=0.02).

In summary, while autistic children can resolve pragmatic inferences similar to their TD peers, they have greater difficulty immediately recalling the mapped words. For retention, unlike in TD. there is no apparent facilitation of pragmatic inference in ASD. However, protective memory mechanism may be in place – particularly for directly mapped words – that allows for recovery and retention.

Cell-Acquiring Fallopian Endoscope for early detection of ovarian cancer

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Ovarian cancer is the deadliest gynecological cancer, with most cases of high-grade serous ovarian carcinoma originating as serous tubal intraepithelial carcinoma (STIC) lesions in the fallopian tube epithelium. The Cell-Acquiring Fallopian Endoscope (CAFE) was designed to optically detect these STIC lesions and collect cells from the suspicious site for further analysis. While approximately 0.93 mm in diameter, the CAFE is able to perform multispectral fluorescence imaging (MFI), white light imaging for navigation, and cell collection. Each of these modalities is useful to locating potentially pathological areas. To find these regions, the CAFE looks for alterations of the autofluorescence of the tissue. Upon identification of a potential STIC lesion, a scrape biopsy collects cells from the region of interest. The prototype CAFE achieved an imaging resolution of 8.77 Î¼m at a 1.01mm distance, and 55.27° full field of view in air. When tested on ex vivo porcine tissue, hemocytometry counts determined that on the order of 10^5 cells per scrape biopsy could be collected. Current progress on the CAFE includes cell collection testing on ex vivo porcine and human tissue, improvements in the imaging resolution, and endoscope assembly.

A Dynamic Bayesian Network Approach To Modeling Engagement And Walking Behavior: Insights From A Year Long Mirco-Randomized Trial (HeartSteps II)

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Introduction: Mobile health (mHealth) technologies such as wearable activity trackers (FitBit, Garmin, Apple Watch, etc.), often in conjunction with digital applications (apps), may be particularly useful for encouraging behavior change among individuals. These technologies can deliver mini-interventions (or prompts) such as anti-sedentary reminders, motivational messages, or opportunities for self-monitoring and reflection. However, despite the potential upside of this message-based approach, the literature provides mixed evidence of their effectiveness to impact sustained behavior change. In addition to prompt or message delivery, mHealth technologies and sensors can also be leveraged to detect contextual conditions (e.g., location, temperature, psychological sate) in order to identify when the participant's conditions are optimum for effective behavior change. Furthermore, there is a lack of modeling tools that can handle missing data, which is pervasive in digital health studies, that can also provide insights into the underlying process of behavior change that respond to ever-changing contextual conditions.

Methods: The primary aim of this study was to apply a Dynamic Bayesian Network (DBN) approach to modeling the ideographic (individual) dynamic relationships between a participant's daily app engagement and walking behavior, with contextual variables including the daily average temperature (in Fahrenheit), daily HeartSteps II intervention messages received by the participant (walking suggestions and anti-sedentary prompts), and day of week (weekend vs. weekday), in conjunction with psychological state variables perceived restedness and perceived busyness. Additionally, this study aims to identify differences in the dynamic relationships of DBN models described above between Hispanic/Latino participants and non-Hispanic/Latino white participants.

Results: Data from 10 participants in the HeartSteps II study (n=5 Hispanic/Latinos

and n=5 non-Hispanic/Latino whites) was used to estimate the DBN models. The average age of the participants in this sample was 46.30 (SD= 7.35). Across participants selected for this study, the average steps per day at baseline was 6,239.16 steps (SD=3,266.15). Across all participants (100%, n=10), there was a strong positive effect of the number of messages/prompts received by the participant on daily application page views with Bayesian Credible Intervals (BCIs) ranging from a mean of 0.22 (SD=0.05, 95% BCI: 0.14-0.31) to a mean of 0.60 (SD=0.05, 95% BCI:0.52-0.68). However, the hypothesized connection between intervention messages and walking behavior was not meaningful. Additionally, among the majority of Hispanic/Latino participants (n=4/5, 80%), there was a strong positive relationship between daily app page views and walking behavior with BCIs ranging from a mean of 0.18 (SD=0.07, 95% BCI: 0.07-0.30) to 0.23 (SD=0.09, 95% BCI: 0.09-0.38). Among both Hispanic/Latino and non-Hispanic/Latino white participants, there were significant idiographic differences in the effects of temperature on walking behavior. For example, one Hispanic/Latino participant had a significant positive relationship between temperature and daily Fitbit steps (mean = 0.12, SD = 0.05, 95% BCI: 0.04-0.21) and another had a significant negative relationship between temperature and steps (mean = -0.2, SD = 0.08, 95% BCI: -0.32 - -0.05). A similar phenomenon can be seen with the relationship between perceived busyness and steps. There were significant idiographic differences among non-Hispanic/Latino white participants with one participant having a negative relationship between the two (mean = -0.17, SD=0.06, 95% BCI: -0.25 - -0.06) and another having a strong positive relationship (mean= 0.58, SD=0.05, 95% BCI: 0.5-0.66), for example. These results would have not been identified without an idiographic modelling approaching and have significant implications for adaptive DBCIs.

Conclusions: This study presents a novel idiographic modeling approach using Bayesian methods to gain a deeper understanding of the complex interrelationships between intervention prompts, DBCI app engagement, and physical activity, given an individual's psychological state, and respective contextual and environmental conditions. Given the pervasiveness of data loss in longitudinal studies, the results from this study also provide a use case for imputing segments of missing time series data using the BayesLDM toolbox. Lastly, the significant differences in DBN models found between individual participants highlights the importance of an idiographic approach, particularly when it comes to identifying individual tailoring

variables in pursuit of adaptive and effective prompt- and message-based interventions.

Characterization of Genetic Markers Involved in the Regulation and Composition of Follicle Cell Ring Canals

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Ring canals are conserved cytoplasmic structures that allow adjacent cells to connect with each other and allow the flow of cytoplasmic contents. Among many species, fecundity is reliant on the formation and stability of these cytoplasmic structures. In this research, we use the Drosophila ovary as our model to study ring canals. In the germline, many proteins have been well characterized with their involvement in the regulation and composition of germline ring canals. However, little is known about ring canals in the ovarian follicle cell lineage. Here, we identify potential markers that may regulate the formation and composition of follicle cell ring canals.

Developing a Chemically-Controlled RAS Toolset

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Using a chemically inducible activator of RAS (NS3a-CIAR), we can directly activate WT-RAS mediated signaling. NS3a-CIAR is composed of protein-peptide interaction between the hepatitis C virus protease (NS3a) and apo NS3a reader (ANR), respectively, that can be disrupted using clinically approved drugs, e.g. Danoprevir. We engineered intracellular and extracellular oligomers fused to NS3a-CIAR to investigate how formation of activated oligomers affects RAS-downstream signaling.

Pseudomonas aeruginosa Displays Enhanced Surface Motility Towards Staphylococcus aureus MRSA in Response to Elevated Calcium Levels

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In patients with cystic fibrosis (CF), Pseudomonas aeruginosa (Pa) is the leading cause of life-threatening lung infections. Its rapid adaptation to the CF lung environment is in part facilitated by virulence factor production. Pa produces numerous virulence factors, some of which affect its ability to interact with other pathogens present in the infected lungs. Staphylococcus aureus (Sa) is a second predominant pathogen in CF lungs and is detected simultaneously with Pain up to 50% of CF patients. The interactions between these two pathogens can shape their impact on the host and exacerbate the disease. Therefore, understanding these interactions at the molecular level will aid in developing novel effective treatments. Calcium (Ca^{2+}) is an important messenger that is present at abnormally high levels in the nasal and lung secretions of CF patients. Previous studies showed an increase in the production of virulence factors by Pa at elevated Ca^{2+} concentrations similar to those commonly detected in the lungs of CF patients. These factors included rhamnolipids and pyocyanin that both have been reported for their importance in the interactions between Pa and Sa. In order to understand how the interactions between the two organisms are affected by Ca^{2+}, we performed an inhibition assay using methicillin-resistant Sa strain MRSA and Pa PAO1. Supporting previous observations, growth of MRSA was inhibited by PAO1. Next, we assessed motility of Pa and Sa when plated next to each other using a clinically relevant Synthetic Cystic Fibrosis Medium (SCFM) with and without added 5 mM Ca^{2+}. In the presence of Ca^{2+}, PAO1 displayed enhanced and directed motility towards MRSA. To determine which component of the earlier elucidated Ca^{2+} regulatory network in Pa plays a role in this Ca^{2+}- dependent motility, we tested a series of deletion mutants lacking individually deleted genes encoding EfhP, CalC, CarP, CarR, and PcrP. Deletion of carR encoding a Ca^{2+} dependent two-component transcriptional regulator abolished PAO1 motility towards MRSA. This suggests that the two-component system CarRS plays a role

in the Ca2+ dependent motility of Pa towards Sa. Current studies aim to elucidate the involved molecular mechanisms of this regulation.

Unraveling ECM Stromal Microenvironment Signaling in TNBC via the Sonic Hedgehog (SHH) Pathway

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Breast cancer remains a leading cause of death in the US. Among breast cancer types, triple-negative breast cancer (TNBC) is associated with a poorer prognosis due to its rapid growth, invasiveness, and high rate of metastasis. Additionally, TNBC has a higher likelihood of relapse compared to other breast cancer subtypes. While the role of Sonic Hedgehog (SHH) signaling in cell fate patterning is well-known, the impact of its graded activity on tumor progression and therapy response remains less understood. Recent studies underscore the critical role of tumor-associated extracellular matrix (ECM) in cancer development and relapse mechanisms. Within the ECM, matrix-bound nanovesicles (MBVs) play a significant role, yet their impact on breast cancer and TNBC mechanisms remains poorly understood.

Properly processed ECMs can serve as naturally derived biomaterials, providing a relevant microenvironment for cellular and mechanistic research with a proven clinical safety record. Tissue-specific ECM hydrogels hold great promise for clinical applications, particularly in the context of precision medicine. Our hypothesis posits that extracellular matrix components act as oncogenic seeds, modulating cellular behavior and impacting drug test models. Our research aims to investigate how stromal ECM and associated MBV components influence a cancer cell model for invasions as a function of the Hedgehog pathway, potentially offering relevant models for drug-testing treatments in breast cancer. We were able to demonstrate that acellular ECM triggers the invasion phenotype, suggesting that ECM preserves oncogenic factors that can recapitulate invasion in vitro.

Anti-CRISPR Delivery for Precision Genome Editing

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Precision control of the dosage of Cas9-based technologies is essential as off-target editing, genotoxicity, and immunogenicity are observed at elevated levels and with prolonged activity of Cas9. Phage-encoded anti-CRISPR (Acr) proteins have been shown to inhibit Cas9, but their size (10-20 kDa) makes them impermeable to the cell membrane. Moreover, existing Acr delivery methods are slow and long-lived (e.g., vectors) or not applicable in vivo (e.g., nucleofection). To address these limitations, we developed the first cell-permeable Acrs: LFN-AcrIIA4 and 6—NLS-AcrIIA4. LFN-AcrIIA4 is a fusion of the nontoxic N-terminal domain of lethal factor protein (LFN) and an Acr (AcrIIA4). Delivery of LFN-AcrIIA4 is mediated by the two-component PA/LFN delivery system derived from anthrax toxin, where protective antigen (PA) proteins bind anthrax receptors widespread in human cells, forming an endosomal pore that LFN binds and uses to translocate the Acr into the cell. We engineered 6—NLS-AcrIIA4 by fusing SV40 nuclear localization sequences (NLS) to the N- and C-termini of AcrIIA4. The cationic nature of the NLS tags mediates the delivery of 6—NLS-AcrIIA4. The main advantages of LFN-AcrIIA4 and 6—NLS-AcrIIA4 are their fast (<20 min) and efficient (<1 μM) cellular entry, short-lived exposure in cells, and in vivo-compatibility. We transfected human cells with Cas9 RNP or Cas9-gRNA plasmids and incubated them with LFN-AcrIIA4 (10 pM-1 μM) and PA (20-100 nM) or with 6—NLS-AcrIIA4 (0.1-4 μM) and validated Acr delivery by monitoring Cas9-mediated knockout, knock-in, and transcriptional control via EGFP and luciferase reporter assays, observing up to 95% inhibition of Cas9 and no cytotoxicity. Next-generation sequencing showed that timing the delivery of LFN-AcrIIA4 or 6—NLS-AcrIIA4 increases the specificity (i.e., the on-target to off-target ratio) of Cas9 and Cas9 base editors up to 1.6-fold at therapeutically relevant sites. PA-mediated delivery of LFN-AcrIIA4 (EC50 100 pM) is generally more efficient than 6—NLS-AcrIIA4 delivery (EC50 500 nM). The receptor dependency of PA/LFN-AcrIIA4 also allows cell-specific delivery when using retargeted PA variants. As a one-component system that does not target cellular receptors, 6—NLS-AcrIIA4 becomes more practical when receptors are inaccessible (e.g., when

Cas9-lipofection reagents cover the cell surface). Ongoing work is focused on using LFN-AcrIIA4 and 6—NLS-AcrIIA4 to increase the specificity of Cas9 in vivo and validate Acrs as co-therapies for the treatment of genetic disorders.

Investigating interstitial fluid flow and increasing substrate stiffness on LECs permeability and morphology

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The lymphatic system is crucial in dietary fat absorption, facilitating trafficking and movement of immune cells, the maintenance of fluid balance and transport of solute and cells. Lymphatic vessels throughout the body are mainly known for transporting solutes, fluids, and cells from peripheral tissues into systemic circulation via a network of lymph nodes. Lymphatic endothelial cells (LECs) line lymphatic vessels, and their type of intercellular junction dictates their permeability. Two lymphatic vessels exist (1) initial and (2) collecting lymphatic vessels. Lymph flow begins in the initial lymphatic vessel that merge into the larger collecting lymphatic vessel, from which lymph is transported into systemic circulation via a network of lymph node.

In initial lymphatic vessels, LECs connect through button-like cell-cell junctions and a discontinuous basement membrane, aiding in the uptake of solutes and fluid. Collecting vessels are larger lymphatic vessels with tighter zipper-like cell-cell junctions and valves that facilitate unidirectional transport1. Collecting vessels have minimal permeability to prevent the leakage of lymph content into the extracellular environment2.

Lymphatic vessels have a unique feature: during static conditions, lymphatic vessels are closed, but when there is interstitial flow (which is constantly experienced by lymphatics), the lymphatic vessels expand, allowing fluid and materials to be pushed into the vessel and increasing permeability.

Methods: In vitro models have been developed to study LEC barrier properties3,4. These models represent in vivo collecting vessel structure and can be used to replicate inflammatory conditions within the body. We use an in vitro transport assay to mimic fluid flow stimulation to measure solute transport when treated with interstitial fluid flow to measure lymphatic permeability. We used a local in vitro permeability assay to assess specific areas where cell-cell junctions are

permeable. I used these assays to study transport across LECs and their proliferation.

Results:

-We found that IFF increases nanoparticle transport by lymphatics more than 5-fold within only a few hours but does not affect monolayer integrity. A

-Additionally, comparing various flow speeds on lymphatic transport, we see a flow-dependent change in transport

-Inhibiting paracellular transport in the presence of fluid flow decreases lymphatic transport of tracers.

-LECs on softer substrate (870Pa) vs stiffer (7kPa) substrate seen with higher total cell count in the soft substrate.

Conclusions: Interstitial flow drives fluid and materials into lymphatic vessels, and fibrous material around collecting lymphatic vessels are associated with increased lymphatic permeability, respectively 5. This suggests that these mechanical cues can affect lymphatic vessel morphology and permeability. Additionally, lymphatic vessels can sense changes in mechanical cues in their microenvironment. However, we still do not understand the underlying cellular mechanisms driving these morphological and cell-cell junction changes in LECs.

Effect of Host Phylogenetics and Host Traits on Microbiome Assembly

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Phytoplankton, a group which consists of eukaryotic microalgae and cyanobacteria, are essential organisms for life on earth. Making up 1-2% of the carbon biomass, phytoplankton contribute over 50% of the total photosynthesis performed annually. Phytoplankton span an unusually large phylogenetic distance, across two domains of life, and have an exterior microbiome amenable to experimental manipulation. Previous studies have outlined the potential effect that host phylogenetics has on the taxonomic makeup of a microbiome. With this background knowledge, I hypothesize that microbiome composition and function will be most similar among phytoplankton species that are closely related, and more divergent among host species that are distantly related. To test this, we obtained 31 species of axenic phytoplankton from various culture collections. Cyanobacteria represented the prokaryotes and the eukaryotic organisms consisted of green algae, red algae, and yellow green algae. Axenic strains were inoculated in sterile jars at a cell density of either 2,000,000 cells/mL or 50,000 cells/mL and covered with a 3.0 µm filter which allows bacteria to pass through and associate with the phytoplankton. The jars were submerged in aquarium tanks filled with mixed freshwater sourced from Lake Miramar and Rancho Penasquitos Creek in San Diego County. After 72 hours, the jars were removed from the tanks for processing, preserving biomass for analysis of the bacterial absolute abundance and community composition. Little is known about the mechanisms by which hosts recruit microbes from their environment. This study asks this question from an evolutionary perspective, and the results will clarify how hosts and their microbiome have co-evolved over evolutionary history.

Integrative Analysis of Cardiac Isoforms of Drosophila Myosin

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All muscle myosin heavy chain (MHC) isoforms expressed in Drosophila melanogaster arise from unique alternative splicing events from a single gene, Mhc. To determine the MHC isoforms present in adult cardiac tubes, we used RT-PCR of isolated heart transcripts. We found that isoforms CardM1 and CardM2 are most prominently expressed. These isoforms differ in their ATP pockets, their relay helices and their converter domains, suggesting they possess unique biochemical and/or biophysical properties. We expressed each of these MHC isoforms in the indirect flight muscles (IFMs) and the tergal depressor of the trochanter (TDT or jump) muscles via transformation with the corresponding cDNA transgenes. Each isoform successfully assembles into normal myofibrils in IFMs and TDTs. Preliminary data suggest that both CardM1 and CardM2 yield impaired flight and jump abilities, with CardM2 having the greater loss in both. Mechanical assays on isolated TDT fibers show that CardM2 is comparatively more capable of eliciting stretch activation, i.e., enhanced force production dependent on mechanical forces applied to the actomyosin bridge. We are now focusing on elucidating the biochemical and structural differences between the cardiac isoforms. CardM1 has been initially observed to have decreased in vitro motility and ATPase activity relative to the fast IFM isoform. Future work will include cryo-EM of actomyosin complexes to observe structural differences and mutagenesis of the cDNA constructs to study biochemical and structural effects of clinically relevant dilated cardiomyopathy mutations previously seen to induce arrhythmia and increased diameters when expressed in the adult cardiac tube.

Regulation of the Mitochondrial Pyruvate Carrier by the Wnt/beta-catenin pathway

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Metabolism is one of the most tightly regulated cellular processes important for the maintenance of homeostasis. The mitochondrial pyruvate carrier (MPC) is a transmembrane protein that resides in the inner mitochondria membrane and facilitates the entry of pyruvate, which is necessary in several metabolic reactions such as TCA cycle. Our lab has previously shown that MPC expression is negatively correlated with Wnt/beta-catenin target genes; however, the molecular mechanism behind the regulation of the MPC by the Wnt/beta-catenin signaling pathway is not known. Based on our preliminary observations, we hypothesize that beta-catenin acts as a repressor of the MPC. To investigate this regulation, we first performed a Fluorescence-activated cell sorting (FACS)-based genetic screening assay in liver cancer cells and found that beta-catenin acts as a genetic repressor of the MPC. Furthermore, we treated liver cancer cells for 48h with GSK3 inhibitors (CHIR99021 or LY2090314) to activate the Wnt/beta-catenin pathway, and MPC protein was analyzed by western blot. Interestingly, we found that MPC protein was reduced in cells treated with CHIR99021 or LY2090314 when compared to untreated cells. To investigate whether MPC expression was affected at the transcriptional level, we performed RT-qPCR and found that MPC1 transcripts were decreased in liver cancer cells treated with CHIR99021 or LY2090314. Overall, our results suggest that activation of beta-catenin results in decreased MPC transcripts and protein levels. Taken together, we proposed a model where beta-catenin acts as a repressor of the MPC in liver cancer cells. Future studies will be focus on characterizing the mechanism by which MPC expression is regulated by the Wnt/beta-catenin pathway and the significance of this regulation in cell metabolism and cell fate.

Visualization and Immunoprecipitation of distinctively tagged PCH-2 strains in C.elegans

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Mitosis and Meiosis are both essential for proper segregation of chromosomes. Defects in mitosis or meiosis produce aneuploid cells and are associated with cancer progression, birth defects and infertility, underscoring their importance to human health. In this experiment, we used C.elegans to better understand the meiotic and mitotic checkpoint protein, pachytene checkpoint protein (PCH-2). PCH-2's role in preventing aneuploidy is complex in both meiosis and mitosis Therefore, in order to determine PCH-2's role, we conducted immunoprecipitations on distinctively tagged PCH-2 strains to identify protein interactors and post-translational modifications that may regulate its function.
We will use strains that our lab constructed which have C-terminally or N-terminally tagged PCH-2. We will also use mutations in PCH-2 thColmenarezat are expected to bind substrates better (EQ mutants). All of the strains were tagged with Green Fluorescent Protein (GFP) and 3X Flag (3XF) in order to visualize the protein in vivo and immunoprecipitate it. We have shown that C-terminally tagged PCH-2 is functional during mitosis but not meiosis. I tested whether the N terminally tagged PCH-2 (3XF::GFP:: PCH2) was functional during meiosis: I assessed meiotic recombination in 3XF::GFP::PCH2, control and pch-2 null mutants and found that 3XF::GFP::PCH2 exhibited no defects in recombination, similar to control worms and different than pch-2 mutants, indicating that this tagged protein is functional for meiosis. Thus, we have two tagged versions of PCH-2, one functional in mitosis, one functional in meiosis. By completing these experiments, we will be able to see if PCH-2 has post-translational modifications or interacting proteins that specifically affect its function throughout mitosis and meiosis.

Elucidating RhlR promoter selection mechanisms in Pseudomonas aeruginosa quorum sensing

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Pseudomonas aeruginosa is an opportunistic human pathogen that is associated with infections in immunocompromised individuals and individuals with pulmonary disorders. Expression of virulence factors is regulated by quorum sensing (QS), a mechanism of bacterial cell-to-cell communication that relies on the production, detection, and response to extracellular signaling molecules, termed autoinducers (AI), to govern the transition of individual behaviors to group behaviors within a microbial community. The regulatory QS network in P. aeruginosa is organized into a complex hierarchy with two canonical LuxI/R pairs: LasI/LasR, and RhlI/RhlR, which produce and detect 3OC12-homoserine lactone (3OC12 HSL), and C4-homoserine lactone (C4HSL), respectively. While RhlR is regulated by the RhlI-synthesized HSL, previous findings have shown that PqsE, a metallo-Î²-hydrolase, increases the affinity of RhlR for the promoters of genes under its control. In some cases, PqsE binding to RhlR is absolutely required for DNA binding to occur. To determine what makes a PqsE-dependent RhlR promoter, we utilized luciferase gene reporter assays in Escherichia coli and electrophoretic mobility shift assays (EMSA) on RhlR-dependent genes hcnA, lecB and a sRNA with unknown function. Luciferase assays showed that transcription is enhanced for hcnA, lecB and sRNA in the presence of PqsE and that this enhancement is independent of C4HSL. Electrophoretic mobility shift assays confirmed that the presence of PqsE increases the affinity for RhlR binding to hcnA, lecB and sRNA. However, except for lecB, PqsE is not required for RhlR binding to RhlR-dependent promoters. Additionally, testing affinity on shortened promoter sequences showed that affinity of RhlR and the RhlR-PqsE complex is decreased, implying that other key structural elements within these promoters alongside the rhl box optimize binding of RhlR. In addition, nucleoid-associated protein (NAP) Fis appears to participate in regulation of RhlR-dependent genes by binding directly to the promoter sequence and effectively blocking RhlR activation.

Modeling expectation-driven endogenous analgesia in mice

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The experience of pain is highly modifiable by an individual's expectations, which can induce placebo analgesia. Placebo analgesia is an expectation-driven reduction in pain perception that can be attributed to a treatment context or operant-induced internal states, such as suggested safety after escape. Some current placebo models in mice and humans rely on a pharmacological conditioning design, where an administered drug, such as an opiate, is paired with cues over many days. Such designs can confound interpretations of reward motivation versus analgesia and may induce compensatory changes related to opioid-induced hyperalgesia, tolerance, or alterations in opioid peptide expression.

Here, we developed a novel non-pharmacological placebo model that utilizes instrumental conditioning to drive expectation-mediated analgesia. In our optimized endogenous analgesia conditioning (EAC) paradigm, mice are placed in an open two-chamber maze with distinct visual contexts and a temperature-programmable floor plate, with continuous free access to explore either chamber. During the instrumental conditioning, the experimental context plate is set at a noxious temperature for EAC-conditioned mice, or an innocuous temperature, for non-conditioned controls, while the other plate remains stably innocuous to serve as the expected safe/pain-free environment. Following multiple within and across-day conditioning sessions where mice can instrumentally escape to the innocuous context, both context plates are set to noxious temperatures and multiple cameras record exploration, motor actions, and nocifensive behaviors as subjects freely explore the inescapable noxious contexts.

We found that EAC mice prefer to spend significantly more time in the formerly innocuous paired context and display attenuated nocifensive behaviors, compared to non-conditioned controls. We are now exploring the role of multiple neuromodulatory systems in the learning and expectation phases of the EAC paradigm, as well as the specific neural circuits involved in the analgesic effects. Collectively, the EAC model demonstrates that context-based nociceptive

conditioning can be used as a paradigm to induce endogenous analgesia in mice and may serve as a strong platform to investigate the malleable nature of pain perception in preclinical pain models.

Investigating the role of Rab GTPases in extracellular vesicle biogenesis and glial uptake

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Secreted extracellular vesicles (EVs) are membrane-enclosed structures that transfer bioactive proteins, RNAs, and metabolites to recipient cells. Released from most, if not all cell types, EVs play critical roles in physiological processes and pathological conditions. In C. elegans, EVs are shed from primary cilia, specialized microtubule-based organelles that protrude from the dendritic tips of sensory neurons, and are environmentally released through a cuticle pore. EVs can also bud from the periciliary membrane of the ciliary base and be phagocytosed by surrounding glia (1). Loss of the G-protein RAB-28 results in EV buildup within the extracellular space between the cilia and surrounding glia, suggesting its function as an EV shedding regulator (2). Release of EVs containing the TRPP channel PKD-2 from the cilium distal tip is unaffected in the rab-28 mutant. However, we have shown that male ciliated sensory neurons shed multiple different EV subpopulations, and that EVs containing the CLHM-1 ion channel are non-distal tip derived (3). RAB-28 may discriminately regulate biogenesis of specific EV subpopulations. Using Total Internal Reflection Fluorescence (TIRF) microscopy and Imaris spot detection, I found that the number of CLHM-1-containing EVs released into the environment increased in rab-28 (gk1040) mutants. Thus, I hypothesize that the CLHM-1 EV subpopulation may accumulate within the extracellular space in rab-28 mutants and could therefore be phagocytosed by the glia. I used spinning-disk confocal microscopy to visualize tdTomato-tagged CLHM-1 expressed at single copy and observed colocalization of neuron-derived CLHM-1::tdTomato with pmiR-228::GFP-labeled glial cells of the male tail sensilla. Currently, I am determining the impact of the rab-28 mutation on CLHM-1 uptake by the glial cells. Characterizing mechanisms of EV biogenesis and the consequential effects on target cells is imperative to understanding this mode of intercellular signaling.

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Single Walled Carbon Nanotube (SWCNT) Detection of Interleukin 1 Î² (IL-1Î²)

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Single walled carbon nanotubes (SWCNTs) are known for their fluorescent properties in the near-infrared region, as well as their photostability and emission within the biological transparency window. This makes them useful as nanosensors for in vivo signal detection. The stability of SWCNTs also allows for repeated, long-term measurements. Implantation of these sensors in vivo allow for detection of biomarkers of specific diseases. Interleukin 1 Î² (IL-1Î²) is a pro-inflammatory cytokine involved in pleiotropic cellular processes. IL-1Î² is commonly found at elevated levels in cancerous tissues and other chronic diseases. We are working to design an IL-1Î² nanosensor by evaluation of optical band gap changes in the presence of the desired antigen. This is accomplished by conjugating an antibody of interest to the SWCNT wrapped with TAT6-NH2 DNA oligonucleotide and passivating the surface of the SWCNT with bovine serum albumin (BSA). This causes the SWCNT's hydrophobic surface to be less available for non-specific protein interference, creating a more functional sensor. We observed significant changes in the sensor's center wavelength and change in fluorescence intensity in the antigen's presence, serving as an indication of the functionality of the nanosensor. We are also working on studying the capability of fluorescent dyes to exacerbate the wavelength shift seen in SWCNTs. This is accomplished by conjugating the dye to a nanosensor and introducing its complimentary antigen that will remove the dye from the SWCNT surface, intensifying its shift in wavelength. The overall goal of developing this type of nanosensor is to use it in point-of-care scenarios to measure biomarker levels in patient samples or in vivo, allowing for detection of chronic diseases that may be difficult to diagnose early on.

Di-berberine conjugates as chemical probes of Pseudomonas aeruginosa MexXY-OprM efflux function and inhibition

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The Resistance-Nodulation-Division (RND) efflux pump superfamily is pervasive among Gram-negative pathogens and contributes extensively to clinical antibiotic resistance. The opportunistic pathogen Pseudomonas aeruginosa contains 12 RND-type efflux systems, with four contributing to resistance including MexXY-OprM which is uniquely able to export aminoglycosides. At the site of initial substrate recognition, small molecule probes of the inner membrane transporter (e.g., MexY) have potential as important functional tools to understand substrate selectivity and as a foundation for developing adjuvant efflux pump inhibitors (EPIs). Here, we optimized the scaffold of berberine, a known but weak MexY EPI, using an in-silico high-throughput screen to identify di-berberine conjugates with enhanced synergistic action with aminoglycosides. Further, docking and molecular dynamics simulations of di-berberine conjugates reveal potentially unique contact residues that can explain observed differences in sensitivity of MexY from distinct P. aeruginosa strains. This work thereby reveals di-berberine conjugates to be useful probes of MexY transporter function and potential leads for EPI development.

TRIM9 modulates cytoskeletal dynamics, membrane remodeling, and motility in melanoma

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Cell shape change and motility are essential in metastasis and involves remodeling of the actin cytoskeleton and plasma membrane. How these cytoskeletal and membrane remodeling are regulated and coordinated is unknown. We previously identified TRIM9 as a regulator of netrin-dependent actin dynamics and exocytosis in developing neurons. Deletion of murine Trim9 impairs neuronal migration, axon turning, axonal and dendritic branching, VASP ubiquitination, and VASP dynamics at filopodia tips, and increased exocytosis and filopodial stability. TRIM9 is expressed in other motile cells, but the non-neuronal role of TRIM9 remains unknown. TRIM9 has been identified as a possible prognostic biomarker in melanoma and high expression correlates with low patient survival. We hypothesize that TRIM9 coordinates actin dynamics and exocytosis in melanoma cells. Here we examine the role of TRIM9 in regulation of focal adhesions, exocytosis, and migration in melanoma. We show that TRIM9 protein is enriched in several human melanoma lines. Genetic loss of TRIM9 increased random migration velocity but altered directional persistence. Interestingly, we find that TRIM9 plays a role in regulating bleb-like morphology transitions and the ability to durotax on shallow gradients on soft substrate. Fluorescence recovery after photobleaching (FRAP), Total internal reflection fluorescence microscopy (TIRF), and widefield microscopy have revealed that loss of TRIM9 results in increased focal adhesions, cell size, and altered dynamics of focal adhesion proteins VASP and paxillin dynamics. In addition, TRIM9 knockout cells have reduced filopodial length and density and altered filopodial localization of VASP. In-gel zymography indicates that TRIM9 knockout cells also display an increased degradative capacity. Current studies are investigating how loss of TRIM9 alters parameters of lamellipodia, invadopodia, and exocytosis to define the role of TRIM9 in melanoma motility. Together these findings suggest TRIM9 is a potent regulator of melanoma adhesion, secretion, migration, and potentially metastasis.

Synthetic Access to 4,5-Aminoglycoside Antibiotics

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The aminoglycoside antibiotics (AGAs) are potent broad-spectrum antibiotics against aerobic, gram-negative pathogens. Gram-negative pathogens contain an impermeable membrane which provides an intrinsic barrier and resistance to many antibiotics. AGA usage is limited by significant side effects, namely ototoxicity or AGA-induced permanent hearing loss and nephrotoxicity, the rapid erosion of kidney function. Evolved resistance to these compounds further complicates issues with their use. Despite these challenges, these essential antibiotics present an opportunity to tailor their activity through molecular design. My work focuses on the synthesis and biological evaluation of novel aminoglycoside derivatives against gram-negative ESKAPE pathogens, a family of multidrug resistant bacteria. Synthetic AGAs will undergo screening for inhibition of bacterial and eukaryotic ribosomes, representative of antibacterial activity and toxicity, respectively. The results of these assays will be used to inform the design and synthesis of next generation AGAs. The goal is to deliver a set of validated advanced AGAs that display broad and potent antibiotic activity against wild type and multidrug resistant gram-negative bacteria, with much reduced toxicity, suitable for future development.

The Role of BET Proteins in KDAC Transcription Regulation

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Transcription is a key step in the cellular replication pathway and, if disrupted, can lead to various diseases including cancer. Epigenetic drugs such as lysine deacetylase (KDAC) inhibitors (KDACi) and bromodomain and extraterminal domain (BET) protein inhibitors (BETi) are being introduced in clinical settings to aid in treating these conditions. Traditional models depict KDACs as transcription corepressors, and while this is a canonical role, our lab has found that KDACs are required co-activators of glucocorticoid-induced transcription in a gene specific manner. I wanted to investigate whether inhibition of BET proteins affected induced transcription in a similar manner to KDAC inhibition. BET proteins have two bromodomains: BD1 & BD2. To account for these, I used both JQ1 and GSK046, which inhibit both and one BRD domain(s), respectively. I found that JQ1 repressed signal-induced transcription at select genes. Additionally, inhibition via GSK046 resulted in even fewer genes being repressed. Taken together, the data so far suggests that BET proteins are required for glucocorticoid-induced transcription in a gene-specific manner, though not to the same extent that KDACs are required.

Biophysical Studies of a Novel Interaction Partner of Human Ribonucleotide Reductase

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Genomic instability is a common trait in many human cancers and can be driven by imbalances in deoxyribonucleotide triphosphate (dNTP) pools. A major regulator of the dNTP pools in humans is ribonucleotide reductase (RNR), the sole enzyme capable of de novo generation of deoxyribonucleotides from ribonucleotide substrates via a radical mechanism. Eukaryotic RNRs are composed of two subunits: the catalytic a subunit and the radical-generating b subunit. The two subunits must physically interact to form an a2b2 holocomplex competent for radical transfer to initiate catalysis. Enzymatic activity is controlled by the binding of ATP or dATP to an N-terminal region in the a subunit termed the cone domain. The binding of these effectors results in the formation of morphologically identical, but differentially stable a6 hexamers, where the b subunit only breaks apart a6-ATP, and not a6-dATP. Non-nucleotide effectors of activity, namely the 60 kDa IP3R binding protein released by IP3 (IRBIT), have been identified in metazoans. Previous studies have found that IRBIT binds to human RNR, but specifically in the presence of dATP, enhancing the inhibition induced by dATP. Additionally, the interaction strength appears to be modulated by phosphorylation of IRBIT. To understand the molecular details of this interaction, we use protein expressed in insect cells to carry out mass photometry (MP) and electron microscopy (EM) studies. Mass photmetry results suggest that a >1 MDa co-complex is formed upon the addition of IRBIT to a subunit samples pre-treated with dATP. Transmission electron microscopy studies suggest the formation of a complex unique from those previously vizualized for metazoan RNRs. Understanding the IRBIT:HsRNR complex would open doors to non-nucleotide regulation of RNR activity and provide a new target for anticancer drugs.

Investigating the cell and tissue-scale movements that drive avian foregut morphogenesis

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The vital functions of respiration and digestion are carried out by organs that arise from an embryonic structure known as the gut tube. How the definitive endoderm is internalized and transformed from an epithelial sheet to a cylindrical tube remains remarkably understudied in amniotes. The anterior segment of the tube, the foregut, is initiated when an invagination known as the anterior intestinal portal (AIP) forms in tight coordination with the head-fold. Posterior descensus of the AIP has been the focus of foundational studies describing foregut formation (Bellairs 1953, Seidl & Steding 1978), but how one-dimensional movement of the AIP forms the three-dimensional foregut tube is not well understood. Here, we describe efforts to determine the dynamics of foregut elongation in the chick embryo, combining embryological and molecular approaches with live in vivo imaging of cell movements in the definitive endoderm. We observed that AIP movement becomes a poor correlate of gut length as development proceeds, and that the elongating foregut tube changes shape dramatically as it elongates. These and other observations suggest that AIP descensus on its own may not fully explain foregut elongation, and behaviors intrinsic to the forming foregut may be important. By live in vivo imaging of endoderm cell movements, we identified a previously unappreciated population of cells that migrate into the foregut through the AIP, mirroring cell movements that drive hindgut folding (Nerurkar et al. 2019). In the hindgut, this movement is driven by conversion of a long-range FGF gradient into an active force gradient. However, no such gradient exists at the forming foregut. Ongoing efforts are focused on studying the role of Fgf8 expressed locally at the AIP in foregut morphogenesis, and ultimately, whether a unifying mechanism can be identified that relates foregut, midgut, and hindgut tube morphogenesis. Funding: Blavatnik Family Foundation (OP), NIH R21HD099529 (NN).

Visible Light-Mediated, Diastereoselective Epimerization of Morpholines and Piperazines to More Stable Isomers

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We report a photocatalyzed epimerization of morpholines and piperazines that proceeds by reversible hydrogen atom transfer (HAT). This strategy provides an efficient editing of the stereochemical configurations of these saturated nitrogen heterocycles, which are prevalent in drugs. More stable morpholine and piperazine isomers are obtained from the more synthetically accessible but less stable stereoisomers, and a broad scope is demonstrated in terms of substitution patterns and functional group compatibility.

Examining Racial Discrimination, Insula Cortex Connectivity and Dissociation During Attention to Threat in A Trauma-Exposed Black American Women Population

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Racial discrimination (RD) is known to have potent negative effects on mental health outcomes in Black American communities. Reports show Black Americans have a higher prevalence of stress-related disorders including clinically significant dissociation. Dissociation is linked to disruptions within interoceptive and emotion-processing networks, and both dissociation and RD are linked to attentional control impairments, particularly in the face of emotion. However, little is known about how RD may affect connectivity in brain networks associated with attentional control and interoceptive awareness. We used data from (n=72) Black American women from the Grady trauma project and those recruited as part of the parent grant "Neural Mechanisms of Vibroacoustically Augmented Breath Focused Mindfulness for Dissociative Traumatized People." Using the CONN toolbox, we conducted a generalized psychophysiological interaction analysis to examine associations of RD with attentional control and interoceptive functional connectivity (FC) during performance on the affective Stroop task. During attention to threat trials, RD was significantly associated with decreased Insula-Prefrontal cortex FC. We extracted these values and ran correlation analysis with multiscale dissociation inventory (MDI) total mean scores and found that our FC values were negatively associated with MDI scores ($r = -.305$, $p = .014$). These findings show that associations between RD and decreased FC within interoceptive networks may be the underlying mechanism by which Black Americans are at an increased risk for dissociation which highlights the importance of considering racial experiences when treating dissociative symptoms in Black populations.

The Intersection of Latinx MSMs' Cultural, Racial, and Sexual Identities on Friendship Network Dynamics, Mental Health, and PrEP Stigma, PrEP Awareness, and PrEP Discussions in South Florida

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In 2021, 4,708 Floridians were diagnosed with human immunodeficiency virus (HIV). Latinx men who have sex with men (LMSM) were disproportionately diagnosed, representing 61% of HIV cases in Florida, 74% in Miami-Dade County (MDC), and 56% in Broward County (BC). However, LMSM are not engaging in pre-exposure prophylaxis (PrEP), a highly effective biomedical HIV prevention option. PrEP stigma is a barrier that impacts an individual's social interactions, health, and health behaviors. My project explores the relationship between LMSM's friendship networks, mental health, PrEP stigma, PrEP use, and PrEP discussion. My project uses data collected for Dr. Mariano Kanamori's R01 PrEParados (R01MH125727). From February 2022 to July 2023 PrEParados recruited 523 LMSM from MDC and BC. The Social Contagion Theory is used as a framework at the network level to contextualize PrEP stigma and PrEP discussions. The Theory of Intersectionality is used at the individual level to explore the relationships between Latinx racial heterogeneity, cultural values, PrEP stigma, and PrEP discussions. Beyond the important public health implications this work is an important milestone for my professional development. Lessons learned from my project will be leveraged in the submission of a career development award (e.g., K-award) focusing on the development of an intervention that addresses LMSM's ongoing experiences of stigma, discrimination, and health disparities (e.g., HIV and poor mental health outcomes).

Exploring the influence of the socio-ecological environment on the Queen Club program for young women in Tanzania

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Background: Malkia Klabu ("Queen's Club"), is a youth-centered loyalty program in northern Tanzania designed to reshape drug shops' role in delivering HIV self-test (HIVST) kits and sexual reproductive health (SRH) products to adolescent girls and young women (AGYW) through convenient access at private drug shops (ADDOs). The built environment or human-made physical structures within the community could inhibit or facilitate access to neighboring ADDOs, which may enhance privacy (via anonymity) for AGYW. We applied a socio-ecological lens to investigate whether drug shops' proximity to other salient structures (e.g., schools, places of worship, markets, centers) and the visible location of SRH products and HIVST kits within ADDO shops influences AGYW utilization at these shops.

Methods: We analyzed eight months of shop sales data from a subsample of 76 out of 159 ADDO shops using random generator in MS Excel by region (Mwanza and Shinyanga) and from shops enrolled in a cluster-randomized controlled trial (NCT05357144). We drew from an observational survey of closed-ended items that geo-located structures and recorded their Euclidean distance to each participating ADDO shop. Using network analyses in ArcGIS Pro and negative binomial regressions in R Studio, we estimated the rate ratios for shop-level product outcomes (HIVST kits, oral and emergency contraception, condoms, pregnancy tests) in relation to product visibility inside drug shops, adjusting by study arm and region. Ongoing analyses are being conducted to examine whether physical proximity to features surrounding the shop is related to AGYW drug shop sales.

Results: Among within-shop features, 74% of shops had at least one of the contraception products or pregnancy tests visibly displayed (no difference by study arm). About 15% of shops placed these products in a somewhat visible or partially hidden spot; none of these products were visible in 11% of shops. About half of the shops (47%; 49% intervention, 45% control) did not have HIVST kits visible, but more intervention shops had HIVST kits in visible locations than control shops

(42% vs. 36%).

Data from July 2022-March 2023 indicated that comparing the SRH product visibility from intervention (N=44) to control (N=32) shops showed somewhat suggestive findings that greater visibility was associated with higher sales to AGYW customers (2 shops excluded as 1 was dropped and 1 on pause during trial period). Increased shop product sales were associated with any of these SRH products being physically visible inside the shop compared to when products were out of sight (RR 2.03; 95% CI 1.06, 3.85).

Conclusions: Interpreting the present study's findings are limited, as cluster-adjusted standard errors were not accounted for in the regression analysis and results are possibly underpowered due to a small sample size. The sample was randomly generated by region, rather than by arm. Understanding the role of the social-ecologic environment on service provision at these critical access points could inform future studies that consider similar youth-friendly interventions to increase AGYW's engagement at drug shops. The localized features within each drug shop, specifically the visible placement of SRH products, could be more relevant than the surrounding exterior community features. However, further research is needed to obtain conclusive findings.

Racial and Ethnic Disparities in Barriers to Mental Health Treatment in United States College Students

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Background: College students suffer from increasingly high rates of mental disorders and students of color report lower rates of mental health treatment utilization compared to their White peers despite similar or greater disorder prevalence. Disparities in treatment access result in poor outcomes, particularly for Black and Latinx individuals and produce a large financial cost. While both attitudinal and structural barriers have been shown to play roles in disparate treatment access, less is known about how these factors differ across racial/ethnic groups. Understanding unique and common barriers could aid in targeted intervention approaches to promote health equity.

Objectives: In a nationally representative sample of college students, this study aimed to 1) Examine the prevalence of mental health disorders by race and ethnicity; 2) Evaluate whether attitudinal and structural barriers vary by race and ethnicity within the context of relative disease burden by racial/ethnic groups.

Methods: The present study draws on a large survey (N = 32,000) conducted across 26 US colleges and universities to examine barriers to treatment among students. Chi-square tests and one-way ANOVAs with post hoc comparisons were used to examine structural barriers (e.g., financial reasons, not enough time, not sure where to go, difficulty finding an available appointment), attitudinal barriers (e.g., perceived need and importance of treatment; willingness, intention, and readiness to seek help), and personal preferences (e.g., dealing with mental health problems with social support) by race and ethnicity (White, Asian, Black or African American, Multiracial, and Hispanic/Latinx).

Results: Black individuals perceived a high need for treatment, endorsed a strong readiness to seek help, and had higher help-seeking intentions compared to White individuals; yet they faced significantly greater financial barriers to treatment. A similar pattern of results was found for Hispanic/Latinx individuals, but a unique

barrier that emerged was a lower perceived importance of mental health, despite perceiving need for treatment. Asian American individuals endorsed a stronger preference for dealing with issues on their own or with support from family/friends, and lower readiness and intentions to seek help, in addition to endorsing greater financial barriers compared to their White peers.

Conclusions: Disparities in unmet treatment need may arise from both distinct and common barriers and point to the potential benefits of tailored intervention approaches to address unique needs of students of color from varying racial/ethnic backgrounds. Addressing barriers to treatment may require interventions that cater to the unique needs of different racial/ethnic groups.

Exploring Neural Correlates of Reward and Punishment Learning in Depression using 7-Tesla MRI

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Major Depressive Disorder (MDD) is a prevalent, heterogeneous disorder, featuring overlapping symptom profiles including anhedonia and anxiety. Parsing discrete symptom domains is imperative to the development of more targeted, precise interventions. Preclinical research suggests divergence in the neural correlates of anhedonia and anxiety-related phenotypes, predominantly stemming from the ventral tegmental area (VTA) (Morrel et al., 2022). However, these neural circuits are difficult to discern in humans in part due to methodological barriers with 3-Tesla (3T) MRI. To address this, we used a probabilistic instrumental learning task during ultra-high field 7T functional MRI, which we have previously used to demonstrate VTA network changes in MDD that were not detectable at 3T. During the task, N=55 (27 Healthy; 28 MDD) participants chose between pairs of stimuli to maximize reward and minimize punishment. A Q-learning algorithm was used to explore reward and punishment prediction error (PE) signaling, combined with enhanced precision for examining VTA via 7T MRI. Preliminary results indicated that reduced prediction error signal in VTA was associated with worse anhedonic symptoms in MDD (N=19 MDD: R=0.494 p=0.032; N=22 HC: R=-0.12 p=0.105).

Using Thematic Analysis to Explore Barriers and Facilitators to mHealth Utilization: Preliminary Analysis

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Background: Mobile health (mHealth) technologies hold tremendous potential to revolutionize healthcare delivery, offering innovative solutions for accessing health information and services remotely. However, the successful integration of mHealth into healthcare systems relies on understanding the various barriers and facilitators that influence its utilization. This study explores factors that hinder and enable the adoption and use of mHealth services across diverse populations.

Method: We recruited 32 caregivers in South Florida who were part of a larger study examining parent-child relationships using smartphones and wearables. Qualitative assessment of challenges and supports to utilizing mHealth tools was explored using 45-minute semi-structured interviews adapted from the Cultural Formulation Interview. A group of 6 coders independently open-coded 10 interviews until saturation to create a codebook. After achieving group consensus on the codebook, 4 coders coded 22 interviews. Cohen's Kappa was used to calculate interrater reliability. When discrepancies occurred, researchers discussed and reached consensus.

Data Analysis Plan: Two thematic analyses will be performed. Initial themes will be extracted from participants' responses to the questions: "Are you willing to use a mobile health app to manage your mental health problem? If so, why? If not, why?" The author will generate initial themes, verify their relevance, and arrive at a final set of themes.

Preliminary Theme Exploration

32 participants provided 290 responses (118 barriers; 172 facilitators). Of the sample, 52.1% of participants were racial/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. When examining overall responses visually, three focal points emerge for barriers, and 2 focal points emerge for facilitators. For mHealth barriers, we see categories surrounding knowledge (e.g., privacy and security concerns, tech literacy, and resistance), structural issues (e.g., cost, technology accessibility), and human interaction concerns (e.g., lack of engagement, lack of interaction, quality, and personalization). For mHealth facilitators, we see two motivators including incentivization (e.g., gamification, financial incentives) and convenience (e.g., personalization, accessibility, performance/reliability).

Further analyses are required to verify the relevance of the themes. However, based on these initial categories, we consider 3 points that could reduce barriers and increase the facilitation of mHealth tools:

-Affordability and Incentives:

-Consider making digital mental health tools affordable or provide financial incentives to encourage usage.

-Human-Centric Design:

-Ensure digital tools offer a personal touch that provides both customized and effective care.

-Explore incorporating convenience as a factor to enhance user experience.

-Education and Empowerment:

-Prioritize educating users on how to effectively use digital tools.

-Teach users about privacy and security concerns, empowering them to engage confidently.

-Promote general digital literacy to foster autonomy and agency in using these tools.

Discussion: Findings may identify enhancements to mHealth tools to facilitate the receptivity of diverse or under-resourced caregivers. Understanding these

mechanisms will inform client-centered, health-literate digital strategies and reduce disparities in mental health care in high-need populations.

Sex-differences in proteasome-dependent K48-polyubiquitin signaling in the amygdala are developmentally regulated

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Background: Sex differences have been observed in several brain regions for the molecular mechanisms involved in baseline (resting) and memory-related processes. The ubiquitin proteasome system (UPS) is a major protein degradation pathway in cells. Sex differences have been observed in lysine-48 (K48)-polyubiquitination, the canonical degradation mark of the UPS, both at baseline and during fear memory formation within the amygdala. Here, we investigated when, how, and why these baseline sex differences arise and whether both sexes require the K48-polyubiquitin mark for memory formation in the amygdala.

Methods: We used a combination of molecular, biochemical and proteomic approaches to examine global and protein-specific K48-polyubiquitination and DNA methylation levels at a major ubiquitin coding gene (Uba52) at baseline in the amygdala of male and female rats before and after puberty to determine if sex differences were developmentally regulated. In a separate experiment, we ovariectomized female rats before puberty and examined sex differences in global K48-polyubiquitination and Uba52 DNA methylation in young adulthood. We then used behavioral and genetic approaches to test the necessity of K48-polyubiquitination in the amygdala for fear memory formation.

Results: We observed developmentally regulated baseline sex differences in Uba52 methylation and total K48-polyubiquitination, with sexual maturity altering levels specifically in females, and these sex differences were altered in young adulthood if females were ovariectomized prior to puberty. K48-polyubiquitination at specific proteins changed across development in both males and females, but sex differences were present regardless of age. Lastly, we found that genetic inhibition of K48-polyubiquitination in the amygdala of females, but not males, impaired fear memory formation.

Conclusions: These results suggest that K48-polyubiquitination differentially

targets proteins in the amygdala in a sex-specific manner regardless of age. However, sexual maturity is important in the developmental regulation of K48-polyubiquitination levels in females. Consistent with these data, K48-polyubiquitin signaling in the amygdala is selectively required to form fear memories in females. Together, these data indicate that sex-differences in baseline K48-polyubiquitination within the amygdala are developmentally regulated, which could have important implications for better understanding sex-differences in molecular mechanisms involved in processes relevant to anxiety-related disorders such as post-traumatic stress disorder (PTSD).

Neuroimmune mechanisms underlying chronic stress-induced reward deficits

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Major depressive disorder (MDD) is a leading cause of disability worldwide, creating an immense burden on countless individuals and the greater global population. One prominent symptom associated with MDD, is anhedonia: the loss of interest for hedonic stimuli. In parallel, several clinical and pre-clinical studies have linked exposure to stress and MDD, a stress-related psychiatric disorder, with alterations of the peripheral immune system. However, the neuroimmune mechanisms that lead to brain circuit alterations and ultimately to behavioral alterations are not well understood. Here, we investigate the impact of stress on blood-brain barrier (BBB) integrity, peripheral immune marker infiltration into the brain, and subsequent effects on the physiology of neurons in brain reward centers controlling anhedonia. We then assess reward deficits in a novel mouse version of the probabilistic reward task (PRT) after chronic exposure to social defeat stress (CSDS) and with or without manipulating BBB integrity or peripheral immunity. This task, first developed in humans, is well-positioned to extract stress-induced reward deficits relevant to MDD. We found that in mice exposed to CSDS, alterations of the BBB lead to infiltration of peripheral monocytes into the ventral striatum. We also found that a direct consequence of this infiltration is a perturbation of the neuronal function in the Nucleus Accumbens and subsequent reward sensitivity deficits in the PRT. Namely, following CSDS, susceptible mice showed a blunted response bias but not unstressed control or resilient mice. Next, we found that artificially opening the BBB using a viral knock-down of the Claudin-5 tight junction protein led to an exaggerated impact of a sub-threshold stressor on reward sensitivity in the PRT. Our results indicate that chronic stress causes systemic inflammation and that alterations of the BBB lead to an infiltration of immunity markers into the Ventral Striatum, altering neuronal function and, ultimately, causing major reward deficits. To bring a translational dimension to this project, we work with the Depression and Anxiety Center at Mount Sinai on using human PRT datasets and blood samples to inform our preclinical findings through an existing liquid biomarker pipeline established by our groups. This study will

provide a critical understanding of the neuroimmune influence on reward deficits in MDD and advance the development of new personalized immune-based therapeutics to treat depression.

Associations of Posttraumatic Stress, Opioid Use, and Pain among Individuals with Probable PTSD and Chronic Pain: Investigating the Role of Health Literacy

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Chronic pain is one of the most common reads adults seek medical care and impacts an individual's physical and emotional functioning. Individuals with chronic pain may be at increased risk for mental health concerns, including posttraumatic stress disorder (PTSD) and opioid use disorder (OUD). Further, comorbidity rates between PTSD and OUD are high. As such, it is imperative to understand individual difference variables related to pain and opioid use among individuals with PTSD and chronic pain. Health literacy is one variable that impacts these associations. The purpose of the present study was to examine the indirect effects of health literacy in the association between PTSD and opioid misuse, opioid dependence, pain intensity, and pain disability. The sample included 163 adults reporting chronic pain and opioid use. Results demonstrated that PTSD symptom severity had an indirect effect on (a) opioid misuse, (b) opioid dependence, and (c) pain intensity via health literacy. This research has the potential to inform intervention strategies among individuals with co-occurring PTSD, chronic pain, and OUD.

Pursuit of a Reliable Working Memory Biomarker Characterizing the High Schizotypy Population

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Background: Individuals with schizophrenia spectrum disorders (SSD) exhibit working memory (WM) impairments. These impairments may be associated with symptom severity rather than the onset of psychosis. We searched for a biomarker of WM deficits in individuals high in schizotypy traits (non-clinical population). We predicted that individuals high in schizotypy would perform worse and have lower event-related potential (ERP) amplitudes than controls in a WM task.

Methods: Individuals were recruited based on their Schizotypal Personality Questionnaire – Brief Revised scores from a separate screening questionnaire. 21 controls and 14 individuals high in schizotypy completed an auditory 2-back WM task, while EEG was recorded (Biosemi). Participants responded with key presses whether number stimuli (0-9) presented over earphones matched or mismatched stimuli presented two numbers prior. ERPs were used to compare amplitude differences in the auditory P300, a neural signature reflective of WM processes, between controls and individuals high in schizotypy.

Results: Individuals high in schizotypy had lower accuracy (M=.83) than controls (M=.91, p=.04). Although not statistically significant individuals high in schizotypy responded slightly faster (high M=1.28 ms, controls M=1.31 ms, p>.05). The P300 was not significantly reduced in amplitude for individuals high in schizotypy compared to controls (p=.76, Mann-Whitney test). Although, there are other ERP differences present (ps<.001).

Conclusions: Our ERPs do not show a diminished P300 in individuals high in schizotypy compared to controls, although P300 reduction is classically found in people with schizophrenia. However, there are group differences along the ERP and WM is impaired in individuals with high schizotypy. Our results are trending towards the perspective that WM biomarkers may be associated with symptom

severity. Identifying a biomarker of schizotypy may help with early identification of SSD.

Eating Disorder Prevalence, Related Psychopathology, and Treatment Need Among Rural, Suburban, and Urban Americans

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Background: Rural areas are disproportionately affected by socioenvironmental health determinants that are associated with increased risk for some eating disorders (EDs) and mental illnesses highly co-morbid with EDs. EDs have been regarded as urban, affluent diseases, however, and are vastly understudied in rural America. The current study aims to evaluate and compare rates of EDs and their related behaviors, treatment receipt and intentions, and suicidality across a nationally-representative sample of rural, suburban, and urban participants.

Method: Data were collected from a validated online screener hosted by the NEDA website from 6/1/2020-6/1/2022. Federal data was used to classify Respondents' ZIP codes into three levels: rural area (RA), suburban area (SA), and urban area (UA). Pairwise chi-square tests with Holm corrections were conducted to compare between levels the prevalence rates of nine ED diagnoses, including subclinical and clinical Anorexia nervosa (AN), subclinical and clinical Bulimia nervosa (BN), subclinical and clinical binge ED (BED), purging disorder (PD), unspecified feeding or eating disorder (UFED), avoidant/restrictive food intake disorder (ARFID), and not at risk for an ED; treatment-seeking intentions and receipt; and suicidality. NOVA tests and pairwise t-tests with Holm corrections were performed to compare between levels the average episodes of ED behaviors, including binge eating, vomiting, diuretics/laxatives use, excessive exercise, fasting).

Results: 155,427 respondents screening for an ED from RAs (n=4331), SAs (n=9625), and UAs (n=43228), were included in analyses. AN and BN rates in RAs were comparable (AN, 4.7%, BN, 30.8%) to rates in SAs (AN, 4.0%, BN, 29.7%), and UAs (AN, 4.5%, BN, 30.8%) respectively. Differences in BED prevalence were negligible in RAs (14.4%) versus UAs (12.4%, $p<.001$, Cramer's $V=.010$), and UAs versus SAs (12.8%, $p<.001$, Cramer's $V=.002$) respectively, and PD in RAs (2.3%) versus UAs (1.6%, $p<.001$, Cramer's $V=.01$). Sub-threshold

EDs (at risk) were more prevalent in UAs versus RAs and SAs. UFED and ARFID rates were comparable between RAs, SAs, and UAs. RAs experienced more average episodes of diuretic and laxative use, fasting, and bingeing versus UAs, and more episodes of binging versus SAs. Episodes of fasting were negligibly more frequent in SAs vs. UAs. Current treatment rates were marginally higher in UAs versus SAs. There were no differences in treatment intentions. Suicidality intention was higher among RAs and SAs as compared to UAs.

Conclusion: This study provides evidence of comparable rates of ED diagnoses, related behaviors, suicidality among RAs, SAs, and UAs and comparable intentions to seek help. Treatment receipt is suboptimal across all areas. Greater work is needed to draw attention to the under-evaluated burden of EDs in rural communities, and motivate development of interventions that may improve access of ED care and services.

Examining the Effects of Emotional Abuse from Family Members and Peer Victimization on the Development of Negative Inferential Style during Adolescence

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Background: Extant literature indicates that a negative inferential style, a significant cognitive vulnerability for depression, forms and stabilizes during adolescence. Despite hypotheses that relational victimization from peers and emotional abuse and neglect from family members contribute to an individual's inferential style, little prospective research investigates the effect of these stressors on the development of negative inferential style during adolescence.

Method: Using a multi-wave study of community adolescents (N = 335, mean age at Time 1 = 14.08 years, 54% Female) and multilevel models, we addressed this gap by examining the effects of peer victimization and emotional abuse and neglect from family members in the development of negative inferential style.

Results: Experiences of emotional abuse and neglect ($\hat{I}^2$ =.04, t = 2.02, p < .05), but not experiences of peer victimization ($\hat{I}^2$ =.03, t = 1.20, p =.230), had significant within-person associations with negative inferential style. These effects were not moderated by sex ($\hat{I}^2$y < .07, ts < 1.48, ps > .139).

Discussion: Our findings suggest that instances of emotional abuse and neglect during adolescent development serve as a potent risk factor for negative inferential style formation. Implications for theory, research, and practice, particularly in relation to depression, will be discussed.

Development of Suicide Ideation and Attempts among LGBTQ Youth of Color: An Integrative Data Analysis

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This study utilized integrative data analysis (IDA) to examine developmental trends in suicide thoughts and behaviors among LGBTQ youth of color (YOC). Individual-level data were harmonized across 18 prevention trials (N=1,615) to compare intersectional groups by gender identity, sexual orientation, and race/ethnicity. Findings revealed peak suicide attempt rates at age 13 for transgender youth; higher attempts for females and more ideation for males; and distinct patterns among Asian, Black, and Multiracial LGBTQ-YOC. The study elucidates developmental variations in suicide risk among LGBTQ-YOC subgroups. Findings can inform targeted, culturally informed prevention programs and highlight the utility of IDA for advancing health equity research on multiply marginalized populations.

Molecular compensation by neurodevelopmental-risk genes Foxp1 and Foxp2 in the striatum

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Transcription factors Foxp1 and Foxp2 have both been implicated as risk genes for autism spectrum disorders (ASD) as well as speech and language disorders. Loss of Foxp1 and Foxp2 in mouse striatum causes ASD-like phenotypes, including deficits in communication and social behavior. Here, I show that both transcription factors can compensate for the loss of the other at a transcriptional, behavioral, and electrophysiological level in striatal D1 SPNs where they are both co-expressed. To begin to unravel the mechanism for Foxp1 and Foxp2 compensation, I use CUT&RUN data to identify the common and direct binding targets of Foxp1 and Foxp2. From these direct binding targets, I propose shared and unique functions of Foxp1 and Foxp2.

Systematic Review of Research among Black Queer Cisgender Women Living with HIV

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Background: Cisgender Black women accounted for 54% of new HIV diagnoses in the US in 2019 amongst all ciswomen (CDC, 2022). These statistics fail to account for the sexual identity of ciswomen and our own work has suggested between 15% and 20% of cisgender BWLWH may be queer (Reid & Dale, 2023; Dale et al., 2023). The experiences of Black cisgender queer women who are living with HIV have not received much attention from the scientific community. Methods: We conducted a systematic review guided by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) to find studies on Black queer cisgender women who are living with HIV (BQWLH). The search was performed utilizing Google Scholar, PsycINFO, and PubMed databases. The word search included terms regarding HIV status, being a Black queer woman, and experiences of sexism, racism, homophobia, and biphobia. To be included in the final review articles had to meet the following criteria: (1) Black queer ciswomen living with HIV, (2) original research articles, (3) published in the year 2003-2023, (4) available in English, (5) and sample size is 18 years or older. Exclusion criteria were (1) books, thesis, and dissertations, (2) systematic reviews, (3) articles without cisgender queer Black women in the sample, and (4) articles on youth populations. Results: Our initial search generated a total of 13,622 articles, books, and dissertations that met search word terms. Based on our inclusion and exclusion criteria, 10 articles were included and reviewed in-depth. The studies were conducted in Canada, Namibia, South Africa, Zimbabwe, the US, and the UK. Most of these studies addressed the health needs of a multitude of ethnic and racial populations, in addition to cismen, transwomen, and transmen. Only 3 out of 10 of the articles were focused solely on cisgender BQWLH. Within the articles, they found that multilevel forms of discrimination (e.g., racism, sexism, homophobia) and HIV-related stigma correspond to barriers to healthcare and negative psychosocial outcomes (Dustin, 2022; Logie et al., 2018; Logie et al., 2012; Logie

et al., 2011). Additionally, a phenomenon called “corrective rape” has been documented within South Africa, Zimbabwe, and Namibia among sexual minority ciswomen, this gender-based violence is rooted in homophobia and is used to “correct” ciswomen by sexually abusing them due to their lack of conformity to gendered and sex-based norms (Lock Swarr, 2012; Matebeni et al., 2013; Naidoo et al., 2012). Conclusions: The initial word search indicated that the bulk of HIV research has been centered on men-loving men communities and there is a dearth of information on cisgender BQWLH. Among the 10 articles included in our review, only 3 centered the experiences of Black sexual minority ciswomen, however, they all indicated that societal barriers and discrimination prevent them from accessing HIV-related care and elevate negative mental health outcomes (e.g., depression, post-traumatic stress disorder). Our review suggests the need for research among this community, which will allow us to develop interventions to address the unique intersectional needs of cisgender BQWLH who stand at the margins.

Parental Incarceration and Children's Health

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More than 2.7 million children—disproportionately Black and Hispanic—have a parent in jail or prison in the United States. Parental incarceration can have a profound effect on children (e.g., traumatic separation, residential instability), increasing their risk of physical health problems. Yet, few studies have examined the relationship between parental incarceration and children's health. Our study addresses this pressing public health issue. We interviewed participants from the Northwestern Juvenile Project—a longitudinal study of 1829 newly detained youth, now median age 41—and their oldest biological child ages 10 to 18 years. Nearly 40% of parents were incarcerated during their child's lifetime. For these preliminary analyses, we first examined three areas of children's health in the entire sample: (1) Health conditions; (2) Health behaviors (e.g., tobacco use); and (3) Health service use. Overall, about half of children had a health condition and nearly one-fifth endorsed having fair or poor health. Some physical health conditions (e.g., asthma) and health behaviors (e.g., tobacco use) were more prevalent than among children in the general population. We next compared the health of children whose parent was incarcerated during their lifetime to children whose parent was incarcerated only before they were born. There were no significant differences. We plan to conduct subsequent analyses to assess health disparities by children's sex, race, and ethnicity. We will also assess the relationship between the "dose" of parental incarceration (e.g., duration, setting) and children's health.

Congenitally blind and sighted adults use causal object history to assign colors

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People naturally talk about objects as if they have an intrinsic color (e.g. polar bears are white) The color perceived can change, however, based on the environment (e.g., day/night) and observer (e.g., human vs. bee) (Cohen, 2009, 2010; Pautz, 2006; Brogaard, 2010). How do speakers decide which is the color of an object? The most straightforward possibility is that speakers treat the color they most frequently experience as â€˜intrinsic' to the object. I call the sky blue because I usually see it as blue. Evidence from sighted and blind speakers suggests, however, that there's more to color labels than what we've seen most frequently (Cohen, 2004; Hasen, 2011; Kim et al., 2019; 2021). By measuring color labeling in sighted and congenitally blind speakers we tested an alternative hypothesis, that speakers use abstract mental models to choose causally relevant colors. Specifically, those seen during ideal viewing conditions (i.e., in daylight) and relevant to the object's function. Blind and sighted speakers alike prefer daytime over nighttime colors and these preferences flip to nighttime colors when objects are â€˜intended' for night (e.g., animals that are nocturnal). This data suggest first person experience of seeing is not necessary to develop abstract and flexible intuitive theories of vision. These â€˜intuitive theories' are used to assign generic object colors, flexibly taking into account the canonical viewer (sighted human), the canonical viewing conditions (daylight) and the causal history of the object being labeled (made for day or night). The data further suggest that when labeling an object's color, speakers describe not just what people are likely to see, but rather communicate deeper causally-relevant object properties.

Elucidating the role of Ankyrins during synapse formation in the outer retina

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Proper synaptic connectivity between photoreceptors and their respective partners is essential for normal visual transmission and processing. The inability of photoreceptors to form synaptic connections during development underlies many vision-related neurodevelopmental disorders. Thus, elucidating the developmental mechanisms that mediate appropriate photoreceptor connectivity will provide insights into devising therapeutic approaches to treat patients with retinal diseases. In the mammalian retina, the different types of photoreceptors synapse selectively to distinct postsynaptic targets: horizontal cells and bipolar neurons. During development, photoreceptors first make contacts to horizontal cells (referred to as first synaptic contact) and then to bipolar neurons (i.e. second synaptic contact). Although the timing and patterns of connections have been well-described for photoreceptors, little is known about the early molecular events that coordinate the selective wiring of photoreceptors to their respective targets. Here, we investigate a new role for the cytoskeletal scaffolding proteins, Ankyrins in mediating the early developmental events involved in photoreceptor connectivity. We found Ankyrin-B (AnkB) and Ankyrin-G (AnkG) to be differentially expressed in both a spatial and temporal manner in the developing retina. AnkB is highly expressed in horizontal cells at early time points when the first synaptic connection between photoreceptors and horizontal cells is being established, whereas AnkG is expressed at later stages in bipolar neurons when the second synaptic connection is formed. Moreover, our initial data reveals that loss of AnkB and AnkG results in phenotypes consistent with synaptic connectivity defects between photoreceptors and their synaptic targets. Additionally, we also found impaired retinal responses in animals with disruption of AnkB and AnkG compared to controls. Taken together, we uncovered a new role for Ankyrins in wiring photoreceptors to their postsynaptic partners during development. Future work will focus on disseminating the developmental mechanisms as well as the cell-type specific requirements of Ankyrins in photoreceptor connectivity. This work will have broad

significance as it may reveal new potential targets that can be used to restore photoreceptor connections in patients suffering from vision loss.

Analyzing the CHARGE-like phenotype caused by mutations of chd7 in Danio rerio

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Analyzing the CHARGE-like phenotype caused by mutations of chd7 in Danio rerio

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Gene expression is regulated by the complex molecular crosstalk between DNA methylation (DNAme) and other chromatin features, such as histone post-translational modifications (PTMs) and chromatin-associated proteins (ChAPs). Changes in the chromatin landscape can have a profound impact on DNAme patterning (and vice versa) in both development and disease. However, an understanding of how DNAme co-occurs and co-ordinates with other chromatin features to control gene expression is limited by a lack of reliable genomic tools.

EpiCypher has partnered with New England Biolabs (NEB) to develop Targeted Enzymatic Methylation-sequencing (TEM-seqTM), an ultrasensitive multiomic genomic mapping technology that delivers high-resolution DNAme profiles at epitope-defined chromatin features. This multiomic workflow integrates EpiCypher's quantitative high-sensitivity CUTANA CUT&RUN assay for genomic mapping together with NEB's enzymatic methyl-seq (EM-seq) for unbiased DNAme analysis (Fig. 1A). CUT&RUN uses antibodies to locally tether protein A/G-micrococcal nuclease (pAG-MNase) to chromatin in intact cells or nuclei, followed by controlled activation of the MNase to cleave nearby DNA. EM-seq leverages enzymatic conversion of DNAme to generate high resolution, unbiased DNAme profiles with less sample.

We demonstrate that TEM-seq is highly reproducible, specific, and efficient (<10% off-target binding, >90% enzymatic conversion of DNAme, <0.5% conversion of unmethylated DNA). TEM-seq is also incredibly sensitive, requiring only 5-50M sequencing reads per assay, demonstrating the disruptive potential of the assay to offer a low-cost solution for targeted DNAme analysis. We are currently working to validate additional targets (focusing on ChAPs) and continuing to develop our controls, including spike-in DNA for 5hmC DNA.

TEM-seq: an ultrasensitive multiomic platform for epitope-targeted DNA methylation mapping

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Gene expression is regulated by the complex molecular crosstalk between DNA methylation (DNAme) and other chromatin features, such as histone post-translational modifications (PTMs) and chromatin-associated proteins (ChAPs). Changes in the chromatin landscape can have a profound impact on DNAme patterning (and vice versa) in both development and disease. However, an understanding of how DNAme co-occurs and co-ordinates with other chromatin features to control gene expression is limited by a lack of reliable genomic tools.

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Utilizing short sequences missing from the genome to identify and functionally characterize gene regulatory cancer driver mutations in liver cancer

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Liver cancer is the seventh most common form of cancer, affecting over 800,000 people worldwide, while 700,000 patients pass away each year. While several gene coding mutations have been found to be associated with liver cancer, we lack an understanding of gene regulatory driver mutations that could also lead to liver cancer. Using short sequences, 16 base pairs in length, termed neomers, that are generally absent from the human genome but appear in liver cancer, we identify numerous potential causative gene regulatory mutations. We identify noncoding neomers that are unique to liver cancer, several of which are enriched in numerous patients and are thought to regulate cancer associated genes. We also characterize specific one kilobase regions in the human genome that are enriched for noncoding neomers and reside near many relevant liver cancer genes. To functionally characterize these sequences in a high throughput manner, we carried out a massively parallel reporter assay (MPRA) for over 40,000 sequences, finding several neomers that affect regulatory activity. Combined, our studies identify novel mutations in gene regulatory elements that could be key regulators of liver cancer drivers, providing a basis for potential downstream diagnosis and therapeutics applicable to liver cancer.

Intervention Mapping to Adapt an Evidence-Based Physical Activity Program for Rural Latinas

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Physical inactivity poses a significant health risk, especially among Latinas, who are at higher risk of chronic diseases such as cardiovascular disease. Faith-based organizations (FBOs) play a crucial role in the Latino community and offer a promising setting for health promotion interventions. Faith in Action, an evidence-based physical activity (PA) intervention, has shown effectiveness in urban/suburban settings. However, its adaptation and implementation in rural areas are essential to address the disparities in PA and chronic disease risk among rural Latinas.

The study will focus on recruiting 3 FBO, with an emphasis on churches serving a significant proportion of Latino families. These churches will be located in a rural, desert, and border region along the U.S.-Mexico border, specifically in Imperial County, where approximately 86.1% of the population is Hispanic/Latino, 30% are foreign-born, and only about 15.2% of adults aged 25 and older have a bachelor's degree or higher. The population of interest includes Latina churchgoers, FBO leaders/staff, and potential community health workers (promotoras) who will implement the intervention.

The proposed study is guided by the Practical, Robust Implementation and Sustainability (PRISM) model to address contextual factors for understanding translation of Faith in Action onto the Imperial Valley community. The IM-Adapt process for this study involves six key steps to adapt the Faith in Action intervention for rural Latinas :(1) Conduct a needs assessment and assess organizational capacity: Barriers and facilitators to physical activity among rural Latinos will be identified through literature review, interviews with FBO leaders/staff, promotoras, and focus groups with church members. A logic model for intervention strategies will be developed. (2) Choose intervention and assess basic fit: Faith in Action, designed for Latina women and adaptable to rural settings and hot climates, is selected as the base intervention. (3) Assess

intervention fit: Brainwriting premortem activity will gather feedback on Faith in Action's perceived feasibility and usability through interviews with FBO leaders/staff, promotoras, and focus groups with church members. (4) Make adaptations: Program documents and content will be adapted based on stakeholder feedback, with pre-testing in focus groups with church members and interviews with promotoras. (5) Plan for implementation: Facilitators, outcomes, and a facilitation plan will be identified to guide the intervention's delivery. (6) Plan for evaluation: Evaluation questions, data collection, analysis, and reporting plans will be created to evaluate the adapted intervention's impact on promoting physical activity among rural Latinas in the Imperial Valley.

By using the IM-Adapt framework, this research aims to optimize the effectiveness of Faith in Action in rural settings and contribute to reducing disparities in PA and chronic diseases among Latinas. The study's findings will have implications for scaling up evidence-based interventions in diverse FBOs across the United States, promoting PA, and improving the health of Latina communities.

Log2Lose: Incenting weight loss and dietary self-monitoring in real time to improve weight management among adults with obesity

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Research Objectives: Log2Lose is a weight management behavioral clinical trial at the University of Wisconsin-Madison and Duke University. We conducted interviews with Black and African American study participants and community members to identify strategies to improve the representation of Black and African American people in weight management studies. The primary goal of the qualitative interviews is to identify strategies to make the content more culturally appropriate and inclusive.

This work aligns with my goals to become a physician interested in community engagement and inclusivity in patient care.

Methods

Design of interview: Interview guides were developed using the Cumulative Model of Patient Complexity by Shippee et al. This model focuses intervention engagement through the lens of patient workload and patient capacity. We created interview questions about barriers that participants and community members may face regarding weight management and attempting to lose weight. Other questions related to making the intervention more appealing to others in the community by assessing goals, values and interests.

Sample: Interviewees were 1) Community members (n=11) from Madison, WI who are involved with the African American community either through organizations or work 2) study participants (n=20) who identified as black or African American and had direct experience with the study content.

Preliminary Findings: Analyses are ongoing. Preliminary findings suggest that Black and African Americans desire programs to assist them with weight management. They highlighted the importance of accountability, social support, and long-term maintenance. They also highlighted the need for more representation of minorities on the study team and when engaging with the community. Participants also highlighted the need to be sensitive to previous exploitation of the Black and African American community by medical researchers.

Discussion: When analyses are complete, we will have a list of suggestions for the study team to engage with the community. We will also have some practical suggestions to modify the intervention to address the needs of African Americans.

I am beginning a post-baccalaureate program in August. During my program, we will continue to code interviews and write up the results for publication. My long-term goal is to apply to medical school and serve the Black and African American community through patient-centered care and advocacy.

DCTN4-018 on Lung Epithelial Cells Impacts on Alveolar Immunity

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The alveoli of the lower respiratory system in lungs are responsible for gas exchange with the circulation and function in tissue repair and defense against infection. However, in many lung diseases, the alveolar space is destroyed or filled with fluid or tissue thereby impairing these critical functions. Indeed, patients with pulmonary fibrosis, Cystic Fibrosis (CF), or Acute Respiratory Disease Syndrome (ARDS) have both reduced pulmonary function and susceptibility to life-threatening lung infections. However, the critical defense mechanisms in the alveoli that bar infection and are impaired in lung disease have not been elucidated. Recently, a single-nucleotide polymorphism (SNP) in dynectin-4 (DCTN4) was identified as a genetic marker in CF and ARDS patients that associates with susceptibility to infection. To assess the function of SNP (DCTN4-018), we used the CRISPR/Cas9 system to knock-in DCTN4-018 in human bronchial epithelial (HBE) cells and found it significantly impaired autophagy, apoptosis, antimicrobial activity, and secretion of the antimicrobial protein, secretory leukocyte peptidase inhibitor (SLPI). Using flip excision switch (FLEX-Cre) system, we plan to create airway (Sox-2) specific DCTN4-018 expressing mice to evaluate the functional roles of DCTN4-018 in immune regulation and bacterial clearance of P.aeruginosa in a future in vivo model. Our data suggests that DCTN4 and the dynein/dynectin complex functions in the innate immune response of the lung mucosa. These findings can potentially aid in the development of therapies specifically geared towards patients expressing the variant DCTN4-018 gene.

Overview: Structural racism and cardiovascular disease risk in pregnant women and their infants-Supplement to the Mother and Infant Determinants of Vascular Aging Study (MIDAS)

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In the United States, cardiovascular disease (CVD) is the leading cause of mortality in women, and the prevalence of cardiometabolic risk factors and cardiometabolic-related pregnancy complications are greater in Black and underrepresented women than White women. Race is a social construct and not biological, thus ethnicity/race alone cannot explain the disparities in cardiometabolic complications in pregnancy. These inequalities in cardiometabolic diseases during pregnancy may be explained in part by structural racism, which includes neighborhood environment and residential segregation. Yet, it is not known how structural racism is associated with cardiometabolic risk in pregnancy, postpartum period, and disparity in adverse infant outcomes. Therefore, the objective of this project is to examine structural racism and the trajectory of arterial stiffness, a measure of cardiovascular disease risk, in pregnancy to postpartum in women and their infants. This project supplements the Mother and Infant Determinants of vascular Aging Study (MIDAS; R01HL157075). The MIDAS study will enroll 840 racially/ethnically diverse healthy and medically complicated mother/infant dyads between 34-40 weeks' gestation. At 34-40 weeks' gestation, within 48 hours of delivery, and at 6 and 12 months postpartum.

The Role of Sex Chromosome Complement and Gonadal Hormone in Lung microRNA Expression in a Mouse Model of Allergic Inflammation

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Epigenetic alterations such as dysregulation of microRNAs (miRNAs) have been reported to play important roles in interactions between genetic and environmental factors. In this study, we tested the hypothesis that induction of lung inflammation by inhaled allergens triggers a sex-specific miRNA regulation that is dependent on chromosome complement and hormonal milieu. We challenged the four core genotypes (FCG) model through intranasal sensitization with a house dust mite (HDM) solution (or PBS as a control) for 5 weeks. The FCG model allows four combinations of gonads and sex chromosomes: 1) XX mice with ovaries (XXF), 2) XY mice with testes (XYM), 3) XX mice with testes (XXM), and 4) XY mice with ovaries (XYF). Following challenge (n=3/group), we assessed expression of 84 inflammatory miRNAs in lung tissue using a PCR array and cytokine levels in bronchoalveolar lavage fluid (BAL) by a multiplex protein assay. Our results showed higher levels of the chemokine, KC (an Il-8 homologue) and IL-7 in BAL from XYF mice challenged with HDM. Additionally, IL-17A was significantly higher in BAL from both XXF and XYF. Furthermore, XYF mice had higher levels of miR-135a and miR-155, XXM mice had higher levels of miR-29b, XXF mice had lower levels of miR-26b and 23 other miRNAs, and no miRNAs reached statistical significance in XYM mice. Regulatory networks of miRNAs identified in this study were implicated in pathways associated with airway inflammation, immunity, and asthma. Female gonadal hormonal effects may alter miRNA expression and contribute to higher susceptibility of females to asthma.

CaMK4 is a Novel Regulator of Myeloid Phenotype and Function in Atherosclerosis

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Chronic inflammation is a major driver of atherosclerotic cardiovascular disease, and therapeutics that target inflammation reduce clinical cardiac events beyond levels seen with conventional strategies targeting cholesterol alone. Recent work by our group and others has demonstrated that advanced atherosclerosis is also characterized by the failure of an active repair process termed â€˜inflammation resolution'. Resolution is an active program that leads to the production of pro-resolving mediators, efficient phagocytosis of dead cells from injured tissue, and the curtailing of inflammatory cell recruitment. Strategies that boost resolution and break the cycle of chronic inflammation promote plaque stability. Our review of publicly available RNAseq data revealed an increase in expression of Calcium/calmodulin-dependent protein kinase 4 (CaMK4) in (1) advanced/unstable regions of plaque within human carotid arteries as well as in (2) myeloid cells derived from atherosclerotic murine aortas. Therefore, we hypothesized that CaMK4 promotes inflammation and impairs resolution.

Sex-specific Valvular Myofibroblast Activation in Response to Nano-scale Stiffness Cues

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Clinical evidence suggests aortic valve stenosis (AVS) progression is sexually dimorphic in disease presentation and outcomes. For example, male aortic valves tend to develop a calcified phenotype while female valves exhibit a distinct fibrotic phenotype. The calcified phenotype is characterized by stiff, spherical calcium-phosphate nanoparticles, where particle size and abundance increase with disease progression. Previous work also suggests that genes and epigenetic modifiers that escape X-chromosome inactivation (XCI) may contribute to sex dimorphisms in valve disease. We hypothesize XCI escape genes partially regulate sex-specific myofibroblast activation in response to nano-scale stiffness cues in the valve tissue microenvironment. To test this hypothesis, we describe a bioinspired hydrogel cell culture platform to interrogate how nanoscale stiffness cues modulate the valvular interstitial cell (VIC) to myofibroblast transition in male and female cells. We observed significant increases in myofibroblast activation in males relative to females in response to nano-scale stiffness cues. After showing that VICs are mechanosensitive at the nanoscale, we then sought to investigate changes in our male and female VICs at the transcriptional level. We showed that methylated lysine states in male VICs in response to the nano-scale stiffness cues were significantly less relative to female VICs. Ongoing work seeks to identify whether the Y-chromosome may contribute to sex-specific responses to nanoscale stiffness cues. We seek to target Y-chromosome specific regulators of methylation, such as SRY or KAP-1, and investigate longer timepoints to assess persistence in the male methylation state.

Cathepsin K cleavage of Angiopoietin-2 creates Tie2 antagonist fragments in sepsis

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Elevated Angiopoietin-2 is associated with diverse inflammatory conditions including sepsis, a leading global cause of mortality. During inflammation, Angiopoietin-2 antagonizes the endothelium-enriched receptor Tie2 to destabilize the vasculature. In other contexts, Angiopoietin-2 stimulates Tie2. The basis for context-dependent antagonism remains incompletely understood. Here we show that inflammation-induced proteolytic cleavage of Angiopoietin-2 converts this ligand from Tie2 agonist to antagonist. Conditioned media from stimulated macrophages induced Angiopoietin-2 secretion. Unexpectedly, this was associated with reduction of the 75 kDa full-length protein and appearance of new 25 and 50 kDa C-terminal fragments. Peptide sequencing proposed cathepsin K as a candidate protease. Cathepsin K was necessary and sufficient to cleave Angiopoietin-2. Recombinant 25 and 50 kDa Angiopoietin-2fragments (cANGPT225, cANGPT250) bound and antagonized Tie2. Cathepsin K inhibition with the Phase-3 small molecule inhibitor odanacatib improved survival in distinct murine sepsis models. Odanacatib's benefit was reversed by heterologous cANGPT225. Full-length Angiopoietin-2enhanced survival in endotoxemic mice administered odanacatib and conversely increased mortality in the drug's absence. Septic humans accumulated circulating Angiopoietin-2 fragments, which were associated with adverse outcomes. These results identify a novel proteolytic mechanism for the conversion of Angiopoietin-2 from Tie2 agonist to antagonist with therapeutic implications for inflammatory conditions associated with Angiopoietin-2 induction.

Serum IgA and Gd-IgA1 levels associate with pulmonary phenotypes. The Multi-Ethnic Study of Atherosclerosis (MESA) Lung Study

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Background: Immunoglobulin A (IgA) protects mucosal surfaces against pathogens. IgA deficiency is associated with impaired lung function, and small airway remodeling; and is highly heritable. Subclinical levels of IgA in plasma could have similar correlations. IgA1, an isotype of IgA, is susceptible to inheritable deficits in O-glycosylation; the resulting galactose-deficient IgA1 (Gd-IgA1) is pathogenic in IgA-related vasculitis and nephropathy. Plasma Gd-IgA1 levels have been less investigated in pulmonary diseases. We hypothesized that there was a causal relationship between serum IgA (all isotypes) and lung function, airway wall thickness, and emphysema.

Methods: The Multi-Ethnic Study on Atherosclerosis (MESA) recruited participants free of cardiovascular disease in 2000-02. IgA was measured in serum using ELISA assays. The MESA Lung Study performed spirometry and chest CT, upon which percent emphysema (-950 HU) and airway dimensions were measured. Linear regression models were used to analyze percent emphysema controlling for age, sex, genetic ancestry, smoking, pack-years, height, weight, and education. Generalized estimating equations were used to account for multiple airway measures per participant controlling for the covariates previously described. Mendelian randomization analyses were also conducted using MR MiSTERI. Variants associated with serum total IgA and Gd-IgA1 levels were extracted from the Trans-Omics for Precision Medicine (TOPMed) database for analysis.

Results: Among 1,296 participants of diverse genetic ancestries with serum IgA and serum Gd-IgA1 levels and segmental CT measures, there were significant positive associations between log normalized serum Gd-IgA1 levels and segmental airway average minor inner diameter and average wall thickness ($\hat{I}^2$=0.067; p = 0.034 and $\hat{I}^2$=0.12; p = 0.012, respectively). In addition, there was a statistically significant negative association of percent emphysema with log normalized serum

IgA levels (n=5,397; Î²=-0.08; p = 0.0045) and log normalized serum Gd-IgA1 levels (n=2,811; Î²=-0.089; p = 0.019). Lastly, MR analyses of emphysema and serum total IgA showed a causal association of the biomarker on the phenotype (n=4,153; Î²=-0.08; p = 0.01).

Conclusions: In one of the first cohort studies analyzing serum Gd-IgA1 levels, we identified associations between reduced serum IgA concentration with percent emphysema (both total IgA and Gd-IgA1) and lower airway abnormalities (Gd-IgA1). In addition, MR analyses indicated a causal impact of serum total IgA on emphysema but not serum Gd-IgA1. These findings support the protective role of IgA and Gd-IgA1 in lung host defense and COPD pathogenesis.

Coparenting QualityÂ is Associated with Healthier Home Food Environments in Preschool-Aged Children

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Positive coparenting, or the ways in which parents relate to each other in their caregiving roles, is well known to support children's socioemotional development. While there is strong reason to hypothesize that positive coparenting also contributes to home environments that promote children's healthy weight, the role of coparenting in childhood obesity has rarely been considered. The objective of this study was to examine cross-sectional associations between couples' coparenting quality, generally and specific to child feeding, home food environments, and young children's body mass index (BMI). The SPROUT study enrolled 332 mothers (mean age = 35.9, 32.8% non-White race/ethnicity) and their 3-year-old children, of which 288 mothers (87%) identified having a coparent, defined as a significant other in the household whom they parent the child with. Mothers completed the Coparenting Relationship Scale, Feeding Coparenting Scale, measures of the home food environment, and sociodemographics. Children's weight and height were measured by trained staff and used to calculate BMIz scores. Preliminary analysis using adjusted regression models showed that mothers who reported more positive coparenting overall and greater alignment with their partner's views and values in child feeding also reported more availability of healthy foods in the home and less use of food as a reward for children ($p=0.02$ and 0.04, respectively). No associations were observed between aspects of coparenting and child BMI; however, children with coparents who more strongly encouraged their child to eat had a lower BMIz ($p=0.02$). Coparents' alignment of views and values, and overall support of each other in parenting roles, may promote healthier eating in preschool-aged children.

Cardiac Fibroblast-MHCII Contributes to Cardiac Pathophysiology in Doxorubicin-Induced Cardiomyopathy

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Cancer patients receiving Doxorubicin (Dox), one of the most widely used chemotherapy agents, often develop cardiomyopathy and heart failure (HF) due to the cardiac toxicity of Dox. Prominent hallmarks of Dox cardiomyopathy include the development of cardiac fibrosis, cardiomyocyte death, and contractile dysfunction. In other etiologies of HF, cardiac T cell inflammation and fibrosis co-exist, and cardiac fibroblasts (CFBs) expressing the major histocompatibility complex II (MHC-II) in response to T cell-released IFNÎ³, contribute to cardiac fibrosis and contractile dysfunction. We hypothesized that CFB-T cells crosstalk contributes to cardiac inflammation and fibrosis through CFB-MHC-II in Doxorubicin-induced cardiomyopathy. Wild type (WT) and T cell-deficient (Tcra-/-) mice were treated with PBS or Dox intraperitoneally (i.p.) (5 mg/kg/week) for 4 or 8 weeks. We investigated the surface expression of MHC-II on CD31-/CD45-/MEFSK4+ CFBs from enzymatically digested hearts using flow cytometry. We found enhanced MHC-II expression on CFBs from Dox-treated WT mice compared to those from PBS-treated WT controls. In contrast, CFBs from Tcra-/- mice treated with Dox did not express MHC-II, and echocardiography determined that they developed less systolic dysfunction than Dox-treated WT mice. 3-day in vitro treatment of primary cultured CFBs with Dox (0.1ug/mL and 1.0ug/mL) reduced CFB viability compared with untreated cells, yet, interestingly, did not induce expression of surface MHC-II protein or gene expression, analyzed by flow cytometry and qPCR, respectively, compared with IFNÎ³ treated CFBs, used as a positive control. To investigate the functional role of CFB-MHC-II in Dox-induced cardiomyopathy, we treated Tcf21iCre/+ MhcIIfl/fl male and female mice with tamoxifen (TMX) (75mg/kg/daily, or vehicle for 5 days, i.p) to reduce MHC-II expression specifically in CFBs (CFB-MhcII-/-) prior to starting Dox treatment. CFB-MhcII-/- mice were compared with vehicle-treated littermate controls (CFB-MhcII+/+) after 8 weeks of Dox treatment. Wheat-germ agglutinin (WGA) staining determined larger cardiomyocyte size in CFB-MhcII-/- male mice compared with CFB-MhcII+/+ littermate controls receiving Dox. Strikingly, Dox-treated CFB-

MhcII-/- male mice showed decreased collagen deposition determined by picrosirius red staining and preserved systolic function, analyzed by echocardiography, compared with Dox-treated CFB-MhcII+/+ controls. CFB-MhcII+/+ and CFB-MhcII-/- female mice, however, showed no changes in systolic function after 8 weeks of Dox compared to PBS treated mice. Together, these data demonstrate that Dox induces MHC-II expression on CFBs in a T cell-dependent manner and not through the direct actions of Dox on CFBs. Our results also identify a novel contributing role for CFB-MHC-II in the development of cardiac fibrosis, cardiomyocyte atrophy, and systolic dysfunction in Dox cardiomyopathy in male mice. Our data also demonstrate that unlike male mice, female mice do not develop systolic dysfunction with the cumulative dosing of Dox.

The association between anxiety and Alzheimer's disease plasma biomarkers across stages

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Cancer patients receiving Doxorubicin (Dox), one of the most widely used chemotherapy agents, often develop cardiomyopathy and heart failure (HF) due to the cardiac toxicity of Dox. Prominent hallmarks of Dox cardiomyopathy include the development of cardiac fibrosis, cardiomyocyte death, and contractile dysfunction. In other etiologies of HF, cardiac T cell inflammation and fibrosis co-exist, and cardiac fibroblasts (CFBs) expressing the major histocompatibility complex II (MHC-II) in response to T cell-released IFNÎ³, contribute to cardiac fibrosis and contractile dysfunction. We hypothesized that CFB-T cells crosstalk contributes to cardiac inflammation and fibrosis through CFB-MHC-II in Doxorubicin-induced cardiomyopathy. Wild type (WT) and T cell-deficient (Tcra-/-) mice were treated with PBS or Dox intraperitoneally (i.p.) (5 mg/kg/week) for 4 or 8 weeks. We investigated the surface expression of MHC-II on CD31-/CD45-/MEFSK4+ CFBs from enzymatically digested hearts using flow cytometry. We found enhanced MHC-II expression on CFBs from Dox-treated WT mice compared to those from PBS-treated WT controls. In contrast, CFBs from Tcra-/- mice treated with Dox did not express MHC-II, and echocardiography determined that they developed less systolic dysfunction than Dox-treated WT mice. 3-day in vitro treatment of primary cultured CFBs with Dox (0.1ug/mL and 1.0ug/mL) reduced CFB viability compared with untreated cells, yet, interestingly, did not induce expression of surface MHC-II protein or gene expression, analyzed by flow cytometry and qPCR, respectively, compared with IFNÎ³ treated CFBs, used as a positive control. To investigate the functional role of CFB-MHC-II in Dox-induced cardiomyopathy, we treated Tcf21iCre/+ MhcIIfl/fl male and female mice with tamoxifen (TMX) (75mg/kg/daily, or vehicle for 5 days, i.p) to reduce MHC-II expression specifically in CFBs (CFB-MhcII-/-) prior to starting Dox treatment. CFB-MhcII-/- mice were compared with vehicle-treated littermate controls (CFB-MhcII+/+) after 8 weeks of Dox treatment. Wheat-germ agglutinin (WGA) staining determined larger cardiomyocyte size in CFB-MhcII-/- male mice compared with CFB-MhcII+/+ littermate controls receiving Dox. Strikingly, Dox-treated CFB-

MhcII-/- male mice showed decreased collagen deposition determined by picrosirius red staining and preserved systolic function, analyzed by echocardiography, compared with Dox-treated CFB-MhcII+/+ controls. CFB-MhcII+/+ and CFB-MhcII-/- female mice, however, showed no changes in systolic function after 8 weeks of Dox compared to PBS treated mice. Together, these data demonstrate that Dox induces MHC-II expression on CFBs in a T cell-dependent manner and not through the direct actions of Dox on CFBs. Our results also identify a novel contributing role for CFB-MHC-II in the development of cardiac fibrosis, cardiomyocyte atrophy, and systolic dysfunction in Dox cardiomyopathy in male mice. Our data also demonstrate that unlike male mice, female mice do not develop systolic dysfunction with the cumulative dosing of Dox.

Associations between Neighborhood Environment & Computerized Cognitive Performance among Black Adults

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Background: Studies have suggested that neighborhood quality is associated with health outcomes beyond individuals' socioeconomic characteristics (Kind & Buckingham, 2018; Ludwig et al., 2011). Limited research has explored how subjective neighborhood quality is associated with cognitive functioning, particularly among socioeconomically diverse Black adults. The current study examined the cross-sectional association between perceived neighborhood characteristics and cognitive functioning among Black adults.

Methods: This study included 1,079 community-dwelling, socioeconomically diverse Black adults (Mage = 56.64, SDage = 9.14; 59% female) from wave 4 of the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study. Neighborhood quality was assessed using three scales about built environment disorder (e.g., litter, graffiti, and crime), social cohesion (e.g., close-knit neighborhood, neighbors can be trusted), and social control (e.g., neighbors take action for children disrespecting adults). Cognitive functioning was assessed using the computerized Joggle battery. Multivariable linear regression analyses were conducted with each cognitive measure as the outcome measure. Models were adjusted for age, sex, education, reading literacy, poverty status, and depressive symptoms.

Results: In the adjusted models, higher built environment disorder was associated with slower performance ($p < .05$) on an attention and visual orientation measure and fewer correct responses (p's $< .05$) on attention, executive function, and speed measures. High social cohesion was associated with faster performance ($p < .05$) on attention, visual orientation, executive function, and speed measures. Additionally, high social cohesion was associated with greater numbers of correct responses ($p < .05$) on an attention and executive function measure. Interestingly, high social control was associated with slower performance on executive function. Significant interactions were observed between neighborhood quality and

covariates. For example, higher perceived social cohesion was associated with slower average reaction time on memory and attention, particularly for individuals with higher depression total scores. In contrast, higher perceived social cohesion was associated with faster average reaction time on executive functioning, particularly for men.

Conclusions: These findings underscore the importance environmental characteristics for cognitive performance among Black adults. Further, these findings support the continued assessment of contextual factors as mechanisms or avenues for understanding how to improve cognitive functioning.

More Exposure to Everyday Discrimination Increases the Odds of Chronic Pain Among Hispanic and Non-Hispanic but not Non-Hispanic Black Middle-Aged and Older adults

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Chronic pain disproportionately affects middle-aged and older adults in the United States. Everyday discrimination is associated with worse pain outcomes and is more prevalent among adults from racial/ethnic minoritized groups. Yet, there is limited evidence on relationships between everyday discrimination and chronic pain among middle-aged and older adults, particularly how discrimination and racial/ethnic identity may interact to influence this relationship. We used the 2018 Health and Retirement study to evaluate associations between exposure to everyday discrimination and odds to experience any, severe, and high impact chronic pain among 5,314 Hispanic, non-Hispanic Black, and non-Hispanic White adults over the age of 50. Logistic regression was used to evaluate the effects of everyday discrimination on the odds of chronic pain (any, severe, and high impact) across racial/ethnic groups. Results showed that Hispanic and non-Hispanic Black middle-aged and older adults had a higher, unadjusted prevalence of severe and high impact chronic pain and reported more exposure to everyday discrimination compared to non-Hispanic White middle-aged and older adults. In addition, study findings showed that any exposure to discrimination increased the odds of chronic pain among Hispanic and non-Hispanic White respondents, but had no significant effect on non-Hispanic Black respondents' odds to experience chronic pain. Findings underscore the influential role of everyday discrimination on the chronic pain experiences of middle-aged and older adults, as well as differential effects across racial/ethnic groups.

Functional Decline among Nursing Home Residents with ADRD during the COVID-19 Pandemic

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Introduction: During the COVID-19 pandemic, changes in nursing home (NH) processes disrupted NH resident care and daily routines which may have affected residents' activities of daily living (ADL) performance. Disruption to routine and changes in staff interactions may have had greater effect on functional decline in residents with ADRD than those without.

Objective: To examine functional decline in ADLs during the COVID-19 pandemic among long-stay NH residents with a diagnosis of ADRD compared to residents without ADRD.

Methods: Using Michigan MDS data, we identified long-term NH residents with at least one NH assessment pre-COVID and during COVID. We excluded residents missing demographic and ADL information, admitted from hospice, or with ADL score â‰¥ 26 (worst function). Residents with ADRD were identified based on ICD-10 codes and diagnosis check boxes on MDS assessments. Functional decline was defined by a decline of â‰¥ 3 points on the long-form ADL Self Performance Scale in MDS . We compared functional decline between residents with and without ADRD using unadjusted descriptive statistics. Kaplan-Meier estimate was used to test for the association of an ADRD diagnosis with risk of functional decline. Cox proportional hazards regression model was used to test for the independent association of ADRD diagnosis and functional decline adjusted for resident characteristics.

Results: We included a total of 24,055 Michigan long-stay NH residents, of whom 14,245 (59%) had a diagnosis of ADRD. The mean age was 78.3 years, 63% were â‰¥ 75 years old, 65.6% were female, and 18.4% were Black. Overall, the mean total ADL score was 15.4 (SD=5.9), 15.6 (SD=6.0) for residents with ADRD and 15.2 (SD=5.9) for residents without ADRD ($p<0.001$). ADL decline of â‰¥ 3 points was identified in 36% of residents with ADRD and 30% of residents without

ADRD ($p<0.0001$). Having a diagnosis of ADRD was associated with higher risk of ADL functional decline compared to those without ADRD after adjusting for age, sex, race, health instability and comorbidities (HR=1.19, 95%CI [1.13-1.24]).

Conclusion: Long-term NH residents with a diagnosis of ADRD were associated with higher risk of functional decline (â‰¥ 3 points in ADL score) compared to those without ADRD during the COVID-19 pandemic.

Parent factors associated with BMI, diet, and physical activity of adolescents with intellectual and developmental disabilities

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Background: Adolescents with intellectual and developmental disabilities (IDD) experience overweight and obesity (OW/OB) up to 1.8 times the rate of their typically developing peers. Parents may influence adolescent weight management behaviors in this population, but the association between parent factors and adolescent weight management behaviors is unclear.

Objective: To examine the associations between parent BMI and sociodemographic characteristics with adolescents' BMI, diet quality, daily energy intake, moderate to vigorous physical activity (MVPA), and sedentary behavior.

Methods: This study analyzed baseline data from an 18-month randomized controlled weight loss trial for adolescents with IDD. We assessed parent BMI (kg/m2) and sociodemographic factors, and adolescent BMI z-score, MVPA, sedentary time, daily energy intake, and diet quality. Associations between parent and adolescent factors were assessed with Pearson, Spearman or Kendall Tau-b correlations; mean differences for categorical outcomes were assessed with independent samples t-tests/Mann-Whitney U tests or ANOVA/Kruskall-Wallis tests.

Results: Ninety-five adolescent and parent dyads were included. Parent BMI was positively correlated with adolescent BMI z-score (n=94: rs= 0.37, p<0.01). Household income was inversely correlated with adolescent BMI z-score (n=95: Tb= -0.18, p=0.02). Parents with less than a bachelor's degree had adolescents with higher BMI z-scores than those with bachelor's or higher (2.1 ± 0.5 vs. 1.8 ± 0.5, p=0.02) as well as higher sedentary behavior (n=28, 515.2 ± 102.6 mins/day vs. n=40, 463.9 ± 148.1 mins/day, p=0.02).

Conclusion: We found parent BMI, income, and education associated with

adolescent BMI z-score. These findings contribute to the sparse literature on parental factors associated with OW/OB in this population.

Survival Methods in Electronic Health Records Data in the Presence of System Migration

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Electronic health records (EHRs) have grown in popularity for healthcare research in the recent decade. While they provide cheap access to large amounts of observational health data, they also come with a long list of potential biases, including but not limited to confounding bias, selection bias, informed-presence bias, and misclassification bias. An unstudied bias results when patients utilize multiple healthcare systems (referred to as "system migration" hence forth). Since system migration patterns are typically unknown in EHR data, the time of disease onset can be unclear for a given patient. This bias is of particular concern in, for example, studying risk factors of the time-to-dementia onset in the Indian Health Services (IHS) EHR data. Many American Indian and Alaskan Native (AI/AN) patients using the IHS also have access Medicare, Medicaid, and/or private insurances, which offer them flexibility in choosing a healthcare system. This flexibility results in two different patterns of system-usage: 1) patients who routinely use IHS and 2) patients who migrate between systems. Ignoring the system migration of select patients will bias effect estimates toward the null. We propose extending the Cox proportional hazards model—a regression method for analyzing the relationship between covariates and the time-to-event in censored survival data setting—to (1) identify potential cases of system migration and (2) map migrated patients' IHS diagnosis times to a routine system visit time. Simulation studies and an application to IHS data highlight how our model reduces bias and is instrumental in producing valid and replicable research in AI/AN healthcare.

Midlife Neuropsychological Profiles and Associated Vascular Risk: The Bogalusa Heart Study

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Background: Individuals with Alzheimer's disease (AD) often present with coexisting vascular pathology that is expressed to different degrees and can lead to clinical heterogeneity.

Objective: To examine the utility of unsupervised statistical clustering approaches in identifying neuropsychological (NP) test performance subtypes that closely correlate with carotid intima-media thickness (cIMT) in midlife.

Methods: A hierarchical agglomerative and k-means clustering analysis based on NP scores (standardized for age, sex, and race) was conducted among 1,203 participants (age 48 Â± 5.3 years) from the Bogalusa Heart Study (BHS). Regression models assessed the association between cIMT > 50th percentile and NP profiles, and global cognitive score (GCS) tertiles for sensitivity analysis.

Results: Three NP profiles were identified â€“ Mixed-low performance [16%, n=192], scores >1 SD below the mean on immediate, delayed free recall, recognition verbal memory, and information processing; Average [59%, n= 704]; and Optimal [26%, n=307] NP performance. Participants with greater cIMT were more likely to have a Mixed-low profile [OR=3.10, 95% CI (2.13, 4.53), P <0.001] compared to Optimal. After adjusting for education and cardiovascular (CV) risks, results remained. The association with GCS tertiles was more attenuated [lowest (34%, n=407) vs. highest (33%, n=403) tertile: adjusted OR=1.66, 95% CI (1.07, 2.60), P = 0.024]

Conclusion: As early as midlife, individuals with higher subclinical atherosclerosis were more likely to be in the Mixed-low profile, underscoring the potential malignancy of CV risk as related to NP test performance, suggesting that classification approaches may aid in identifying those at risk for AD/vascular dementia spectrum illness.

NLRP3 Gene Loss Exacerbates LV Hypertrophy and M2 Polarizes Left Ventricle Resident Macrophages in the DAHL/SS-Nlrp3em2Mcwi Rat Model

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Background: The Nod-like receptor pyrin domain-containing protein (NLRP3)-3 inflammasome is a multimeric protein complex that is activated in response to pathogen and damage associated molecular patterns (PAMPs & DAMPs) to initiate inflammatory responses through IL-1ÃŸ, IL-18, and pyroptosis. Pharmacological inhibition of NLRP3 attenuates inflammatory responses in rodent models of myocardial infarction and hypertension. However, the effect of NLRP3 gene loss on left ventricle (LV) morphology and basal macrophage and monocyte populations in the Dahl/SS rats is unknown. We hypothesized that NLRP3 gene loss would not impact LV morphology or alter macrophage/monocyte phenotype under basal conditions. Methods: CRIPSR was used to induce a 14 base pair deletion in exon 1 of the NLRP3 gene in the DAHL/SSJrHsdMcwi strain to produce DAHL/SS-Nlrp3em2Mcwi rats (NLRP3 KO). WT and NLRP3 KO rats were studied at 4-6 mo. Teklad 7034 low salt (0.12% NaCl) diet was provided ad libitum since weening. Morphological characteristics that include body weight, LV weight, tibial length (TL), spleen length and weight, visceral adipose weight, and body weight were measured. CD45+ CD11b+ macrophage/monocytes were characterized for polarization status using proinflammatory M1 marker CD68+ and anti-inflammatory marker M2 CD206+ in the LV and PBMCs. CD68 immunohistochemistry was performed on LV formalin-fixed paraffin-embedded samples. LV morphology was assessed using H&E and Masson's Trichrome staining. Results: Sex ($p<0.05$) and genotype ($p<0.05$) main effects were observed in several morphological characteristics. As expected, male rates displayed significantly increased right tibial length (TL), rat (RW), kidney, adipose and LV weights, and LVW/TL vs females. Surprisingly, NLRP3 KO rats exhibited significantly increased ($p<0.05$) LV weight, LVW/TL, and LVW/BW ratios compared to WT rats. In the LV, the % of M2-like MÎ¦s was significantly increased ($p<0.05$) in NLRP3 KO vs WT (n=3-5/group). WT PBMCs macrophages/monocytes exhibited significantly increased ($p<0.05$) CD68 and

CD206 protein expression compared to KO (n=3-5/group). There were no significant differences in LV morphology between all groups. Conclusion: Flow cytometry data suggest that NLRP3 gene loss on a Dahl salt sensitive background induces M2 polarization of resident LV MÉ¸s, which may be associated with increased LV mass. Additionally, NLRP3 gene loss prevented MÉ¸ polarization in PBMCs. We hypothesize that NLRP3 may possess an inflammasome independent role in regulating LV hypertrophy and MÉ¸s polarization under basal conditions in our model. Project supported by: NIH (NIA) Grant R21AG070723 and NIH (NIGMS) Grant T32GM108563. Animal protocols were approved by the Penn State IACUC #43534.

Action Plans Increase Advance Care Planning Documentation and Engagement Among English and Spanish-speaking Older Adults

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Background: Advance Care Planning (ACP) has been reconceptualized as a health behavior. Action Plans (APs), or patient-directed mini contracts, have been shown to increase behavior change in exercise and nutrition. However, no prior studies have assessed whether creating an ACP AP can increase ACP documentation and engagement.

Methods: English and Spanish speaking older adults with two or more serious or chronic illnesses were included from public and VA outpatient clinics in San Francisco. Participants were in the intervention arm of the PREPARE for YOUR Care trial, which included asking participants to choose 1 of 5 APs at baseline (choose a surrogate, ask a surrogate, choose flexibility for a surrogate, tell others about their medical wishes verbally/in writing, and ask clinicians questions). At 6 months, we assessed whether participants completed their AP and if completion was associated with participant characteristics, ACP documentation in the EMR, and the validated 5-point ACP Engagement Survey scores. We used t-tests, Chi-squared, and multivariate analysis adjusted for baseline ACP and clustering by physician. We also used qualitative thematic analysis to explore reasons for not completing an Action Plan.

Results: The mean age of the 586 participants was 65 ±10 years; 44.0% were women, 45.9% were Spanish-speaking, 31.4% had limited health literacy, and 42.5% of people completed an AP at 6 months (the top 3 APs were choose a surrogate (57.6%), ask a surrogate (16.4%), and tell others about their medical wishes (15.8%)). Of participant characteristics, those with limited vs adequate health literacy were less likely to complete AP (25.4% vs. 35.9%, p=0.01). Completing an AP was associated with greater ACP EMR documentation 49.8% vs 35.6%, p <0.001, (Adjusted Odds Ratio: 2.06; 95% CI (1.43-2.97) and engagement (adjusted 5-point Engagement Scores (3.69; 95% CI 3.57-3.81 vs 3.10; CI: 2.98-

3.21), p <.001). Qualitative themes for non-completion included not being ready and logistic issues (e.g. family out of country, partner dying, surrogate refusing.)

Conclusions: Among English and Spanish speaking older adults, creating an ACP Action Plan resulted in greater ACP EMR documentation and self-reported engagement. Action Plans may help facilitate ACP behavior change as part of effective ACP interventions. Additional support may be needed for patients with limited health literacy and those facing logistic barriers.

Facing Trustworthiness: Age-group differences in trust-related decision-making & learning

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Facial impressions and repeated experiences contribute to trustworthiness evaluations. Older adults are particularly vulnerable to violations of trust. Using the Social Iowa Gambling Task, we examined age differences in the impact of facial trustworthiness on decision-making. Advantageous decks were represented by trustworthy (congruent, CS-IGT) or untrustworthy faces (incongruent, IS-IGT). Younger (n=143) and older (n=129) participants completed the standard IGT, CS-IGT, or IS-IGT. Both groups preferred trustworthy faces at first, indicating age-related similarities in approaching trustworthy faces. Older, compared to younger, adults performed worse on the IS-IGT, suggesting incongruent cues interfered with performance for older adults. Though both groups initially performed worse in the IS-IGT than the CS-IGT, older adults performed worse in all tasks over time. Findings suggest that when facial cues are paired with repeated unfavorable outcomes, older adults are less able to adapt their decision-making strategies, which aligns with older adults' reduced sensitivity to negative social reputations.

Exploring Sex-Specific Impact of Alzheimer's Disease-Related APP Mutations on Colitis-Associated Colon Cancer

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Cancer and Alzheimer's disease (AD) are age-associated conditions and leading causes of mortality in the United States. Despite numerous epidemiological studies revealing an inverse relationship between cancer and AD, the molecular mechanisms of this negative correlation remain largely unexplored. Sex-related distinctions are believed to contribute to the heterogeneity of both diseases. Notably, approximately two-thirds of AD patients in the US are female, while certain cancers, such as colorectal cancer, exhibit higher incidence and mortality rates in males. Emerging evidence from several investigations suggests an association between cancers and elevated levels of amyloid precursor protein (APP) or its N-terminal fragment, sAPP, implying a potential mechanistic interplay between APP biology and cancer progression. In our current study, we aimed to replicate the inverse relationship between AD and cancer by utilizing a mutant APP amyloidosis model and an azoxymethane (AOM)/dextran sodium sulfate (DSS) colitis-associated colon cancer model, thus facilitating a comparative assessment of disease progression.

We treated male and female C57BL/6 wild type, AppNL-G-F, and App-/- mice with or without IP injection of AOM and 1 week of oral DSS administration, followed by a recovery period spanning 17 weeks. Immunohistochemical analyses revealed prominent APP expression within normal and cancerous epithelial cells, as well as enteric neurons in the colons of both wild type and AppNL-G-F mice. Immunoreactivity associated with Î²-secretase (BACE1) and AÎ² peptide was also detected within the normal and cancerous epithelium of AppNL-G-F mice, indicative of APP processing within these cell populations. The mortality rate of App-/- male mice was dramatically increased by AOM/DSS-induced colon cancer compared to the other lines. This heightened mortality rate was not observed in female App-/- mice. However, despite mortality differences, no significant changes in tumor count or area were observed between App-/- male mice and their wild type counterparts. In contrast, male AppNL-G-F mice showed a significant

increase in both tumor count and area compared to wild type mice. Conversely, female AppNL-G-F mice demonstrated remarkable resistance to colon cancer, exhibiting significantly reduced tumor count and area when compared to wild type controls.

These data support a sex-selective relationship between AD and colon cancer, with AD-associated APP mutations potentiating and attenuating cancer in males and females, respectively.

Senescence-Based Drug Screening for Alzheimer's Disease Using Multi-Organ Models

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Cellular senescence is a factor of cell aging linked to age-associated neurodegenerative conditions like Alzheimer's disease (AD). In cells, senescence presents with markers of cell cycle arrest, persistent DNA damage response (DDR), senescence-associated Î²-galactosidase (SA-Î²-gal), and senescence-associated secretory phenotype (SASP). Preventing or reducing cellular senescence could contribute to treatment and mitigation of AD. AÃŸ is a protein known to contribute to pathogenesis of AD, and AÃŸ oligomers have recently been shown to induce cellular senescence. Tacrine is an acetylcholinesterase inhibitor used to treat AD, and testing its effects on senescent cells could contribute to further AD drug discoveries. In this study, we developed brain and gut-liver-kidney organ models on the OrganRXTM to assess AÃŸ-induced senescence and to test drug effects of tacrine on senescence-induced cells. Results from SA-Î²-gal staining of brain and gut-liver-kidney organ models suggest decreased cell senescence with increased concentration of tacrine.

Neuronal Integrity of Hippocampal Granule Cells in Primary Progressive Aphasia due to FTLD-tauopathies

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Background: Primary progressive aphasia (PPA) is a dementia syndrome that is characterized by an isolated and progressive language impairment with relative sparing of memory. PPA can be due to several underlying neuropathologies, including the tau form of frontotemporal lobar degeneration (FTLD-tau). Tau can be expressed in different isoforms, with either 3 or 4 repeats in the microtubule binding domain (3R or 4R). FTLD-tau subtypes are characterized by whether pathologic tau inclusions contain either 3R tau (Pick's Disease; PiD) or 4R tau (cortical basal degeneration, CBD; or progressive supranuclear palsy, PSP). Previous studies based on stereologic quantitation of tau inclusions show that the dentate gyrus (DG) of the hippocampus is differentially vulnerable to FTLD-tau inclusion formation, where PiD show highest densities compared to other species. The objective of this study was to examine neuronal size as a proxy for cellular integrity across FTLD-tauopathies within the DG.

Methods: Twenty-five right-handed participants with clinical diagnoses of PPA and an autopsy-confirmed tauopathy (PiD, N = 9; CBD, N = 8; PSP, N = 8) as the primary pathologic diagnosis were included in this study. Paraffin-embedded sections of the left posterior hippocampus were stained with 1.0% Cresyl Violet to visualize granule neurons. Digital images of 3 slides per region/hemisphere were obtained at 20x and the dentate gyrus was annotated using the QuPath digital pathology software. Average neuronal area (Î¼m2) of granule cells was calculated per group. Unpaired t-tests were used to compare average neuronal size (area) between 3R- and 4R-tauopathies, and ANOVA to compare average neuronal size between all three tauopathies. Pearson correlations were used to calculate the relationship between neuronal size and tau-positive inclusion densities (count/mm3), the latter data previously quantitated stereologically.

Results: PSP participants had significantly higher age of onset ($p<0.01$) and age at

death ($p<0.05$) compared to PiD. Average granule cell size was significantly smaller in 4R tauopathies (M=91.69 μm2, SD=13.68) compared to 3R tauopathies (M=103.3 μm2, SD=11.22) ($p<0.05$). CBD cases showed significantly smaller dentate cells than PiD ($p<0.05$). There were no differences between PSP vs PiD or CBD. Within the 4R tauopathies (CBD + PSP), there was a significant negative correlation between neuronal size and tau inclusions ($r=-0.7023$; $p<0.005$); this appeared to be driven by the CBD group where a strong significant negative correlation between granule cell size and tau inclusions ($r=-0.9414$; $p<0.0005$) was apparent.

Conclusions: Preliminary findings showing that granule cells are significantly smaller in the DG of 4R-FTLD-tau compared to those with 3R-FTLD-tau suggest that FTLD-tau species have a unique pathogenic cellular profile. Tau inclusions in CBD appear to be related to neuronal shrinkage; this contrasts with PiD where despite a high density of DG inclusions, neuronal size appears to be relatively larger. Future studies will more closely examine the relationship between proxies of neuronal integrity and tau in other anatomic regions and will include a cohort of cognitively-normal specimens for relative comparison.

Internal Mechanical Stress May Cause Stochastic Release of Large Neuronal Vesicles

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Our lab recently discovered that *Caenorhabditis elegans* adult neurons can package and expel protein aggregates, mitochondria, and other cytoplasmic contents from the soma within large (~5µm diameter) vesicles called exophers. Exophergenesis may represent a novel branch of µm diameter) vesicles called exophers. Exophergenesis may represent a novel branch of m diameter) vesicles called exophers. Exophergenesis may represent a novel branch of neuronal proteostasis and mitochondrial quality control relevant to human neurodegenerative diseases. We have uncovered a potential role of mechanical stress in the formation of exophers in mechanosensory neurons of *C. elegans*. The frequency of formation of exophers in these neurons strongly correlates with the position of the neuron within the worm, particularly its proximity to the uterus, with mechanosensory neurons located further away from the uterus (e.g. the PLMs) producing fewer exophers than those neurons closer to the uterus (e.g. the ALMR). While removal of oocytes/fertilized eggs (via genetic disruption of germline maturation or sperm function) abrogates the ability of neurons to make exophers, disruption of egg laying in infertile worms with a concomitant increase in volume of the worm taken up by oocytes greatly increases the frequency of exophers. Additionally, microinjection of fluid into the worm's body cavity near the ALMR also induces exophergenesis from the ALMR. Together, these findings suggest the possibility of mechanical stress triggering the production of exophers, although further work is needed to confirm this. Planned work includes the use of a tension/compression biosensor expressed in neurons to directly measure physical stresses on the neuron, as well as the use of a microfluidic platform to apply localized pressure to different parts of the worm to directly test the correlation between exophergenesis and physical stress on the neuron.

Wide Field of View Non-Mydriatic Biomarker Detection Portable Fundus Camera

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We report a large field of view FOV low-cost smart fundus camera with non-mydriatic and spectral band illumination features for the purpose of diagnosing central nervous system diseases such as Alzheimer's disease. By combining a customized optical lens group, ring light emitting diode light source, Li-battery, and Raspberry Pi we can port the spectrally selected light source through a 4 mm diameter pupil. Taking advantage of the open hardware platform and Linux operating system, we integrate two narrowband filters explicitly selected based on the unique spectrums of biomarkers (580nm and 660nm) with a Raspberry Pi camera module to obtain high quality images for the purpose of enhancing the visibility of retinal vasculature and nerve fiber layer.

Estrogen replacement therapy restores cerebral glucose uptake and attenuates cognitive decline in ovariectomized Bri-AÎ²42 mice

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Two thirds of individuals with late onset Alzheimer's disease (AD) are women, with advanced age and menopause being strong risk factors. Chronic decline in cerebral metabolic rate of glucose (CMRglc) precedes and, in individuals with mild cognitive impairment, predicts onset of AD cognitive decline and brain atrophy. Postmenopausal estrogen depletion may also contribute to deficiencies in CMRglc in women, thus increasing susceptibility to AD-associated cognitive decline. In the Bri-AÎ²42 (AÎ²-Tg) mouse model, we previously reported that young female mice are protected against metabolic and cognitive decline compared to males. Thus, we hypothesize that estrogens in female AÎ²-Tg mice protect against amyloid-induced pathology.

WT and AÎ²-Tg female mice underwent bilateral ovariectomy (OVX) or sham surgery at approximately 6 weeks of age. After 2 weeks, mice were implanted with a timed-release subcutaneous pellet containing 17Î²-estradiol (Î²E2) or a placebo pellet (n=10-13/group). Two weeks after implantation, a subset of mice (n=8) were assessed for long-term spatial memory testing in an IntelliCage (IC). Mice were trained for one week to alternate between two opposite corners of the cage to access drinking water. At 16 weeks of age, a subset of mice underwent glucose tolerance tests (GTT) and a 2-deoxyglucose (2DG) assay of CMRglc.

Sham WT and AÎ²-Tg mice significantly increased the correct pattern rate over 7 days of training in IC (Two-way ANOVA, $p < 0.05$). OVX AÎ²-Tg mice failed to improve over time, but OVX AÎ²-Tg with Î²E2 had significantly greater correct responses as early as Day 2 of training. Analysis of the difference in performance on Day 1 vs. Day 2 showed that WT, AÎ²-Tg, and AÎ²-Tg-OVX-E2Î² mice all significantly improved by Day 2, whereas AÎ²-Tg-OVX only changed by 0.72%. GTT results varied by treatment and not genotype; OVX mice of both genotypes were most impaired, while Î²E2 supplementation protected both genotypes. Finally,

mice were injected intravenously with [3H]2DG to measure cerebral glucose uptake. AÎ²-Tg OVX mice had significantly lower levels of 2DG uptake compared to their WT counterparts (Two-way ANOVA, $p < 0.01$), while OVX WT mice had similar levels as sham-operated WT, indicating interacting effects of estrogen depletion and amyloid accumulation on CMRglc.

Loss of estrogen production during menopause has been shown to precede cognitive decline in AD, while treatment with HRT may attenuate the onset of these symptoms. Here, we show that loss of estrogen in a model predisposed to amyloidosis may affect cognitive function via glucose metabolism.

Falls and Cognition in Adults Aging with Down Syndrome

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Background: Adults with Down syndrome (DS), who are at risk, will show the neuropathological changes of Alzheimer's Disease (AD) within their 40s, partially due to an overexpression of beta-amyloid, which accumulates in the brain across their lifespan. The risk of developing AD in adults aging with DS increases with age and is marked by increased confusion, short-term memory problems, and/or a loss of the ability to complete daily activities. There is limited evidence on the functional changes that happen to adults aging with DS prior to any marked AD symptoms. Growing evidence suggests that functional mobility abnormalities and an increase in falls may precede cognitive impairment and marked AD symptoms. The relationship between falls and cognition in adults with DS has not been thoroughly investigated; and could potentially serve as a less invasive method of assessment.

Methods: The purpose of this study is to assess patterns of falls and functional mobility impairments that occur within an adult population aging with DS and their relationship with cognition. The participant receives an initial home visit, conducted by the PI, who completes and collects data on functional performance, fall risk(s), and the environment. The participant agrees to document any fall incidents using a calendar journal, and completes the monthly check-in calls and/or emails initiated by the study team.

Results: We conducted a correlation analysis with the preliminary data from this cohort of 17 participants, 9 male and 8 female, with a mean age of 38.5 years (SD=8.81). Increased falls were significantly related ($p < .05$) to impaired gait speed ($r = .615$) and balance ($r = -.739$), while cognition ($r = .513$, $p > .05$) was not associated with falls. Increased fear of falling assessment scores were correlated with impaired gait speed ($r = .687$), lower extremity ($r = -.502$) and mean grip strength ($r = -.492$), and balance ($r = -.596$).

Conclusion: We demonstrated that (1) self reported cognitive impairment and falls

were correlated, and (2) fear of falling scores and decreased functional mobility were similar in aging adult with DS populations to older adult (â‰¥ 65 years of age) populations.

PhD Graduate student and Research assistance

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Changes in health status related to central nervous system, sensory, musculoskeletal, and cognitive functions have been associated with changes in driving. As we began a parent study, "In-Vehicle study" we realized there were other changes in addition to cognitive changes that could affect how older adults drive. No appropriate existing measure was found. This study aims to develop and psychometrically evaluate a new self-report measure of change in the physical health status of older drivers that is sensitive to the effects of change in driving behavior. Both deductive (literature review) and inductive strategies (qualitative interviews) were used to generate measure constructs. Open-ended questions were administered to participants of the parent study. Two reviewers conducted content analysis, quantified the responses, calculated frequencies of answers, and drafted a measure containing 30 items related to health changes and driving responses. The scale's first set of 16 items represents health changes related to the manifestation of symptoms, the status of new and old conditions (sensory, musculoskeletal, and central nervous system), emotional changes, medications, and health-related procedures - the second set of 14 items represents potential effects on driving behavior. The domains and related dimensions will provide valuable information on health changes other than cognition that may interfere with driving. The new Scale is being pilot tested on 10 to 15 participants in the parent study. Psychometric evaluation for the scale's dimensionality, reliability, and validity is planned after the scale has been administered to a larger, diverse sample from the parent study.

Investigating sex differences in the neuroanatomy and behavioral roles of neurons expressing the neuropeptide cholecystokinin (CCK) in the bed nucleus of stria terminalis (BNST)

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The bed nucleus of the stria terminalis (BNST) is a heterogeneous brain region considered part of the "extended amygdala" network that is involved in emotional behaviors and consists of many neuronal subpopulations marked by neuropeptide modulators. One subgroup of BNST neurons is defined by expression of the cholecystokinin neuropeptide (CCK) which is traditionally known for playing a role in appetite regulation. Previous studies revealed that CCK-BNST neurons show rapid physiological activation in response to appetitive odorant stimuli, and that optogenetic activation of CCK-BNST neurons drives approach behavior, indicative of their rewarding properties. Yet, these studies were largely conducted in male mice, and the BNST is known to express steroid hormone receptors that regulate sex differences in neuronal function and behavior. Thus, we set out to characterize sex differences in the structural organization, activation patterns, and behavioral functions of CCK-BNST neurons. Using genetically-encoded reporter labeling, viral anterograde tracing, fiber photometry recordings with a new fluorescent neuropeptide sensor, and chemogenetic neuromodulation, we discovered sex differences in the numbers of posterior CCK-BNST neurons. Preliminary tracing studies also revealed sex differences in the projections of CCK-BNST neurons to the medial amygdala (MeA), a previously understudied pathway that may regulate sex-specific social interactions. We tested a new neuropeptide sensor to detect CCK release (GRAB-CCK 2.3) in the BNST in response to social interaction. Finally, we investigated the role of CCK-BNST neurons in feeding behaviors. Our findings indicate that CCK-BNST neurons are a sexually dimorphic cell type in the extended amygdala network, opening up new avenues for research into how these neurons may play roles in social and reward behaviors.

RNA-seq analysis reveals prenatal alcohol exposure is associated with increases in placental inflammatory cells and gene expression

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Introduction: Fetal alcohol spectrum disorders (FASD) are the most common preventable cause of birth defects and neurodevelopmental disorders worldwide. The placenta is the crucial interface between mother and fetus. Prenatal alcohol exposure has been shown to influence bulk tissue placental gene expression, but few studies have examined this relation at the cellular level.

Methods: We leveraged a placenta single-cell RNA-seq dataset to perform cell-type deconvolution of bulk placental tissue RNA-seq data from 34 heavy drinking pregnant women (Mean = 4.4 drinks/occasions on 2.3 days/week) and 34 abstaining/light-drinking (no binge episodes) controls in our prospective birth cohort in Cape Town, South Africa. We used bivariate analyses and adjusted linear regression models to assess the effects of PAE on the RNA-seq inferred placental cell-type proportions. Finally, we examined differential expression of inflammatory response gene markers, adjusting for cell-type proportions.

Results: Deconvolution analyses showed heterogeneous cell-type composition; stromal, endothelial and cytotrophoblasts were the predominant cell-types. Heavy drinking around the time of conception/first trimester was associated with a higher percent of Hofbauer cells (placental macrophages; ß= 0.51, p=0.035) in linear models adjusted for maternal age, infant sex, and gestational age. 35 inflammatory response genes were differential expressed in alcohol exposed placentas (FDR $p<0.05$), with increased expression seen in all but 4 genes.

Conclusion: Our findings suggest that heavy alcohol exposure during pregnancy may induce placental inflammation, as evidenced by increased proportion of fetal placental macrophages (Hofbauer cells) and expression of inflammation-related genes. Larger studies are needed to further characterize these effects and to assess potential mechanistic roles of placental inflammation in FASD.

Hem1, Autoimmunity, and B Cell Tolerance

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NCKAP1L and its associated protein HEM1 make up a hematopoietic cell specific member of the WAVE regulatory complex (WRC), which is critical for normal F-actin formation and related processes (migration, adhesion, phagocytosis, etc)1. Loss-of-function mutations in NCKAP1L have recently been identified as causing severe primary immunodeficiency (PID) and autoimmunity in children2,3. However, the molecular and cellular mechanisms explaining how loss of HEM1 results in disease are unclear. In this study, we investigate how loss of HEM1 in B cells may contribute to B cell hyperactivation and autoimmunity.

We bred Nckap1L floxed mice to Mb1Cre mice, eliminating Hem1 in all B cells beginning at the pre-B stage of development. Our preliminary results suggest that loss of Hem1 results in B cell hyperactivation. In addition, we identified a new population of B cells that under-express Nur77, an important gene for maintaining B cell tolerance4, following loss of Hem1. These results suggest that inefficient expression of Nur77, combined with B cell hyperactivation, may contribute to a loss of B cell tolerance in Hem1 deficient mice and humans.

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Development of New Oxysterol-Binding Protein (OSBP)-Targeting Antiviral and Anticancer Compounds

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Recently, oxysterol-binding proteins (OSBPs) have been demonstrated to be potentially druggable broad-spectrum antiviral and precision anticancer targets. The OSBP protein is required for the proliferation of a range of pathogenic RNA viral pathogens, including Enteroviruses, coronaviruses, Zika virus, Dengue fever virus, and hepatitis. OSBP has also been shown to regulate mTORC1 activity, which would both limit cancer proliferation and viral protein translation. Additionally, OSBP-related protein 4 (ORP4), which is closely related in its sequence to OSBP, is not required for viral proliferation but is selectively expressed in many human cancers, where ORP4 has been shown to drive cancer cell proliferation. The natural product compound OSW-1, isolated from the plant Ornithogalum saundersiae, is a potent anticancer and antiviral agent through targeting OSBP and ORP4, and therefore a starting point for drug targeting of these proteins. The goal of this research project is to produce OSW-1-related compounds for potential drug development, including selective OSBP-targeting antiviral compounds and ORP4-selective anticancer compounds. Currently there are no reported protein structures of OSBP or ORP4. To develop an OSW-1 SAR, a library of OSW-1-related compounds will be produced for in vitro OSBP and ORP4 binding studies and biological evaluation. In this project, two approaches for producing OSW-1-related compounds for SAR studies will be reported. The first approach is done via isolation of these OSW-1-related compounds from the natural plant source, including a new compound that has not been previously reported. The second approach is to modify the existing OSW-1 structure, including through the synthesis of OSW-1 Fluorescent and PROTAC analogs. Determining the binding affinity to OSBP and ORP4 and the biological activity of these related OSW-1 compounds will provide further understanding of OSW-1 SAR and progressively guide the development of new OSBP- and ORP4-targeting compounds for pre-clinical drug development as novel antiviral and anticancer drugs.

Delta-8-Tetrahydrocannabinol ameliorates colitis through suppression of inflammatory myeloid cells

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Colitis is an inflammatory bowel disease that affects 1.5 million people in the United States. The pathogenesis of this disease involves interactions between microbiota, immune response, as well as external environmental factors, in addition to genetic and epigenetic markers. Past studies from our laboratory have shown that Delta-9-Tetrahydrocannabinol (Δ9 THC), a cannabinoid, can attenuate colitis through its anti-inflammatory properties. However, one of the drawbacks of using Δ9 THC is that it is highly psychoactive. In the current study, we tested the efficacy of Δ8 THC, which is less psychoactive than Δ9 THC to determine its efficacy to attenuate the pathogenesis of colitis. Experiments were conducted by administering mice 30 mg/kg of Δ8 THC i.p. every other day for 8 days while being exposed to a 3% DSS solution orally, and mice were euthanized on the 9th day. The data on body weight and colon lengths demonstrated significant mitigation of colitis symptoms as determined by reversal of weight loss and colon shortening in the THC-treated colitis mice when compared to the colitis+vehicle group. In colonoscopies, there was a significant sloughing of cells and overall signs of inflammation in colitis group which also was found to be decreased following Δ8 THC treatment. Histopathological studies also showed that the overall structure of the colonic tissue was disrupted by colitis, and Δ8 THC treatment mitigated these changes. Our studies showed that macrophages recruited to the colon from the bone marrow have a significant role in inflammation during colitis. Interestingly, Δ8 THC treatment caused a significant decrease in the number of CD45+ CD11b+ CCR2+ cells blood monocyte-derived macrophages in the colon. There was also a significant drop in granulocytes (CD45+ CD11b+ Ly6G+) following Δ8 THC treatment when compared to colitis group. Neutrophils were found to be increased in the colitis model and showed a significant decrease when exposed to Δ8 THC. Due to the significant myeloid cell changes found in comparison to lymphoid, further studies are underway to examine myeloid cells. miRNA sequencing analysis found several dysregulated miRNAs that may be responsible for inflammatory response in colitis and how Δ8 THC mitigates colitis. Notably miR-30b, a marker found to be associated with IL-6 and TNFα as well as miR-6238 associated with β-defensin 113 were found to be downregulated in DSS

group, but significantly upregulated in the DSS + Δ8 THC treated mice. Together, the current study demonstrates that Δ8 THC can attenuate colitis by suppressing presence of inflammatory myeloid cells by regulation of miR expression. (This work was supported in part by NIH grants R01AI160896, R01ES030144, P01AT003961, P20GM103641, R01AI123947, R01AI160896 to MN and PN as well as by R0160986 Supplement to JG-S).

Interactions between mycobacterial cell wall regulators may control peptidoglycan metabolism

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The mycobacterial lipid II flippase, MurJ, has uncharacterized regulatory domains that interact with two peptidoglycan regulators, FhaA and CwlM. The physiological functions, interaction sites, and phospho-dependence of these interactions are unknown. We are using in vitro biochemical assays to characterize these protein interactions. We are also using AlphaFold-Multimer to predict the interaction sites and then testing those predictions biochemically. Lastly, we use genetics in Mycobacterium smegmatis to elucidate the physiological functions of these interactions. This work will allow us to understand how lipid II flipping by MurJ is regulated, and how the regulation of peptidoglycan synthesis controls the cell cycle control and helps the cell adapt to environmental stimuli.

Uncovering the Mechanisms of Transcriptional Regulation of ECA Biosynthesis in E. coli K-12

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Nearly 3 million antibiotic resistant infections occur per year in the United States. This problem is especially acute in gram-negative bacteria, where the outer membrane (OM) which surrounds the aqueous periplasm and cytoplasmic membrane acts as a permeability barrier capable of excluding many antibiotics. The OM of Enterbacteriales contains a highly conserved invariant carbohydrate-derived moiety known as enterobacterial common antigen (ECA), which plays an important role in controlling this membrane permeability barrier. ECA has three forms: a surface-bound linear polysaccharide linked to phosphoglyceride (ECAPG), a surface-bound linear polysaccharide linked to LPS (ECALPS), and a cyclic form (ECACYC) found in the periplasm. All three forms of ECA are made from repeating units of three amino sugars: 4-acetamido-4,6-dideoxy-D-galactose (Fuc4Nac), N-acetyl-D- mannosaminuronic acid (ManNAcA), and N-acetyl-D-glucosamine (GlcNAc). Although ECA was first discovered in the 1960s, it's function and regulation remain largely mysterious. However, it is known that many of the genes necessary to make these ECA units, flip them across the inner membrane, and control their polymerization are found in an operon (wec); however, it is not currently known how these genes are transcriptionally regulated. This project aims to identify the specific genes involved in transcriptional regulation of ECA biosynthesis and how different environmental conditions effect ECA promoter activity. In order to identify potential transcriptional regulators, we conducted a promoter analysis which produced a list of potential ECA regulators. We built strains with single gene deletions of each of these potential regulators and transformed them with a plasmid containing the luciferase genes and the promoter region for the wec operon to assay ECA promoter activity in these mutants. We have identified genes that have an effect on ECA promoter activity, some positive and some negative, and these genes will be further investigated to characterize their function in ECA biogenesis regulation. Additionally, the effect of varying environmental conditions on ECA promoter activity was examined and pH was found to have an interesting effect on expression of the wec operon, suggesting

ECA may have a role in acid resistance. Overall, this work seeks to identify genes responsible for transcriptional regulation of ECA in order to determine the conditions in which ECA's activity on the permeability barrier is important and to ultimately advance the initiative to identify new potential targets for antibacterial therapeutics.

Learning from Nature: Pyrococcus furiosus Thioredoxin (PfTrx) Scaffolds Displaying Extracellular Loops of the Treponema pallidum FadL Outer Membrane Proteins in the Search for a Syphilis Vaccine

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Background: The re-emergence of syphilis in the new millennium highlights the need for vaccine development to combat Treponema pallidum (Tp) infection. Ex vivo studies utilizing immune rabbit serum (IRS) and peritoneal macrophages strongly support that an effective vaccine must be antibody (Ab) based, as evidenced by the opsonophagocytosis assay, considered a reliable surrogate of protection. Structural models of Tp outer membrane proteins (OMPs, the Tp OMPeome) provide a 'roadmap' to identify potential vaccine candidates whose prime targets are extracellular loops (ECLs). Tp FadL proteins, orthologs of the E. coli FadL fatty acid transporter, are likely crucial for the survival of Tp due to its fatty acid auxotrophy, representing a potential Achilles heel of the syphilis spirochete. Elucidating ECL-directed Ab responses in the animal models will inform human vaccine development.

Methods: Pyrococcus furiosus thioredoxin (PfTrx) was used as a scaffold to display individual ECLs from all five FadL OMP paralogs in the Tp Nichols strain (TP0548, TP0856, TP0858, TP0859, and TP0865). Sera from five immune rabbits (IRS) were tested for reactivity against 30 PfTrx-scaffolded ECLs and five hatch (plug) domains by immunoblotting and ELISA. Four cohorts of mice and rabbits were immunized with the immunodominant PfTrxTP0856/ECL2&ECL4 and PfTrxTP0858/ECL2&ECL4 antigens. ECL-specific reactivities of the antisera were assessed using a second scaffold, a modified form of transferrin binding protein B (TbpB), displaying the corresponding ECL. The mouse and rabbit opsonophagocytosis assays were performed using rabbit and mouse antisera to scaffolded ECLs using rabbit peritoneal and mouse bone marrow-derived macrophages, respectively. Spirochete internalization was confirmed via confocal microscopy. Lastly, we used a newly developed assay employing in vitro-cultivated Tp to determine the neutralizing activity of TP0856 and TP0858 ECLs 2 and 4

specific antisera.
Results: ECL2 and ECL4 of TP0856 and TP0858, ECL3 of TP0865, and the hatch domain of TP0865 were identified as the antigenic standouts. Mouse and rabbit antisera generated using PfTrx-scaffolded ECLs 2 and 4 of TP0856 and TP0858 detected nanogram amounts of the corresponding ECLs displayed by TbpB. These ECL-specific antisera exhibited robust ‘functional activity’ in opsonophagocytosis assays using rabbit peritoneal and mouse bone marrow-derived macrophages. Moreover, TP0858 ECL2 and ECL4 demonstrated strong in vitro neutralizing activity.
Conclusions: Our findings demonstrated that antigenic analysis of Tp OMP ECLs can identify promising vaccine targets. In this study, ECLs 2 and 4 of TP0856 and TP0858 emerged as top-tier candidates. Furthermore, our results suggest that the murine opsonophagocytosis assay could be a viable alternative to the rabbit assay. The neutralization data also indicates that ECL-specific Abs may protect against Tp via mechanisms other than macrophage-mediated opsonophagocytosis. Finally, this ECL-based strategy could be extended to identify OMP vaccinogens in non-spirochetal diderm bacterial pathogens.

ZnuABC is not essential for Klebsiella pneumoniae virulence

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Nutritional immunity is the process used by hosts to sequester transition metals from invading pathogens. To counteract nutritional immunity, bacteria have developed mechanisms to obtain transition metals, which include ABC transporters and siderophores. Klebsiella pneumoniae, a major cause of hospital-acquired pneumonia and sepsis, encodes the siderophore, yersiniabactin (Ybt) within its genome. Ybt is an important virulence factor used by pathogens such as Yersinia pestis to overcome not only iron (Fe) limitation but zinc (Zn) limitation during infection. Ybt's role in Fe acquisition during K. pneumoniae infection has been well characterized. However, the role of Ybt in K. pneumoniae zinc acquisition during infection has not yet been defined. We sought to define the contribution of Ybt and ZnuABC to K. pneumoniae virulence during pneumonia. Mutants lacking YbtX and the high affinity Zn transporter, ZnuBC, were generated. We infected C57BL/6 mice with wildtype, znuBC and znuBC ybtX mutants and monitored survival and determined bacterial burden in the lungs. Mice infected with the znuBC ybtX mutant had similar survival rates as mice infected with a znuBC mutant and wildtype K. pneumoniae. Furthermore, there was no significant difference in bacterial burden in the lungs of mice infected with znuBC ybtX compared to mice infected with wildtype or znuBC mutant. These data indicate K. pneumoniae encodes an additional zinc acquisition system, and the bacterium uses multiple mechanisms to acquire Zn during infection.

Temperate phage encode regulators of the bacterial SOS response to control phage induction

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Bacteriophages (phages) have a tremendous capacity for shaping the ecology and evolution of bacterial communities. Temperate phages, in particular, have the potential to rewire the biology of their bacterial hosts during lysogenic replication when they take the form of an integrated or episomal prophage. In contrast, temperate phages can act as time-bombs upon induction of lytic replication, leading to the depletion of certain bacterial lineages. There are many unanswered questions about the factors that regulate temperate phage life cycles and as a result, how they balance their beneficial and antagonistic relationships with their hosts. In part, this is because many phage genomes and the functions they encode remain uncharacterized (creating so-called “viral dark matter”). To bridge this knowledge gap, I am probing for molecular modulators of phage life cycles using the P2 temperate phage as a model system. P2 is a DNA-damage inducible phage that encodes a small peptide (which we have named ‘PhiR’) that has homology to a bacterial regulator of RecA and the DNA damage SOS response. In this study, I assess how PhiR may function to buffer the bacterial SOS response through RecA interactions that ultimately time prophage induction. I have also found evidence that PhiR may be involved in regulating RecA-dependent adaptive mutagenesis. Lastly, I uncover how widely distributed PhiR-like homologs are across different bacterial lineages and their potential to enhance bacterial fitness. Ultimately, this work highlights how temperate phage tune into the bacterial SOS response to regulate their life cycles and influence bacterial ecology and evolution.

Dissecting early molecular interactions between typhoidal and non-typhoidal Salmonella with human intestinal cells

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Typhoidal and non-typhoidal Salmonella enterica serovars have distinct disease manifestations. These differences are likely driven, in part, by early serovar-specific interactions with human intestinal epithelial cells. However, it has been difficult to study the pathogenesis of typhoidal serovars since they are human-restricted and lack a robust animal model. Thus, to investigate early interactions required for both typhoidal and non-typhoidal Salmonella to enter and replicate in intestinal epithelial cells, I utilize our 3D human-derived intestinal epithelial organoids as a model system. Our 3D organoids are a robust model since these cells are derived from healthy human tissue, recapitulate the multicellular epithelial population of the intestine, and have controllable polarity (i.e., apical vs. basal surface). During the early stages of infection, Salmonella must migrate and adhere to epithelial cell surfaces. Our data indicate that non-typhoidal serovars actively swim to the apical surface of the intestinal epithelium in a chemotaxis-dependent manner. In contrast, typhoidal serovars migrate to the basal surface. To identify the required genes, I generated clean deletions in all known chemoreceptor genes in Salmonella. My results have identified chemoreceptor genes required for epithelial sensing and attachment in both typhoidal and non-typhoidal serovars. It is anticipated that our research will advance our understanding of how human-restricted pathogens like typhoidal Salmonella infect the human intestinal epithelia and identify serovar-specific signals that trigger their distinct disease manifestations.

Use of Peroxisomal Targeting Sequences in Drug Delivery

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In the parasite Trypanosoma brucei, several essential metabolic pathways, including glycolysis, localize to specialized peroxisomes called glycosomes. Using in vitro assays, we identified small molecule inhibitors of enzymes within these pathways. However, the in vivo activity of some of these inhibitors is lower than in vitro studies predict. One possibility is that these inhibitors are not effectively targeted to the glycosome. Exogenous cargo, including small molecule inhibitors of enzymes, can been targeted to glycosomes by appendage of a type 1 peroxisomal targeting sequence (PTS1), a C-terminal tripeptide motif that targets proteins to these organelles. Building on this concept, we appended a PTS1 sequence to a TbHK1 inhibitor. This modified inhibitor exhibited increased trypanocidal activity. With the goal being optimized delivery of drugs by modification with PTS1 tripeptides, we are quantitating how different PTS1 sequences influence glycosomal targeting. Using fluorescein isothiocyanate (FITC) coupled to different PTS1 sequences, we monitored uptake into trypanosome cells via flow cytometry. We have identified a variety of factors that can impact assay reproducibility including incubation time, presence of FBS in the culture media used during incubations, and compound concentration. Optimization of cytometry protocols, along with microscopy to compare distribution of the label, is ongoing to strengthen our understanding of exogenous cargo uptake in the African trypanosome. We will discuss the assay conditions and specific amino acid sequences that influence PTS-FITC uptake.

Skin dendritic cell positioning and trafficking regulated by Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2)

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Dendritic cells (DCs) are crucial sentinels and an/gen presenting cells of the immune system. Their role is to transmit information about their environment to adaptive immune cells to initiate specific immune responses. DCs in mice can be classified into two types of conventional DCs (cDCs), cDC1 and cDC2. Our lab has previously shown that cDC2 positioning and function in the spleen is dependent on the chemoaPractant receptor, Epstein-Barr Virus induced gene 2 (EBI2) also known as GPR183. EBI2 is a G protein-coupled receptor activated by the ligands, 7α,25-hydroxycholesterol (7α,25-HC) and 7α,27-HC. DCs in the skin are important in initiating responses against pathogens that cross the epidermal barrier. Their function depends on correct positioning in the skin and migration to draining lymph nodes following pathogen encounter.
The factors controlling skin cDC positioning and migration are not fully understood. Here we examine the role of EBI2 in skin cDC positioning and function. Our analysis revealed that EBI2 is highly expressed in cDC2 within the skin. Dele/ng EBI2 specifically in cDCs we can demonstrate that the receptor contributes to cDC2 positioning within the skin and trafficking to the draining lymph nodes. The findings reveal a mechanism for how skin cDC2 migration is controlled and have implications for approaches to therapeutically regulate cDC2 function during skin immune
responses.

Trichomonas vaginalis Extracellular Vesicles Suppress Host Cell IFNε -Mediated Protection

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Trichomonas vaginalis, a common sexually transmitted parasite that colonizes the human urogenital tract, secretes extracellular vesicles (TvEVs) that are internalized by host cells. Host cell uptake of TvEVs has been shown to mediate host cell immune responses and to increase parasite killing of host cells. Our understanding of the underlying mechanisms by which TvEVs interact with and alter host cell signaling to facilitate these effects remains limited. To address this issue, we analyzed the effect of TvEVs on host cell gene expression by treating cells with TvEVs and performing RNA sequencing (RNA-seq). RNA-seq revealed that TvEVs largely down-regulate host cell gene expression and many of the genes that are being suppressed are known to be involved in the host cell immune response. Pathway analysis also revealed that the type I IFN pathway. Interestingly, while most type I interferons were not present in the data set, Interferon epsilon, was strongly downregulated and has been previously shown to be protective against bacterial and viral sexually transmitted infections (STIs). Additionally, we found that TvEVs can strongly block IFNε-mediated activation of STAT1/2, important downstream mediators of the type I IFN response pathway. Finally, we showed that pretreatment of host cells with IFNε is protective against T. vaginalis killing. Together these results show that TvEVs suppress host cell immune responses to increase host cell killing by the parasite and that IFNε is protective against T. vaginalis.

Estimation of radiographic joint space of the trapeziometacarpal joint with computed tomographic validation

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The trapeziometacarpal (TMC) joint exists at the base of the thumb and is defined by the articulation between the first metacarpal and trapezium. X-ray radiography is commonly used to evaluate the joint with respect to the presence of musculoskeletal disorders, such as osteoarthritis. Quantitative determination of radiographic features, such as TMC joint space, can be challenging. The purpose of this study was to formulate a methodology for measuring the radiographic joint space of the TMC joint using computed tomographic (CT) measurement for validation. CT scans were taken of 15 cadaveric arm specimens. Simulated radiographic images were generated from the CT data. TMC joint space was calculated using the 3D models generated from the CT data and using the simulated 2D radiographic images. A correction factor was established by comparing the radiograph-based and CT-based measurements. Leave-one-out validation was used to correct the radiograph-based measurements. The radiograph-based measurements underestimated the joint space by 1.4 mm [95% CI: 0.987-1.858 mm; $p < 0.001$] in comparison to measurements based on CT. The difference between the CT-based joint space and the corrected radiograph-based joint space for all specimens was -0.1 mm [95% CI: -0.635-0.414 mm; $p = 0.669$]. This study provides a novel methodology for estimating the radiographic joint space of the healthy TMC joint and, with continued development, has potential for application in the advancement of quantitative radiographic study of the TMC joint.

Quantification of mitochondrial transfer from mesenchymal stem cells to annulus fibrosus cells and the development of an intervertebral disc degeneration model

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Introduction: Common treatments for early-stage intervertebral disc (IVD) degeneration focus on reducing symptomatic pain, but do not address the underlying pathology.^1 Thus many patients require continued treatment and surgical interventions at later stages of degeneration.^2 In the last decade, stem cell-based therapies have become a promising treatment for IVD degeneration due to their potential to differentiate into IVD-like cells and secrete immunomodulatory factors.^3 We have previously demonstrated that including mesenchymal stem cells (MSCs) in tissue engineering approaches for annulus fibrosus (AF) repair enhances glycosaminoglycan (GAG) retention and tissue hydration in vivo.^4 However, the exact mechanism employed by MSCs to stimulate IVD regeneration remains unclear. Multiple research groups have found that MSCs can donate their mitochondria (MT) to diseased cells experiencing MT dysfunction, salvaging the bioenergetics of recipient cells.^3 Studies show that MT activity, including calcium production and quality control such as mitophagy, is impaired in diseased cells, highlighting MT transfer as a possible rescuing mechanism for IVD cells.^5 The first objective of this study was to establish a protocol to validate and quantify transfer events between AF cells and MSCs from confocal images. Due to the limited availability of degenerative human AF tissue, the second objective of this study was to develop a model of IVD degeneration using the inflammatory cytokine, IL-1Î², which has been shown to be upregulated in degenerative IVDs. Our end goal is to use the tools developed herein to study MSCs and determine a method to optimize MSCs for annular repair strategies by increasing their rate of mitochondrial transfer.

Methods: To quantify MT transfer from MSCs to AF cells, a coculture model was used. Briefly, IVDs and MSCs were harvested from neonatal bovine tails and femoral trabecular bone, respectively. Bovine MSCs were lentivirally transduced with mCherry to allow for MT visualization. 6mm AF explants were taken from the IVDs and stained with Vybrantâ„¢ CFDA SE. Transduced MSCs were then

seeded onto AF explants and cultured for 3 days. Confocal microscopy and Image J were used to quantify transfer events. To establish a model of IVD degeneration, explant culture media was supplemented with IL-1Î² at 0, 0.1, 10, 100, and 300 ng/mL. Media was harvested and exchanged every 3 days for a total of 6 days. A DMMB and Hydroxyproline assay were performed to determine the amount of GAG and collagen content released into the media and within the AF explants.

Results: Confocal imaging displayed mCherry signal within CFDA stained cells, indicating transfer. ImageJ processing highlighted red and green signal colocalization of structures between 0.5 and 1 Î¼m in length, consistent with the size of individual MT. The hydroxyproline assay of AF media exhibited very slight changes in collagen release between the different IL-1Î² concentrations, while the DMMB assay exhibited a possible upwards trend in GAG release as IL-1Î² concentration increased. DMMB and hydroxyproline assays of AF tissue show a decrease in GAG/hydroxyproline ratio as IL-B concentration increases.

Discussion: The objectives of this study were to develop a protocol to validate and quantify MT transfer events and to establish an IVD degradation model that could be used to study MT transfer. From the coculture studies, we confirmed that MT transfer occurs between AF cells and MSCs, as seen from the presence of white colocalized signal in our processed images. Additionally, we established a system for MT transfer quantification using ImageJ. The IL-1Î² experiments indicate that IL-1Î² has a stronger effect on GAG rather than collagen breakdown in AF tissue. Furthermore, GAG release seems to be linearly correlated to IL-1Î² concentration up to 100ng/mL. Cadaveric AF tissue studies have shown around a 25% decrease in GAG/hydroxyproline ratio between Pffirman grades 2 and 3, which may be possible to replicate with IL-1Î² in bovine AF tissue given the results from this preliminary study.^6 A limitation of this study is the low number of experimental replicates (n=2). To confirm the relationship of IL-1Î² to GAG and collagen breakdown more experimental repeats will be performed. However, IL-1Î² has been previously shown to negatively affect GAG content in AF tissue.^7

Significance: MSCs have been shown to have a regenerative effect on a variety of diseased tissues including the IVD. Although MSCs can function in numerous ways, MT transfer has gained attention in recent years due to the important role of MT in cell fate. Severe MT dysfunction is observed in degenerative IVDs. Thus, MT transfer may serve as a strategy to salvage IVD cells experiencing impaired

MT function. This work will help develop a method to study MT transfer between AF cells and MSCs that can later be used to elucidate how MSCs may be engineered to increase their MT donating capacity. This would enable the development of better bioactive repair strategies that are capable of restoring native IVD composition.

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A Mesoscale Model Of Skin To Investigate The Role Of The Dermis-Epidermis Interface On The Tissue Biomechanics

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Understanding and correctly simulating the mechanics of skin is crucial for many medical applications, e.g. planning reconstructive surgery or monitoring wound healing. The field of skin biomechanics has often modeled this tissue as a homogeneous material because its response at finite deformations is governed by its collagen content. However, skin consists of two main layers with different constituents and microstructure: epidermis and dermis. Additionally, there are skin appendages such as hair follicles. Recently, multi-layer models of skin have been developed to study wrinkling and transport. 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 mesoscale.

Synthetic Hydrogels for Muscle Satellite Cell Transplantation in Dystrophic Diaphragms

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Duchenne muscular dystrophy (DMD) is a genetic disorder that causes a lack of functional dystrophin protein in skeletal muscle. As a result, patients suffer from ambulatory disability and cardiorespiratory failure, the latter of which leads to premature death. Currently, there is no cure. Although glucocorticoid steroid treatment has lengthened patients' life span, this form of daily treatment has many negative side effects with short- and long-term use. An alternative strategy is to transplant muscle stem cells, known as satellite cells (MuSCs), to a target muscle to repopulate the stem cell niche and restore dystrophin to the muscle fibers. There are limitations associated with cell delivery, including poor retention at the delivery site and low cell survival upon transplantation. Due to its role in respiration, the diaphragm muscle is an important target for delivery; however, it poses additional challenges due to its thin dimensions and deep-seated location. We seek to leverage hydrogels as a vehicle for localized MuSC delivery to address challenges of low cell viability, retention, and functionality at the diaphragm delivery site. In this work, poly(ethylene glycol) hydrogels were functionalized with the cell adhesive peptide RGD or the inactive peptide (i.e., RDG) to evaluate the effect of peptide specificity on MuSCs in vitro. MuSCs were isolated from 1 – 2-month-old-C57BL/6 mice via FACS then encapsulated in hydrogels. MuSCs cultured in RGD-functionalized hydrogels resulted in larger myogenic colonies as compared to hydrogels with scrambled peptide. These data agree with previously published work demonstrating how specific adhesive molecules in the synthetic matrix are necessary for sustaining and directing MuSCs processes that are important for cell delivery. Additionally, in collaboration with Dr. Stanislav Emelianov's lab, we have demonstrated the advantage of ultrasound imaging shear wave elasticity imaging (US-SWEI) for longitudinal tracking of diaphragms in naïve, dystrophic mice. US-SWEI estimates the speed of shear waves propagating through tissue and provides a map of tissue stiffness. In this work, US-SWEI was used to estimate the shear wave speed (SWS) in diaphragms of naïve healthy (WT) mice, mdx mice (mdx), and mdx mice haploinsufficient for utrophin (mdx-utr) at 6 and 12 months of age.

Diaphragms were then extracted for ex vivo force testing and histological analysis at 12 months of age. Shear wave speed increased (i.e. tissue stiffness increased) with increasing disease severity and with time. A strong correlation was observed between SWS and collagen deposition and between SWS and muscle fiber size. Together, these data demonstrate the ability of US-SWEI to track and predict diaphragm morphology over time. Furthermore, our results highlight an advantage of US-SWEI over ex vivo testing by obtaining multiple measurements in the same subject. Future work involves delivering hydrogel-encapsulated MuSCs to dystrophic mice and using US-SWEI and ex vivo force testing to track diaphragm properties and function, respectively, after treatment. We expect to see increased cell engraftment and re-introduction of dystrophin as well as improved diaphragm function and morphology. This work can provide support for hydrogel-encapsulated MuSC delivery as a strategy for treating dystrophic diaphragm muscle.

Osteoclast-specific Deletion of Î²2-Adrenergic Receptor Limits Trabecular Bone Acquisition in Male, but not Female Mice

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The sympathetic nervous system (SNS) is important for maintenance of bone homeostasis through Î²-adrenergic signaling. Our lab and others found that osteoclasts are direct targets of the SNS, and that Î²-blockers limit osteoclast differentiation. Osteoclasts express both Î²1- and Î²2-adrenergic receptor (Î²2AR), but Î²2AR is more highly expressed. To directly examine the effects of Î²2AR on osteoclasts, we developed an osteoclast-specific Î²2AR knockout mouse model. Adrb2fl/fl mice were crossed with the myeloid lineage specific Lyz2Cre/Cre (a.k.a. LysM-Cre) mice. We tested the efficiency of deletion with Adrb2fl/flLyz2Cre/+ (Cre/+) and Adrb2fl/flLyz2Cre/Cre (Cre/Cre) mice and found deletion of Adrb2 was comparable in both using gene expression and in situ hybridization in the distal femur. Using ÂµCT, we measured the bone microarchitecture of wildtype (+/+) and Cre/+ male and female mice at 8 and 26 weeks of age (N=7-10). In male mice with the deletion, L5 vertebrae trabecular bone parameters (BV/TV, BMD, Tb.Th) were significantly lower. Distal femur trabecular BMD was reduced by 10% and SMI was elevated by 35% in Cre/+ compared to +/+ ($p<0.05$). Cortical bone (Ct.Ar/Tt.Ar, Ct.TMD) tended to be lower in Cre/+ mice ($p<0.09$). Both osteoclast and osteoblast marker genes were reduced in tibia, including Acp5, Alpl, Col1a1 and Tnfsf11 (Rankl). The low bone mass phenotype subsided by 26 weeks, suggesting age-related bone loss is unaffected by Adrb2 deletion in osteoclasts in male mice. Female mice had no significant changes in bone microarchitecture or serum bone remodeling markers at 8 or 26 weeks of age. Although global deletion of Adrb2 has been reported to cause a high bone mass phenotype, our work indicates that Î²2AR function in bone is sex, age, cell-type, and stress dependent. We have also found that Î²2AR deletion in osteoclasts may have indirect effects on osteoblast function, indicated by lower osteoblast marker gene expression. Future studies will examine this using osteoblast culture and co-culture studies with osteoclasts. These studies will aid in our understanding of mechanisms through which stress and Î²-blockers influence bone density, which may inform osteoporosis prevention and treatment strategies.

Aggrecan siRNA Treatment Enhances Collagen Fiber Formation in Tissue Engineered Meniscus

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INTRODUCTION: In the native meniscus, during the development of collagen fibers proteoglycan content is low, and as the meniscus develops, proteoglycans emerge.1 In vitro, fibrochondrocytes (FCCs) produce GAGs early in culture, which can have inhibitory effects on collagen fiber formation during the maturation of tissue engineered (TE) meniscus.1,2 This early over-production of GAGs, specifically those found in large aggregating proteoglycans (~100 GAG chains) such as aggrecan, inhibits the formation of aligned fibers and fiber bundles in TE menisci. Degradation of GAGs in early stages of development improves fiber formation; however, this non-specific degradation of all chondroitin sulfate GAGs from proteoglycans may be limiting the potential for further extracellular matrix (ECM) development by proteoglycans associated with collagen fibrillogenesis, such as small leucine rich proteoglycans (SLRPs).3 Additionally, reincorporation of these GAGs after sufficient fiber development has been achieved would be necessary to ensure a robust TE construct. Small interfering RNA (siRNA) therapies specifically and temporarily inhibit the production of aggrecan without affecting other critical ECM components and without long-term inhibition of beneficial GAG deposition. The use of siRNA therapies to target and orchestrate matrix production for TE applications has not been widely studied. Therefore, the objectives of this study were to: 1) develop methods for specifically inhibiting aggrecan production using siRNA in fibrochondrocytes; 2) demonstrate inhibition of GAG accumulation in 3D culture after aggrecan siRNA treatment; and 3) assess the effect of siRNA-induced inhibition of GAG production on the development of collagen fibers in tissue engineered meniscus constructs.

METHODS: Fibrochondrocytes (FCCs) were harvested from juvenile bovine menisci, as described previously.4.5 siRNA/Lipofectamine complex was prepared by diluting siRNA and Lipofectamine RNAiMax into 4500 mg/L glucose DMEM. Components were combined and incubated with FCCs, which were centrifuged to form a pellet. ACAN siRNA was used as the aggrecan target siRNA, siGLO Red

was used as a siRNA control, and untransfected FCCs were used as a control. 2D Culture: Cells were placed into 24-well plates at 0.1x106 cells/well and cultured in complete DMEM media with 4500 mg/L glucose at 37Â°C and 5% CO2. Monolayer cells were cultured for 6 days and media was changed and collected every 3 days for biochemical analysis. 3D Culture: Disc (8 mm in diameter and 2 mm thick) and linear meniscal constructs were made with transfected FCCs at a final concentration of 25x106 cells/ml and 20 mg/ml collagen. Constructs were cultured in complete DMEM media with 4500 mg/L glucose at 37Â°C and 5% CO2. Constructs were cultured for 12 or 30 days and media was changed and collected every 3 days for analysis of GAG and hydroxyproline content. Constructs were either taken for biochemical assessment, used for imaging, or mechanically tested. Second harmonic generation (SHG) imaging and scanning electron microscopy (SEM) were performed to assess collagen organization. Tensile properties were determined using an ElectroForce 5500 System and pulled to failure at a rate of 1.5 mm/s for quasi-static loading.

RESULTS: ACAN siRNA treatment inhibited aggrecan production in both 2D and 3D culture, demonstrating the feasibility of this tool for regulation of ECM production in TE meniscus constructs. The specific inhibition of aggrecan production using siRNA would keep SLRPs intact while removing the primary source of GAGs in the tissue, allowing for SLRPs to further facilitate matrix organization. Additionally, the transfection of siRNA into these FCCs does not affect the production of collagen. Therefore, we used this method to specifically inhibit aggrecan production without affecting other biosynthetic activity. siACAN transfected constructs improved alignment index and displayed a 2 fold increase in fiber diameter, in addition to improved fiber bundling. siACAN transfected constructs showed a 117% and 20% increase in UTS compared to control and Lipofectamine, respectively. Resilience showed a 90%, 16%, and 17% increase of siACAN transfected constructs compared to control, Lipofectamine, and siGLO.

DISCUSSION: This study uses ACAN siRNA to specifically control aggrecan production and manipulate fiber structure in TE constructs. The specific inhibition of aggrecan production using siRNA would keep SLRPs intact while removing the primary source of GAGs in the tissue, allowing for SLRPs to further facilitate matrix organization. Additionally, the transfection of siRNA into these FCCs does not affect the production of collagen. Therefore, we used this method to specifically inhibit aggrecan production without affecting other biosynthetic

activity. Distinct architectural and functional changes are occurring in constructs with suppressed aggrecan production and these data support that a transient decrease in aggrecan promotes the formation of a more robust fiber network and improved mechanical properties.

SIGNIFICANCE: These results show that siRNA therapies have potential to be used to specifically and temporarily inhibit production of proteoglycans by FCCs. Specific inhibition of aggrecan production improved collagen fiber diameter, producing fibers close to native fibers. Further exploration of proteoglycan content in developing TE meniscus constructs is needed to optimize collagen fiber formation.

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Advancing Endometriosis Management and Biodegradable Polymer Innovations Through Entrepreneurial and Scientific Training

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Endometriosis is a chronic, incapacitating condition characterized by the abnormal growth of uterine-like tissue outside the uterus, leading to chronic pain and infertility in affected individuals. Current therapeutic approaches mainly involve anti-inflammatory and hormonal treatments, often failing to provide complete and lasting relief from pain. Sur180 Therapeutics, a women-owned company, specializes in testing therapeutic drug antagonists to address the management of endometriosis-associated pain. The primary objective of this study is to assess the efficacy of different treatments by quantifying and qualifying endometriotic vesicle lesions, histological markers, and inflammatory cytokines using Real-Time PCR and ELISA assays in a rat endometriosis model. Additionally, we seek entrepreneurial mentoring to leverage our expertise in establishing a business-to-business startup company focused on raw polymer development in Puerto Rico. The mentoring process will encompass tasks such as company registration, research group management, business plan formulation, administrative expertise acquisition, business pitch preparation, and securing external funding for the company's various stages. The long-term goal is to expand the development, optimization, and commercialization of biodegradable polymer blends derived from bacteria, specifically polyhydroxyalkanoate (PHA), along with fermentation methyl levulinate derivatives of considerable commercial interest. These PHA blends possess unique anti-inflammatory and antibacterial properties, making them suitable for applications as internal tissue-drug scaffolds for endometriosis, external wound dressing coatings, and other biomedical uses. By capitalizing on Sur180 Therapeutics' expertise, we anticipate the potential for numerous opportunities, including pursuing future STTR/SBIR grants for further advancement and refinement of our technology across various applications.

Barriers to Pediatric Clinical Trial Recruitment and Retention among Black/African American Children and Families

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Introduction: More than half of drugs used to treat children do not have safety and efficacy data to inform standard dosing and overall use. Clinical trials among diverse populations of children are critical to inform safe and effective treatments in the broad pediatric population. However, over 20% of pediatric clinical trials are discontinued or fail due to not meeting participant enrollment and retention goals and most trials do not include representative samples of Black/African American children. We aim to identify barriers to clinical trial enrollment and retention among Black/African American children.

Methods: We are conducting a cross-sectional study utilizing questionnaires to survey parents of children concurrently enrolled in a double blinded, placebo controlled clinical trial. The parents are asked to complete a survey at study enrollment, midpoint of the study, and at study completion to identify described barriers to study enrollment and retention. We describe some of the early themes identified among families/children and describe potential differences in barriers between children who identify as Black/African American vs. White/Caucasian.

Results: Parents or guardians of children, aged 6-17 years of age, enrolled in a clinical trial completed the surveys. Black/African American participants reported lack of transportation and interference with child's school as barriers to enrollment in a clinical trial. White/Caucasian participants described barriers of long study visit duration, negative feelings towards research and fear of research. Both Black/African American and White/Caucasian participants cited childcare needs of other children as a barrier for retention.

Conclusion: Initial data from this ongoing study reveals that unique barriers to pediatric clinical trial enrollment and retention may exist for Black/African American families. However, common barriers related to childcare of other non-participant children may be more universal. Additional data may lead to findings

that help investigators to implement strategies and supports to meet the unique needs of all families/children participating in pediatric clinical trials for inclusive trials.

Functional Neural Connectivity Associates with Specific Aspects of Sensorimotor Control in Stroke

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Introduction: Approximately 800,000 Americans experience a stroke per year. Moreover, it is common for stroke to induce sensorimotor impairments on one side of the body, referred to as hemiparesis. Hemiparesis frequently presents one of the upper extremities, restricting the completion of activities of daily living. Thus, there is a growing public health need to improve upper extremity motor function in an increasing number of stroke survivors. To understand how upper extremity impairments manifest in stroke survivors, previous studies suggest that altered functional neural connectivity, observed via electroencephalography (EEG), is a potential contributor to these deficits. However, most of these studies have associated functional neural connectivity with standardized motor function tests, such as the Wolf Motor Function Test (WMFT) or the Box and Block Test (BBT), a limitation because though valid and reliable, these tests do not account for the heterogeneity of deficits that may be present after stroke. Understanding the relationships between functional neural connectivity specific aspects of sensorimotor control will likely contribute evidence to support precision stroke rehabilitation and assist in developing personalized rehabilitation strategies for stroke recovery. The current study hypothesized that specific aspects of sensorimotor control would be associated with different measures of functional neural connectivity.

Objective: To investigate the relationship between functional neural connectivity and specific aspects of sensorimotor control in chronic stroke survivors.

Methods: Twelve chronic (greater than 6 months post-stroke) stroke survivors with mild-moderate upper extremity motor impairment participated. Exclusion criteria comprised of: (1) inability to follow instructions from study team and (2) botulinum toxin injection within 3 months prior to study enrollment. Each participant completed a paretic hand grip-and-relax task using 6-axis load cells while EEG data was collected. From this grip-and-relax task, the following

measures of paretic hand sensorimotor function were obtained: (1) reaction time, (2) relaxation time, (3) force magnitude control, and (4) force direction control. Functional neural connectivity was calculated as imaginary coherence within the sensorimotor regions (primary motor cortex, premotor cortex, and somatosensory cortex) within both brain hemispheres. Computations were independently run within the alpha (8-12Hz) and beta (13-30Hz) frequency bands for each grip phase. T1-weighted structural MRI scans were obtained and segmented via FreeSurfer for EEG source localization. Regression was used for statistical analysis.

Results: Each measure of sensorimotor performance was associated with a functional neural connectivity measure. The following results were observed. Reaction time was found to strongly associate with alpha band connectivity within the non-lesioned hemisphere during the grip preparation phase ($r = -0.61$; $p = 0.035$). Relaxation time was observed to strongly associate with beta band connectivity within the lesioned hemisphere during the grip execution phase ($r = 0.75$; $p = 0.007$). Force magnitude control strongly associated with alpha band connectivity within the lesioned hemisphere during the grip preparation phase ($r = 0.66$; $p = 0.021$). Lastly, force direction control strongly associated with alpha band connectivity during grip execution phase between the premotor and primary somatosensory cortices within the non-lesioned hemisphere; however, this association did not reach statistical significance ($r = -0.57$; $p = 0.051$). Associations between connectivity and standardized motor function tests (WMFT & BBT) were not observed.

Conclusion: The results of this proof-of-concept study support further, larger investigations into the relationships held between functional neural connectivity and specific aspects of sensorimotor control of the paretic hand in chronic stroke survivors. Understanding these relationships may prove pivotal in the development of personalized rehabilitation strategies for stroke survivors, targeting specific neural networks deemed responsible for a given motor deficit.

Sensorimotor neuroplasticity and impact on motor recovery in post-stroke, upper extremity rehabilitation

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Background: Many stroke survivors experience chronic motor and sensory impairments. Motor control requires proper working of both the motor and sensory pathways. Despite sensory feedback being integral to functional motor control, stroke rehabilitation research has focused primarily on investigating the corticospinal motor pathway only. The objective of this research is to determine the relative contributions of all three components of the sensorimotor pathways – the sensory pathway, sensorimotor integration, and motor pathway – to motor recovery following stroke and to assess changes of the sensorimotor pathways that occur with therapy.

Hypothesis: The integrity of each of the three components of the sensorimotor pathway at baseline will independently contribute to the extent of motor recovery with upper extremity rehabilitation intervention (Aim 1), and the integrity of the three components of the sensorimotor pathways will improve from pre- to post-upper extremity rehabilitation intervention (Aim 2).

Methods: 76 stroke survivors will undergo standardized hand task practice therapy 3 sessions/week for 6 weeks. Participants will be randomized such that the treatment group receives TheraBracelet subsensory vibration during therapy while the control group receives no vibration. Motor recovery will be quantified as change in the Wolf-Motor Function Test (WMFT) from pre- to post- upper extremity rehabilitation intervention. The three sensorimotor pathway components will be assessed at pre- and post- intervention: (1) sensory pathway integrity will be assessed by somatosensory evoked potential (SEP) using electroencephalogram (EEG); (2) sensorimotor integration will be assessed by cortical sensorimotor connectivity using EEG coherence; (3) motor pathway integrity will be assessed by motor evoked potential (MEP) using transcranial magnetic stimulation (TMS).

Statistical analysis: For Aim 1, multiple regression will be used to determine if the

three components of the sensorimotor pathway integrities prior to intervention (baseline sensory pathway integrity, sensorimotor integration, and motor pathway integrity as independent variables) independently predict motor recovery (reduction in WMFT time from pre- to post- intervention as the dependent variable), after accounting for group (treatment vs. control). For Aim 2, multiple regression will be used to determine if changes in the three sensorimotor pathway integrities from pre- to post- intervention (independent variables) explain motor recovery (dependent variable).

Impact: This study will clarify the importance of considering the sensory pathway and sensorimotor integration in addition to the motor pathway integrity for post-stroke motor recovery and neuroplasticity. Consideration of all neural pathways is expected to enhance prognosis of motor recovery post-stroke. In addition, the complete picture of the changes occurring in the sensorimotor pathways is expected to elucidate the neural mechanisms of motor recovery.

Family Cash Transfers and Maternal and Perinatal Health Outcomes

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Much literature in the US documents an intergenerational transmission of maternal and perinatal morbidity in socioeconomically disadvantaged groups. A separate line of work indicates that family cash transfers may improve life chances of low-income families well into adulthood. By exploiting a quasi-random natural experiment of a large family cash transfer among a southeastern American Indian (AI) tribe in rural North Carolina, we examine whether a "perturbation" in socioeconomic status during childhood improves maternal/perinatal outcomes when they become parents themselves. We acquired birth records on 6,805 AI and non-AI infants, born from 1995 to 2018, in three counties from the North Carolina Office of Vital Records. Difference-in-difference methods tested whether the mother's American Indian (AI) status and exposure to the family cash transfer during her childhood years corresponds with improvements in maternal and birth outcomes. Findings show an increase in age at childbearing (coef: 0.15 years, 95% confidence interval [CI]: 0.05, 0.25) and a decrease in pre-pregnancy body mass index (BMI; coef: -0.41, 95% CI: -0.74, -0.08) with increased duration of cash transfer exposure during childhood. Consistent with the pre-pregnancy BMI findings, infant birthweight is also reduced, yet remains within a healthy range, among AI births whose mother had relatively longer duration of exposure to the cash transfer. We, however, observe no relation with other maternal/perinatal outcomes (e.g., tobacco use during pregnancy, preterm birth). In this rural AI population, cash transfers in one generation correspond with improved maternal and infant health in the next generation.

TLR9 -/- Mice Are Not Protected Against Acetaminophen Induced Lung Injury Despite Altered STAT3 Signaling

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PURPOSE: Acetaminophen (APAP) is the most used analgesic worldwide, yet acute and chronic exposures can cause both lung and liver injury. Early life exposure increases the risk of developing asthma, however the contributing molecular pathways underlying this injury are unknown. While inhibition of proinflammatory TLR9 signaling prevents APAP induced liver injury, whether this pathway contributes to lung injury is unknown.

METHODS: Two strains of male C57BL/6 mice (N=4-6 per condition) were exposed with a dose of APAP (IP,140 mg/kg; 0/6hrs) during the late alveolar stage of lung development (PN14). The mice were wildtype and TLR9 -/-. Blinded histopathological scoring of pulmonary injury was performed. RT-qPCR was used to assess the pulmonary expression of inflammatory and oxidant stress response genes. Western blot was used to assess for activation of pro-inflammatory signaling pathways.

RESULTS: Il6 expression following APAP exposure (140 mg/kg; 6 hours) was significantly reduced in the Tlr9 -/- mice lung in comparison to wildtype mice. Phosphorylated STAT3 protein content was also significantly reduced in Tlr9 -/- mice lung nuclear protein fractions exposed to APAP (140 mg/kg; 6 hours) compared to wildtype samples. However, the only other NFkB target that demonstrated a significant decrease in pulmonary injury was IL1B and it only occurred in male mice ($p<0.05$). Moreover, the blinded Histopathological scoring revealed that APAP exposed samples increased in pulmonary injury, however the difference was not significant.

CONCLUSION: The attenuation of pulmonary STAT3 expression/signaling through Tlr9-/- does indeed reduce APAP induced injury. The potential for Tlr9-/- to serve as a potential therapeutic alternative towards neonatal pulmonary dysfunction is limited. More work is needed in understanding the relationship between Tlr9-/- and the influence STAT3 plays.

Categories and Clustering of Adversity in ABCD

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Early Life Adversity encompasses child maltreatment (CM) and other adversities such as the death of a parent and poverty which lack intentionality from the caregiver and chronicity. Caregiver intent and chronicity are almost always present in CM but lacking in many other ELA. Intent is an important aspect of the perception of threat and chronicity influences long-term cognitive development. Terms used inconsistently lead to inconsistent findings which has significant deleterious effects on biological mechanism development. The intent of this study is to examine how items naturally group using a person based approach to investigate how participants characterize their ELA in a large representative dataset.

Barriers and Facilitators of Implementing a Parent Engaged Intervention Focusing on Teens with a Traffic Citation (ProjectDRIVE)

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Purpose: Limited interventions have focused on high-risk teen drivers, such as those with a traffic citation. This qualitative study aimed to identify barriers and facilitators to the implementation of ProjectDRIVE, a parent communication-based intervention to improve safe driving practices among teen drivers who received a traffic citation.

Methods: We conducted virtual, semi-structured interviews with parents/guardians who completed ProjectDRIVE, a two-component intervention (parent effective communication training and in-vehicle technology feedback system). Participating parents were asked about their experience with ProjectDRIVE, including barriers and facilitators to program implementation. Completed interviews were transcribed verbatim and coded by two coders using a systematic, open, and focused coding process. Data were analyzed using ATLAS.ti to identify major themes and subthemes relating to the barriers and facilitators to the implementation of ProjectDRIVE.

Results: A total of 7 interviews were completed, with 2 males and 5 females. Three themes emerged, (1) scheduling, (2) intrinsic and extrinsic motivation, and (3) implementation. Scheduling acted as a barrier for implementing ProjectDRIVE; parents reported difficulty finding time to participate in the communication training and/or discussing safe driving practices with their teens. Intrinsic and extrinsic motivation were facilitators for study participation. Parents who were intrinsically motivated were more likely to have intentional conversations with their teens about safe driving. Support from the court system also promoted parental engagement. Implementation served as both a facilitator and barrier, depending on the parent's past experience and existing relationship with their teen.

Conclusion: Scheduling, motivation, and implementation had the largest impact on

the implementation of ProjectDRIVE following a teen's traffic citation. Future studies should address these barriers to facilitate successful implementation of interventions tailored to high-risk teen drivers.

Significance/Contribution: Our results contribute to a better understanding of unique barriers and facilitators to implement parent communication-based intervention with high-risk teen drivers who received a citation.

Keywords: Parent communication; Program implementation; Teen drivers; Safe driving; Qualitative

Risk evaluation in early adolescents is associated with striatal and salience network activity during reward anticipation

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Background: Adolescence is a time when risk taking and reward sensitivity are heightened; however, mechanisms underlying their developmental concurrence are not agreed upon. Further investigation is needed to elucidate the connection between adolescent risky behavior and reward hypersensitivity. Here, we examined how neural response to reward anticipation relates to evaluation of risk in early adolescence.

Methods: Participants from the Adolescent Brain and Cognitive Development (ABCD) study with year-two annual follow-up Monetary Incentive Delay (MID) fMRI scans and Social Influence Task (SIT) data were eligible for inclusion (N = 7069; ages 11-12 years). Bayesian multilevel modeling (BML) was used to investigate the relationship between risk evaluation of behavioral scenarios (SIT mean initial risk rating) and reward anticipation (MID task anticipation response in the reward vs. neutral contrast) in salience network regions of interest.

Results: BML demonstrated that higher initial risk ratings were associated with bilateral putamen deactivation (posterior mean beta-weight = -0.0243 (left), -0.0250 (right)) within 95% confidence intervals and bilateral caudate deactivation (posterior mean beta-weight = -0.0184 (left), -0.019 (right)) within 90% confidence intervals. Notably, there were no significant associations between initial risk ratings and activation in neither the bilateral anterior cingulate cortex, insula, nor nucleus accumbens.

Conclusions: These results suggest that decreased activity in the dorsal striatum during reward anticipation is associated with more conservative risk evaluation in young adolescents. This association may relate to later variability in elevated risk taking behavior and reward sensitivity.

Study Protocol for Examining Connection With Recovery Support Services Among Clinics That Provide Medications for Opioid Use Disorder

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The opioid epidemic has seen a major increase in opioid overdose fatalities and Opioid Use Disorder (OUD) within the past year. OUD is a chronic condition best managed with long-term use of medications (MOUDS). The U.S. National Drug Control Strategy highlights the importance of recovery support services in engaging and supporting people throughout all stages of addiction recovery. Recovery Community Centers (RCCs) are centers located in the heart of their respective communities that support multiple pathways to recovery including MOUDs and provide a variety of recovery support services. RCCs can potentially be viable and important allies to clinics providing MOUDs, but many clinics may be unaware of RCCs. This study is aimed to determine to what degree clinics that provide medications for opioid use disorder (MOUDs) are aware of RCCs, the association between proximity to RCCs and services provided among clinics that provide MOUDs and gain feedback from clinic staff about what may be needed to support networking between MOUD-providing treatment settings and RCCs. We focused on the 20 oldest and therefore most established RCCs nationwide, as identified in prior work (1R24DA051988). Using the SAMHSA treatment locator, for each RCC, we identified two clinics: Near; within 5 miles (n=20) and far; at least 50 miles away (n=18; n=2 did not have a clinic further than 50 miles away, but less than 200 miles). Only data collected from the SAMHSA Treatment Locator on recovery support services providing by MOUD-providing clinics were available for analysis. We provide descriptive statistics (i.e., percentages with sample sizes) to summarize use of on-site recovery support services and related indices. We conducted logistics regressions to test if distance to nearest RCC (coded binary, near vs. far) was related to these variables (p-values for statistical significance; r-sq for effect size estimate). Results did not show a significant difference between proximity to recovery community centers and recovery support services offered by MOUD-providing clinics. Few clinics (2.6%) have no connection to recovery support services, only a third (31.6%) of clinics offer

recovery coaching, and a third (31.6%) of clinics utilize linkage procedures (i.e., 12-step facilitation). Data collections are ongoing. Connecting with clinic directors has been significantly more challenging than connecting with RCCs, possibly echoing experiences patients may have as well. SAMHSA treatment locator results support that clinics in general appear to be open to recovery support services; including having community-linkage as part of their treatment approach (i.e., 12-Step facilitation), however few provide recovery coaching, which might make RCCs important allies.

Geospatial Perspective of the Pennsylvania Tobacco Retail Environment

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Introduction. More than 480,000 deaths are caused each year in the U.S. due to cigarette smoking. Quitting smoking reduces the risk of premature death; however, a disparity exists in annual quit rates between African American and White individuals who smoke (4.9% vs. 7.1%). Despite making more quit attempts than White individuals who smoke, African American individuals are less likely to remain abstinent from smoking long-term. Previous literature has shown that psychosocial factors (e.g., stress) and an individual's physical environment contribute to tobacco-related behaviors and inhibit smoking cessation. The aim of this study was to examine the physical environment (tobacco retailer density) of those who smoke and used the text messaging service of the Pennsylvania Quitline.

Methods. We identified tobacco retailers (TRs) who were currently licensed to sell tobacco in Pennsylvania using the Tobacco Products Tax Licenses dataset from the Pennsylvania Department of Revenue. The list is updated monthly with data to include the retailer's name, address (geocode), licensing type, and county.

The American Community Survey 5-year estimates geodatabase was used to derive numbers for rural/urban areas and population. TR density per 1,000 people was calculated. The Behavioral Risk Factor Surveillance System (BRFSS) Survey was used to derive adult smoking prevalence estimates. Results. Overall, there were 14,727 TRs in Pennsylvania's urban (n=9,709) and rural (n=5,018) areas and a mean density of about 1.2 TRs per 1,000 population. Conclusion. The findings suggest that a higher proportion of TRs are in the urban areas of Pennsylvania; however, smoking prevalence is higher in rural areas. Additional analysis is needed to determine whether TRs are in areas with a higher proportion of African Americans. This research will inform future smoking cessation interventions.

ACEs and Alcohol Use Expectancies among Latin Youth: The Role of Resting-State Functional Connectivity (rsFC), Neurocognition, and Parental Familism

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Purpose: Alcohol use patterns in childhood are essentially nonexistent, but for select youth experimentation occurs in the transition to pre-adolescence. This early initiation of alcohol can have long-term adverse effects on academic achievement, behavioral problems, and neurodevelopmental trajectories. Even in cases where alcohol use initiation is delayed until later years, the transition from childhood to preadolescence appears to be characterized by initial thoughts or cognitions regarding alcohol use experimentation and expectations. Prior research has suggested that exposure to high levels of adverse childhood experiences (ACEs) significantly affects alcohol use initiation and misuse among youth. Further, ACEs-based literature has indicated that cultural factors such as familismo and acculturation may decrease or increase alcohol use risks. However, increased risks in the transition to preadolescence are not universal for all youth with distinct neurodevelopmental functioning, exposure to ACEs, or both. Hence, it is essential to understand how and under what conditions ACEs may influence alcohol use susceptibility before adolescence. This investigation aimed to 1) Explore whether neurocognitive functioning (i.e., crystallized intelligence and executive function) and rsFC (i.e., connectivity between the ventral attention network and the left-caudate and connectivity between the frontoparietal and cingulo-opercular networks) mediate the relation between ACEs and negative alcohol use expectancies; 2) Explore whether parents' adherence to familismo values moderates the relation of the aforementioned paths, especially between ACEs and negative alcohol use expectations. Methods: This investigation is a secondary analysis of data involving two time points spanning two years from the ABCD Study (https://abcdstudy.org). The ABCD Study is a 21-site, 10-year longitudinal investigation of cognitive development in a demographically representative sample of U.S.-residing children. Data: Data from 2224 families (parents and children aged 8 through 10 at enrollment) of Latin descent are examined in this investigation. Results: Neither the rsFC nor neurocognitive variables mediated the

relation between ACEs and negative alcohol use expectancies. However, simple moderation effects on several paths were identified. Interestingly, parents with higher levels of acculturation may lower a youth's susceptibility to alcohol use in response to ACEs, while parents with lower levels of acculturation may encourage youth to develop unique cognitive adaptive strategies when facing ACEs.

Conclusion: Determining the effect of ACEs on alcohol use risk factors and the potential risk and protective effects of cultural factors has important implications for etiological models of addiction and preventive-intervention efforts.

Acceptability of biospecimen donation for substance use research among Black and Hispanic/Latinx SGM people

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Background: Black and Hispanic/Latinx LGBTQ+ people are at greater risk for substance use compared to heterosexual and cisgender people of the same racial and ethnic backgrounds (Day et al., 2017; Mereish et al., 2019; Ocasio et al., 2016). Black and Hispanic/Latinx LGBTQ+ people may be less likely to donate biospecimens for substance use research compared to their White counterparts (Scott et al., 2010). The aim of this study was to examine factors affecting willingness to donate biospecimens (i.e., blood, saliva, urine, hair) for substance use research among Black and Hispanic/Latinx LGBTQ+ people regardless of past or current substance use.

Methods: In-depth phone or video interviews were conducted with 22 Black and Hispanic/Latinx LGBTQ+ people from The PRIDE Study, a longitudinal cohort study of LGBTQ+ people living in the United States and its territories. Fourteen participants were sexual minority (i.e., did not exclusively identify their sexual orientation as heterosexual) and eight participants were gender minority (i.e., individuals whose gender identity differs from that often associated with their sex assigned at birth). We used an inductive thematic analysis approach to identify themes and patterns among the transcribed interviews.

Results: Themes included: (1) community benefits, (2) personal benefits, (3) community risk, (4) personal risks, (5) convenience, (6) trustworthiness of the research team, (7) perceived value in donating, and (8) normalization of biospecimen collection. Participants were generally motivated to engage in biospecimen donation for altruistic purposes. The most cited concerns were related to data security, privacy, and misuse.

Conclusion: Researchers must be proactive in building trust with Black and Hispanic/Latinx communities to increase engagement, which can lead to diversification of biospecimen repositories and reduction of health inequities.

Future research involving biospecimens should provide education about biospecimens during the consent process and prioritize participant convenience.

Community social relations and their impact on Black and Latine peoples' substance use recovery journey

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Black/Latine with substance use disorders (SUD)s Dying at Disproportionate Rates. Black and Latinx people with SUDs have seen a 140%/118% increase in death due to fentanyl and xylazine. Racial/ethnic minorities in the US are less likely than whites to seek mental health treatment (including drug and alcohol). Compared with other racial groups, Black and Latine people are less likely to start drug and alcohol treatment. Religion and spirituality enable Black and Latine people to cope with psychological distress (mind stress). In urban Black communities (cities), 65-80% of adults attend church regularly; 75% of Latine populations living in urban communities attend church regularly. Strong social ties among people with alcohol use disorder (AUD) and opioid use disorder (OUD) and the excitement and pleasure associated with drug subculture add to the challenges of social change. Self-stigma and discrimination are also important barriers in this process. In this sense, together with the social determinants of health (SDOH), the complexity of the drug subculture is expressed in the different clusters of cultural elements related to the social networks of Black and Latinx with AUD or OUD. Developed through a community-based participatory process. Learning through Conversations with communities and faith-based leaders and members. Through conversations with the organizing team, using suggestions from communities to modify and develop the faith-based recovery program. Selected a model to combine the 5 Rs (Rowe et al.) and 8 Dimensions of Wellness (Swarbrick) with wrap-around coaches. We hypothesize 1) that the social network of Black and Latine people with AUD and OUD is an essential and measurable moderator of recovery; 2) that the Imani Breakthrough intervention can act as an agent of social network change because it increases participants' social capital. The Imani Breakthrough intervention also triggers the Black and Latine communities to change their views of AUD and OUD and inspires acts of solidarity from community members toward Imani participants; and that Social Network Analysis is feasible, acceptable, safe, and tolerable among Black and Latinx people with AUD or OUD. Preliminary results show that the mean number of people with

whom participants discuss important matters and, at the same time, are helpful with their goals (positive contacts): 5.3 positive contacts per participant. Also, the mean number of people with whom participants discuss important matters and, at the same time, move the participant away from their goals (negative contacts): 1.5 negative contacts. Finally, 54% of participants have had pain today other than common once-in-a-while types of pain, such as headaches or toothaches.

Exploring the Links between Vicarious Racism and Substance-Use: a cross-sectional study

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Racism is a pervasive and persistent threat to social health with differential impact based on race and ethnicity. Considering the continued perpetration and visibility of racism online and in the news, vicarious racism (VR), or "secondhand" racism, is a particularly urgent threat in the context of such disparities and their subsequent consequences for health. In comparison to the literature on interpersonal racism, empirical research on vicarious racism is limited, especially as it relates to substance-use, a potent health behavior. Furthermore, protective factors against the health consequences of VR are unknown. Thus, the current study examines if VR exposure and emotional impact are linked to substance-use, and explores the roles of feelings of social connection and strength of ethnic in-group identification (ethnic identity) in moderating these relationships. In a cross-sectional survey, 504 participants identifying as Black/African American or Latine reported on their experiences with VR and substance-use over the past 30 days. Among the full sample, there were positive associations between reported VR emotional impact and both total substances used and frequency of use. Ethnic identity moderated the association between VR exposure and total substances used in that those reporting lower ethnic identity showed the positive association whereas those higher did not. Taken together, our results indicate that vicarious racism is indeed linked with substance-use behaviors, and suggest that strong ethnic in-group identification may be protective for vicarious racism-related substance-use among these groups. Further research is needed in order to develop interventions and increase awareness as vicarious racism becomes an increasingly common experience among marginalized populations.

Genetically-encoded probes for labeling neurotransmitter-defined synaptic vesicles

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Vesicular transporters represent highly specific targets for the development of molecular tools for mapping brain circuits by allowing precise specification and imaging of synaptic vesicles (SVs) and synapses based on their neurochemical identity. Conventional tools used to visualize neurotransmitter-defined SVs and synapses rely on immunolabeling and have key limitations, e.g., tissue processing reduces antigenicity and the use of detergents needed for bulky antibody permeability damages tissue ultrastructure, making immunolabeling suboptimal for use with some modern electron microscopy (EM) techniques, such as serial block-face scanning 3D EM. Here, we expand on our approach for labeling SVs using genetically-engineered tagged vesicular transporters. This approach is fully compatible with a wide array of light and EM techniques and can be combined with photooxidation to label SVs without the use of antibodies. We developed a suite of genetically encoded probes for identification of glutamatergic, GABAergic, and monoaminergic SVs using both light and EM applications by fusing mini singlet oxygen generator (miniSOG) or HaloTag to intralumenal- or cytoplasmic-facing domains of vesicular transporters for glutamate (VGLUT2), GABA & glycine (VGAT), and monoamines (VMAT2). We packaged genes encoding these tagged transporters into Cre-dependent adeno-associated virus (AAV) vectors and assessed their expression and trafficking by both light and EM, both in primary neuronal cultures and in vivo using appropriate Cre-expressing mouse lines. In particular, we highlight photooxidation labeling of SVs catalyzed by miniSOG and Janelia Fluor HaloTag ligands for visualization by EM. These tools represent a new resource for accessing subcellular localization of SVs in presynaptic terminals defined by their unique neurochemical profiles.

RNA SEQUENCE ANALYSES IMPLICATE IMMUNITY AND FATTY ACID METABOLISM IN PERINATAL OXYCODONE-EXPOSED OFFSPRING DEFICITS

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Perinatal opioid research and its impact on offspring is an emerging field of interest to scientists. Oxycodone (OXY) is a major contributor to the opioid epidemic, particularly during the first stages of the epidemic. Therefore, studying OXY-exposed offspring in the context of adolescence is crucial as many exposed are reaching this age. However, research has not used high-throughput methods to investigate these impacts to provide a detailed characterization of pathways affected in these offspring. This study used two robust perinatal OXY models to address two key natures of opioid exposure, prenatal (IUO) and (PNO) postnatal exposure. We employed RNA sequencing of the prefrontal cortex to analyze pathways enrichment in PNO- and IUO-exposed offspring. Gene Set Enrichment Analysis (GSEA) and Ingenuity Pathway Analysis (IPA) found that genes associated with disruption of synapse homeostasis were significantly enriched. Genes associated with social disorders and long-term depression were found to be upregulated in PNO-offspring, but not IUO-offspring. IUO-offspring were seen to have significant negative enrichment of innate and humoral immunity. Interestingly, oxidative stress and synaptogenesis pathways were significantly impaired according to IPA in both PNO- and IUO-offspring, predicting these pathways to be upregulated on the protein level. Oxidative stress predictions were validated using western blot analysis of purified synaptosomes to find fatty acid synthase and mitochondrial Complex II were upregulated in PNO- and IUO-offspring. These pathways are implicated in the progression of complex psychological disorders such as PTSD, suggesting that impacts of exposed offspring share aspects of pathophysiology. Collectively, our studies suggest that perinatal OXY exposure prime the developmental immune systems to produce previously characterized deficits.

Examining the role of community conditions on coalition functioning for community drug prevention coalitions.

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Multiple community partnership models for health promotion have been shown to influence youth and community health outcomes. Community contextual factors often are underlying conditions that contribute youth and community health and problems. Context is also important coalitions as it can relate to community readiness, and in some cases program and coalition success are sensitive to those contextual factors. In many communities across the U.S., drug and violence prevention coalitions operate in challenging environments that may impede a coalition's capacity to achieve success. Thus, advancing knowledge on how and in what ways community context contributes to coalition capacity can be an important endeavor to help identify and address initial and ongoing barriers to coalition capacity and success, but also to elucidate strengths within community context that can promote coalition functioning.

Sex specific neuroimmune signal suppression in the ventral striatum due to nicotine use differentially regulated by steroid hormones

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Craving and relapse to smoking appear to vary as a function of biological sex, whereby women face unique challenges with use of nicotine-containing products which appear to be critically impacted by ovary-derived steroid hormones. Progesterone (P4), a steroid hormone that is anti-inflammatory, has shown clinical efficacy in promoting smoking cessation in women although the mechanisms of this are unknown. There are clinical signals implicating immune system dysregulation in women who smoke, such as changes in circulating cytokines. Preclinically, it has been shown that neuroimmune signaling plays a vital role in modulating responses through the regulation of neural activity and plasticity in key brain regions within the reward pathway. Here we found that neuroimmune signaling as measured via microglia morphology and cytokine profiles within the nucleus accumbens core (NAcore) are driven by nicotine use during self-administration (SA) in a sex-specific fashion, whereby female rats were more susceptible to nicotine-induced neuroimmune consequences as compared to males which was reversed by P4 only in females. Specifically, we found that females display unique structural changes in NAcore microglia (ANOVAs, p's<0.05) indicating that these cells are stuck in a homeostatic state, unable to respond to injurious stimuli following nicotine SA. P4 was able to reverse this and increase the number of microglia present only in females. Supporting this female-specific structural consequence, we found that nicotine SA altered cytokine profiles within the ventral striatum as compared to saline SA controls whereby TNFÎ±, IL-6, G-CSF, IL-1Î±, VEGF, leptin, among others were suppressed (t tests, p's<0.05). However, fractalkine, which is released from neurons as a "find me" signal to recruit microglial processes for clearance of apoptotic cells and it also regulates glutamate neurotransmission, was significantly increased by nicotine SA. Conversely, specific peripheral circulating cytokines in serum were increased, including TNFÎ±, IL-6, IL-1Î± and IL-18 (t tests, p's<0.05). Together, these results indicate critical sex differences in nicotine-induced dysregulation of the

neuroimmune and peripheral immune landscape, which may have translational implications for novel sex-specific therapeutics for smoking cessation.

Lived Experiences among Suburban Mothers and Pregnant Women who use Opioids and ACCESSED METHADONE Assisted Treatment and Harm Reduction Since COVID-19

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Background: The United States recently experienced two public health emergencies, the COVID-19 pandemic and an ongoing opioid epidemic, which have both compounded and exerted a power shock to individuals with opioid use disorder (OUD) (Harris, 2022; Leppla, 2022). Opioid antagonist medications such as buprenorphine and methadone are known for reducing overdoses and improving drug use and high risk behaviors. Methadone is solely dispensed at opioid treatment programs (OTPs) which are strictly regulated by federal, state, and local regulations. Buprenorphine is prescribed by providers with a controlled substance license which limits them to treat thirty patients. Prior to the pandemic strict regulations and treatment models at OTPs created inflexibility and time consumption for individuals seeking treatment. In March 2020, the Substance Abuse and Mental Health Services Administration (SAMHSA) recognized the public health and safety issues in relation to COVID-19 and adjusted administration requirements by providing permission to OTPs to dispense up to 28 days of take-home to stable patients and 14 days of take homes to unstable patients (Amram, 2021; Frank 2021; Jemberie, 2020; Watson 2022). Telehealth visits became very prominent and were utilized for individualized counseling, groups, and provider visits which allowed clinicians and patients to have flexibility in appointment times (Hughto, 2021). Many individuals benefited from avoiding barriers such as limited clinic hours, location of clinic, transportation limitations, and childcare. However, others also found the changes to be difficult due to their reliance on remaining engaged emotionally and cognitively on in person treatment models.

Aims/Methods: The aims of this study were developed from a parent study, "Suburban Opioid Study-Providing for Opioid-using Mothers and Pregnant Women who need Treatment" (SOS-PrOMPT), a longitudinal study with the aim to gain an in-depth understanding of the lived experiences of suburban women with

OUD who are pregnant or mothers of young children. A mixed methods approach was utilized which incorporated qualitative interviews and surveys conducted with women who are mothers of children or pregnant and living with opioid use disorder in the suburbs of Newark, NJ and New Haven, Connecticut. Members of the research team developed a semi-structured interview guide, a life history trajectory, and surveys which covered demographics, opioid use, treatment, health, social services, and the impact of COVID-19. The analysis primarily focuses on the lived experiences of women who accessed Methadone Assisted Treatment (MAT) and harm reduction services since the COVID-19 pandemic.

Results/Conclusion: The analysis yielded two main themes which diverged into four sub themes: Access: Access to MATs, Access to Syringe Exchange Programs (SEPs) and Barriers: Barriers to MATs, Barriers to SEPs. Together the themes concentrated on the access and barriers to MATs and SEPs during the COVID-19 pandemic. The theme access to MATs was defined as a form of freedom as persons with OUD found less frequent clinic visits accommodating which allowed them to manage their medications independently and utilize their time to provide for their family, work, continue education, and fulfill other leisure activities. On the other hand, barriers to MATs focused on socioeconomic and geographic related issues such as lack of transportation or childcare, limited clinic hours, poor clinical staff attitudes, healthcare inequities, and etc. which destabilized substance use goals for individuals with OUD. Access and barriers to SEPs were a difficult area to obtain data for in our sample since majority of the women were not current injection drug users. A small number of women who were suffered with injection drug use were lost to contact before data collection began for the supplemental data. The management of the opioid crisis during the COVID-19 pandemic has brought forth challenges that vulnerable persons with OUD encounter and thus has been the most concerning among substance use disorders (Dubey, 2020).

Longitudinal Associations Between Alcohol Use and Depressive Symptoms Among Latinx Youth in Rural Communities in the U.S.

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Introduction: For some youth, adolescence can be a period of increases in alcohol use and depressive symptoms. Little is known about these links among Latinx youth in rural communities in the U.S., a growing and underserved segment of the U.S. population. Understanding the longitudinal links between alcohol use and depressive symptoms in this population will be informative for etiological and prevention work. The present study examined alcohol use and depressive symptoms over the course of adolescence among Latinx children of immigrants (COI; foreign-born children and children of foreign-born parents), Latinx children of non-immigrants (CONI; U.S.-born children of U.S.-born parents) and non-Latinx White CONI living in rural or small-town communities.

Methods: Longitudinal data were from youth who participated in the control group of a community-randomized trial of the Communities That Care Prevention system (N = 1386; 47% female; 23% Latinx COI; 11% Latinx NCONI; 66% non-Latinx White NCONI) from 9 rural or small-town communities in five states. We examined links between trajectories of past-month alcohol use frequency and depressive symptoms over the course of adolescence (Grades 6-12).

Results: Parallel process latent growth models were conducted, and all models adjusted for nesting of participants in their communities by including community fixed effects. Covariates were entered in 2 steps: Model 1 included race-ethnicity and immigrant generation status and Model 2 included age, gender, rebelliousness, and parent education. Models 1 and 2 showed analogous findings with respect to race-ethnicity and immigrant generation status. Specifically, Latinx CONI reported

steeper increases in alcohol use over the course of adolescence than Latinx COI. Latinx CONI reported more depressive symptoms and higher levels of alcohol use at the beginning of high school (Grade 9, age: M = 15.08, SD = 0.38) and steeper increases in alcohol use over the course of adolescence than non-Latinx White CONI. Adolescents' reports of alcohol use and depressive symptoms at the beginning of high school were positively associated. Reports of alcohol use and depressive symptoms at the beginning of high school were positively associated with alcohol use over the course of adolescence.

Conclusions: Findings suggest that alcohol use and depressive symptoms are positively associated across adolescence and future research should identify factors that explain disparities in alcohol use and depressive symptoms between Latinx COI and non-Latinx White CONI with Latinx CONI.

Effort-Driven Attentional Capture

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How we direct attention to stimuli in our environment reflects, in part, the value we associate with these stimuli (Anderson et al., 2011). When stimuli serve as predictive signals for reward or punishment, they become prioritized by the attention system. We wondered whether one's experience associating stimuli with the need to exert effort would recruit this same learning-dependent mechanism, and if so, whether one would be biased to direct their attention toward a stimulus associated with high effort (aversive stimulus) or relatively low effort (negative reinforcement). In one experiment (N=44), participants searched for a color-defined target (red or green circle) among differently colored non-target circles. On each trial, after reporting the target, they were required to squeeze a dynamometer (hand grip device) across conditions of high- or low-effort, and critically, each level of effort was linked to a target color. In a subsequent test phase, the search task changed to a search for the unique shape in the array while non-targets were occasionally rendered in the color of one of the effort-associated targets from training (critical distractor). Analysis of response times (RTs) showed that participants were slower to report the target in the training phase when it was associated with high effort, possibly reflecting a reluctance to initiate the corresponding grip requirement. Importantly, there was a main effect of the distractor condition in the test phase, driven by a slowing of responses by the high-effort distractor relative to distractor-absent trials. RT in the low-effort distractor condition was intermediately impacted, differing from neither of the other two conditions statistically. In an ongoing study, we seek to replicate this effect using a paradigm in which stimuli previously associated with high- and low-effort directly compete for attention on some trials, potentially providing a more sensitive measure of differential effort-based attentional priority.

The effects of cannabis use patterns and bipolar disorder on risky decision-making

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Background: Knowledge of detailed cannabis use patterns is important for populations that report using cannabis to treat cognitive symptoms, e.g., people with bipolar disorder (BD+). We recently reported that chronic cannabis use is associated with improved risky decision-making in BD+ participants, but not in healthy comparison participants (HC). Data on these factors may help elucidate the mechanisms underlying the effects of cannabis on cognition.

Methods: We recruited BD+ (n= 27) and HC (n=26) participants who regularly use cannabis. We collected cannabis use data (i.e., reasons for use, potency, route of administration, age of initiation and frequency), and tested participants on decision-making using the Iowa Gambling Task. Relationships between cannabis use factors and risky decision-making were examined.

Results: Most cannabis users reported high Î”9-tetrahydrocannabinol (THC) as their preferred cannabis potency and inhalation as the route of administration; there were no significant differences between BD+ and HC-. HC participants tended to initiate cannabis use at an earlier age, however these data were incomplete. Daily cannabis use was associated with worse risky decision-making, (F (1,6.3), p=.016, ES=.113) irrespective of diagnosis.

Discussion: There were no significant differences in cannabis use patterns between BD+ and HC participants, thus it remains unclear why cannabis use is associated with improved risky decision-making in BD+, but not HC. This separation could indicate that cannabis modulates neurotransmitter release differently in BD+ versus HC. We are testing how cannabis may differentially modulate neurotransmission by measuring neurobiological markers (e.g., anandamide) after acute cannabis administration in BD+ and HC participants.

You are what you vape: Implications of chronic Δ9-THC self-administration in changes in the gut microbiome of rats.

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Vaping of cannabis and its constituents, such as Δ9-tetrahydrocanabinol (Δ9-THC), has been promoted as a safer alternative to smoked Cannabis. Nevertheless, chronic effect of Δ9-THC vaping in gut microbiome modulation and its effect in health and or disease is still largely unknown. Hence, this project intends to study the effect of chronic Δ9-THC vapor self-administration on changes in the gut microbiome of Sprague-Dawley rats. Female rats were randomly assigned to either vehicle vapor (100% propylene glycol [PG]) or Δ9-THC vapor groups and were allowed to self-administer PG or Δ9-THC vapor puffs in operant chambers for one hour on intermittent days for a total of nine months. At the end of this period, these rats underwent behavioral testing to determine anxiety-like behaviors using an open field test. Afterwards, rats were euthanized with isoflurane overdose and rapid decapitation. Feces, metabolic and immune organs, and serum were collected at the time of necropsy. Fecal DNA was extracted, and relative bacterial gut abundance was determined by sequencing the bacterial ribosomal 16S hypervariable region V4. From the pancreas, colon, heart, kidney, liver, colon, mesenteric lymph node, lung and muscle, RNA was extracted and relative expression of genes from the immune and the endocannabinoid system were measured using RT-PCR. Results shows that rats exposed to âˆ†9-THC chronic self-administration show anxiety-like behavior in the open field test, when compared with the PG exposed group. This change in behavior is also accompanied by an imbalance in the Firmicutes/Bacteroides when compared with the PG group. Furthermore, significant differences were found in the relative expression of MX1 in the muscle, CCL2 in the lung, TNF-a in the pancreas and PPARY in the Mesenteric Lymph Node. Protein from these same organs was extracted and currently determination of NF-kB and STAT1 are being determined using Western Blot analyses. This study is one of the first to study Δ9-THC chronic self-administration and its effect in behavior, microbiome and expression of the immune and endocannabinoid systems.

Prenatal Cannabinoid Exposure Leads to 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.

Pregnant dams are exposed to delta9-THC via passive inhalation of vaporized drug solution containing delta9-THC 100mg/mL in PEG400 prenatally from GD5 to GD 21 or 22). The THC detected in dams is consistent with a moderate dose of THC in humans and THC is present in the brain, and plasma of pups immediately after birth. The first aim of our study will assess how pre- and post-synaptic GABAergic signaling is altered in PCE rodents. 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. The third aim will investigate the effect of altered GABAergic signaling in PCE rodents' learning and memory performance. For aim 1, 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. Open Field Test with Novel Object Recognition investigates long-term memory in the hippocampus and the medial prefrontal cortex. Y Maze with Novel Arm will investigate short-term spatial memory and Elevated Plus Maze investigates anxiety-related behavior. 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.

GABA receptor delta subunit expression across the estrous cycle

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Learning and memory appears to be modulated across the menstrual cycle in women, suggesting that circulating hormones such as estradiol and progesterone may play a role. The dorsal hippocampus and ventral tegmental area (VTA) are important for learning and memory, and are also highly sensitive to circulating hormone levels. The expression of GABA receptors, in particular the delta subunit, is regulated by neurosteroids, including estrogen and progesterone. Extrasynaptic GABA receptors mediate a constant tonic inhibition and respond to low and moderate concentrations of ambient GABA found in the extrasynaptic space. Thus, neurosteroids can modulate neuronal activity through their actions at the GABA receptor. To determine if expression of the delta subunit of GABAA receptors was modulated across the estrous cycle, female C57BI/6J mice were perfused in the four phases of the estrous cycle and immunoreactivity for the delta subunit was assessed in hippocampal subregions and the VTA. Expression of the delta subunit is enhanced in the prostrus phase in the dentate gyrus and CA3 subfield, but not CA1 or CA2. Expression of the delta subunit is reduced in estrus in the VTA. Our results raise the possibility that fluctuations in the expression of the delta subunit of the GABA receptor may have implications for estrous cycle-dependent alterations in behavior.